EMJ EUROPEAN MEDICAL JOURNAL

HEPATOLOGY

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INSIDE

Review of

ILC 2014

London, UK

MOMENTS LIKE THIS...

SOVALDI®▼ transforms HCV therapy, allowing more adult patients the opportunity of cure

In clinical trials

- The first approved nucleotide polymerase inhibitor with pan-genotypic activity and a high barrier to resistance[1]
- ≥90% of treatment-naïve adults, across all genotypes are cured with 12 weeks of SOVALDI + Peg-IFN + RBV (No Peg-IFN required for GT 2)[1]
- The first all-oral 24-week option across all genotypes for those unsuitable for IFN (12-24 week treatment duration for GT 2)[1]
- No specific adverse drug reactions identified for SOVALDI within SOVALDI + RBV ± Peg-IFN regimens[1]

SOVALDI is indicated in combination with other medicinal products for the treatment of chronic hepatitis C (CHC) in adults.[1]

SOVALDI®▼ PRESCRIBING INFORMATION
Presentation: Sovaldi film-coated tablet for oral use. Each film-coated tablet contains 400mg of sofosbuvir.
Indications: in combination with other medicinal products for the treatment of chronic hepatitis C (CHC) in adults.
Dosage & Administration: Adults: One 400mg tablet, taken orally, once daily with food. Sovaldi should be used in combination with other medicinal products. Genotype 1, 3, 4, 5 and 6: 12 weeks treatment with Sovaldi in combination with ribavirin & peginterferon alfa is recommended. Genotype 2: 12 weeks treatment with Sovaldi in combination with ribavirin is recommended. 24 weeks treatment option with Sovaldi in combination with ribavirin for Genotype 1,3,4,5 and 6 can also be considered. Please refer to the SmPC for recommended dose & treatment duration for combination therapy. Monotherapy of Sovaldi is not recommended. Dose reduction is not recommended. If a patient has a serious adverse reaction potentially related to peginterferon alfa and/or ribavirin, their doses should be reduced or discontinued. Refer to the individual SmPCs for additional information. If a patient has a serious adverse reaction potentially related to ribavirin, the ribavirin dose should be modified or discontinued, if appropriate, until the adverse reaction abates or decreases in severity. Please refer to the SmPC for guidelines for dose modifications and discontinuation based on the patient's haemoglobin concentration and cardiac status. If the other agents used in combination with Sovaldi are permanently discontinued, Sovaldi should also be discontinued. Renal impairment: No dose adjustment of Sovaldi is required for patients with mild or moderate renal impairment. The safety and appropriate dose of Sovaldi have not been established in patients with severe renal impairment (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m^2) or end stage renal disease (ESRD) requiring haemodialysis. Refer to the SmPC for ribavirin for patients with creatinine clearance (CrCl) < 50 mL/min. Hepatic impairment: No dose adjustment of Sovaldi is required for patients with mild, moderate or severe hepatic impairment (Child-Pugh-Turcotte [CPT] Class A, B or C). The safety and efficacy of Sovaldi have not been established in patients with decompensated cirrhosis. Refer to the SmPC for peginterferon alfa for contraindication in hepatic decompensation. Patients awaiting liver transplantation: The duration of administration of Sovaldi in patients awaiting liver transplantation should be guided by an assessment of the potential benefits and risks for the individual patient. Children and adolescents: The safety and efficacy of Sovaldi in children & adolescents aged <18 years have not yet been established. Elderly: No dose adjustment is warranted for elderly patients.
Contraindications: Hypersensitivity to the active substance or to any of the excipients. When Sovaldi is used in combination with peginterferon alfa/ribavirin or ribavirin, the contraindications applicable to those agents are applicable to combination therapies. Refer to their SmPCs for a list of their contraindications.
Warnings and Precautions: Monotherapy of Sovaldi is not recommended. It should be prescribed in combination with other medicinal products for the treatment of CHC. If the other medicinal products used in combination with Sovaldi are permanently discontinued, Sovaldi should also be discontinued. Consult the SmPCs for co-prescribed medicinal products before starting therapy with Sovaldi. Pregnancy and concomitant use with ribavirin: When Sovaldi is used in combination with ribavirin or peginterferon alfa/ribavirin, extreme care must be taken to avoid pregnancy in female patients and in female partners of male patients. When Sovaldi is used in combination with ribavirin or peginterferon alfa/ribavirin, women of childbearing potential and their male partners must use an effective form of contraception during the treatment and for a period of time after the treatment. Refer to the SmPC for ribavirin for full recommendations. Use with potent P-gp inducers: Medicinal products that are potent P-glycoprotein (P-gp) inducers in the intestine (e.g. rifampicin, St. John's wort *[Hypericum perforatum]*, carbamazepine and phenytoin) may significantly decrease sofosbuvir plasma concentration leading to reduced therapeutic effect of Sovaldi. Such medicinal products should not be used with Sovaldi. Renal impairment: The safety of Sovaldi has not been assessed in subjects with severe renal impairment ([eGFR] < 30 mL/min/1.73 m^2) or ESRD requiring haemodialysis. Refer to the SmPC for ribavirin for patients with CrCl < 50 mL/min. HCV/HBV co-infection: There are no data available. Children and adolescents: Sovaldi is not recommended for use in children and adolescents under 18 years of age because the safety and efficacy have not been established in this population.
Interactions: Sofosbuvir is a nucleotide prodrug. After oral administration of Sovaldi, sofosbuvir is rapidly converted to the predominant inactive circulating metabolite GS-331007 that accounts for greater than 90% of drug-related material systemic exposure, while the parent sofosbuvir accounts for approximately 4% of drug-related material. In clinical pharmacology studies, both sofosbuvir and GS-331007 were monitored for purposes of pharmacokinetic analyses. Sofosbuvir is a substrate of drug transporter P-gp and breast cancer resistance protein (BCRP) while GS-331007 is not. Medicinal products that are potent P-gp inducers in the intestine (e.g. rifampicin, St. John's wort, carbamazepine and phenytoin) may decrease sofosbuvir plasma concentration leading to reduced therapeutic effect of Sovaldi and thus should not be used with Sovaldi. Co-administration of Sovaldi with medicinal products that inhibit P-gp and/or BCRP may increase sofosbuvir plasma concentration without increasing GS-331007 plasma concentration; accordingly, Sovaldi may be co-administered with P-gp and/or BCRP inhibitors. Sofosbuvir and GS-331007 are not inhibitors of P-gp and BCRP and thus are not expected to increase exposures of medicinal products that are substrates of these transporters. The intracellular metabolic activation pathway of sofosbuvir is mediated by generally low affinity and high capacity hydrolase and nucleotide phosphorylation pathways that are unlikely to be affected by concomitant medicinal products. Refer to SPC for full information regarding interactions.
Use in pregnancy and lactation: When Sovaldi is used in combination with ribavirin or peginterferon alfa/ribavirin, extreme care must be taken to avoid pregnancy in female patients and in female partners of male patients. When Sovaldi is used in combination with ribavirin or peginterferon alfa/ribavirin, women of childbearing potential and their male partners must use an effective form of contraception during the treatment and for a period of time after the treatment. Refer to the SmPC for ribavirin for full recommendations. There are no or limited data on the use of sofosbuvir in pregnant women. Animal studies do not indicate direct or indirect harmful effects with respect to reproductive toxicity. Sovaldi should not be used during breast-feeding. See also the SmPC for ribavirin.
Side effects: No adverse drug reactions specific to sofosbuvir have been identified. The following adverse drug reactions have been identified with sofosbuvir in combination with ribavirin or in combination with peginterferon alfa and ribavirin. Frequencies are defined as follows: very common (≥ 10%) or common (≥ 1% and < 10%). Sofosbuvir + Ribavarin: *Very commonly reported adverse events (≥1/10):* haemoglobin decreased, insomnia, headache, nausea, blood bilirubin increased, fatigue, irritability. *Commonly reported adverse events (≥1/100, <1/10):* nasopharyngitis, anaemia, depression, disturbance in attention, dyspnoea, dyspnoea exertional, cough, abdominal discomfort, constipation, dyspepsia, alopecia, dry skin, pruritus, arthralgia, back pain, muscle spasms, myalgia, pyrexia, asthenia. Sofosbuvir + Peginterferon-alfa + Ribavarin: *Very commonly reported adverse events (≥1/10):* anaemia, neutropenia, platelet count decreased, lymphocyte count decreased, decreased appetite, insomnia, dizziness, headache, dyspnoea, cough, diarrhoea, nausea, vomiting, blood bilirubin increased, rash, pruritus, arthralgia, myalgia, chills, fatigue, influenza like illness, irritability, pain, pyrexia. *Commonly reported adverse events (≥1/100, <1/10):* weight decreased, depression, anxiety, agitation, migraine, memory impairment, disturbance in attention, vision blurred, dyspnoea exertional, constipation, dry mouth, gastroesophageal reflux, alopecia, dry skin, back pain, muscle spasms, chest pain, asthenia.
Overdose: If overdose occurs the patient must be monitored for evidence of toxicity. Treatment of overdose with Sovaldi consists of general supportive measures including monitoring of vital signs as well as observation of the clinical status of the patient. Haemodialysis can efficiently remove the predominant circulating metabolite GS-331007 with an extraction ratio of 53%.
Pharmaceutical precautions: No special requirements for use and handling. Store in the original package in order to protect from moisture. Keep the bottle tightly closed.
Legal Category: POM.
Package Quantities: Bottle of 28 film-coated tablets.
Price: UK NHS Price – £11,660.98; Eire Price – €TBA
Marketing Authorisation Number: EU/1/13/894/001
Further information is available from the marketing authorisation holder: Gilead Sciences International Ltd, Granta Park, Abington, Cambridge, CB21 6GT, UK.
Telephone: +44 (0) 1223 897555. E-mail: ukmedinfo@gilead.com

CONSULT THE SUMMARY OF PRODUCT CHARACTERISTICS BEFORE PRESCRIBING PARTICULARLY IN RELATION TO SIDE EFFECTS, PRECAUTIONS AND CONTRAINDICATIONS.

Sovaldi is a trademark

Date of PI preparation: January 2014

HCV1/UK/13-12/MM/1144

▼ Following approval of the Marketing Authorisation, this medicinal product is currently subject to additional monitoring, as indicated by the presence of the inverted black triangle. Any suspected adverse reactions to Sovaldi should be reported to Gilead via email to csafety@gilead.com or by telephone +44 (0) 1223 897500.

For Healthcare Professionals prescribing in the United Kingdom:

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard.

For Healthcare Professionals prescribing in the Republic of Ireland:

Suspected adverse reactions should be reported to the IMB using a Yellow Card obtained either from the IMB, or electronically via the website at www.imb.ie. Adverse reactions can also be reported to the IMB by calling (01) 676 4971.

Reference: 1. SOVALDI Summary of Product Characteristics, January 2014.

GILEAD

March 2014 HCV/IHQ/14-03//1178

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Hepatology

Editor-in-Chief: Prof Dr Markus Peck-Radosavljevic

Professor of Medicine, Vice-Chairman, Department of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria; Fellow of the Austrian College of Physicians; Member of American Association for the Study of Liver Disease; the Austrian Transplant Association; the Austrian Society for Infectious Diseases; Austrian Association for Gastroenterology and Hepatology; Secretary General of the Austrian Association for Internal Medicine; Secretary General of the European Association for the Study of the Liver (EASL), Vienna, Austria.

Welcome

Spring has now sprung, as has our second edition of *European Medical Journal - Hepatology.* The International Liver Congress – the official meeting of the 49th European Association of the Study of the Liver (EASL) – was a remarkable meeting to attend for witnessing first-hand the explosion of data concerning hepatitis C. Prof Graham Foster, Professor of Hepatology, Queen Mary University, London, UK, said: "[We are] in a world where hepatitis C is on the defensive."

This year there was a record number of abstracts submitted, and as there was such an emergence of new treatments for hepatitis C discussed throughout the 5-day event, EASL as well as the World Health Organization (WHO) issued guidelines on how to treat patients effectively.

Although these new treatments bring hope to patients, the enthusiasm can be short-lived. Many of these drugs are expensive and are not available in low-income countries. Dr Mehlika Toy describes in her paper: '*Cost-effective interventions in the control of chronic hepatitis B infection*', the challenges which many health professionals face because the hepatitis B virus is not recognised as a serious health concern. Dr Toy details the challenges of delivering effective and affordable care to patients who need it most. Moreover, affordable diagnostic methods for detecting the disease sooner are also a main point of discussion in the paper.

The theme of affordable treatments continues through to our 'Congress Review' section. The article '*Hepatitis C: eradicated by the year 2020?*' not only explores the idea of hepatitis C becoming a historical virus but also calls for better public awareness of the disease. Additionally it calls for policy-makers, especially the European Union, to become more proactive in conquering this disease, and for cost-effective prevention methods to be implemented. In our 'What's New' section we feature an exclusive interview with Mr Michael Elliott from Gilead concerning their new drug Sovaldi® (sofosbuvir), which has been approved for use in tackling the hepatitis C virus.

Non-alcoholic fatty liver disease (NAFLD) was another hot-topic featured throughout this year's congress. One of the findings outlined how cardiovascular disease and diabetes mellitus are brought on by NAFLD. These findings are significant for the physician as it indicates that these factors need to be taken into account when treating the patient.

Dr Andrie Panayiotou discusses the role of both adipose tissue and insulin resistance in patients with NAFLD, and the interrelationship between this condition and the metabolic syndrome. The most current tools and techniques used to treat this population are also explored.

These were not the only topics of discussion; paracetamol, a common household drug, can have many harmful effects, but, as shown in the Congress, there is hope yet for liver transplant patients as a new allocation model can predict patient outcomes. This model will enable patients to be assessed quickly and will provide them with a personalised mortality risk.

We would encourage all of those with an interest in Hepatology to read this edition of *EMJ-Hepatology*; I hope it to be not only an informative and useful read, but also an enjoyable one.

Spencer Gore

Director, European Medical Journal

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Foreword

Univ Prof Dr Markus Peck-Radosavljevic

Professor of Medicine,
Medical University of Vienna, Austria.

Dear Colleagues,

I would like to welcome you to this new issue of the *European Medical Journal - Hepatology* 2014. This year is an extremely exciting year for Hepatology in general and chronic hepatitis C in particular.

At the International Liver Congress (ILC), the official Annual Meeting of the European Association for the Study of the Liver (EASL) in early April in London, we witnessed an unprecedented line-up of pivotal Phase III studies of all-oral direct-acting antiviral treatments for chronic hepatitis C in a great variety of different patient populations. Alongside this, the healing rates for this once difficult to treat condition were outstanding throughout, with minimal side-effects. This means we are entering a completely new era with an almost universal cure for chronic hepatitis C in all patients that are able to receive treatment.

In addition to these major advances in treating chronic hepatitis C, there are other areas in Hepatology that merit further discussion. Amongst these are the treatment of chronic hepatitis B, the mechanisms and treatment of non-alcoholic fatty liver disease (NAFLD), acute liver failure, and last but not least, liver transplantation. These are the topics which are covered here in the current issue of *EMJ - Hepatology*, and these are the issues which will remain hot topics in the years to come.

> “We are entering a completely new era with an almost universal cure for chronic hepatitis C in all patients that are able to receive treatment.”

I am glad for our patients that we are witnessing these exciting times in the field of Hepatology and I hope you can share my excitement for the dramatic advances that are happening right now. So I am happy to present to you the latest edition of *EMJ - Hepatology*, and I invite you to attend the next ILC in April 2015 in Vienna, Austria. Come and join us at the most important liver meeting in 2015 and celebrate together with us the 50th anniversary of EASL, the premier association for the advancement of liver education worldwide.

Kind regards,

Markus Peck-Radosavljevic

Professor of Medicine, Vice-Chairman, Department of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria; Fellow of the Austrian College of Physicians; Member of American Association for the Study of Liver Disease; the Austrian Transplant Association; the Austrian Society for Infectious Diseases; Austrian Association for Gastroenterology and Hepatology; Secretary General of the Austrian Association for Internal Medicine; Secretary General of the European Association for the Study of the Liver (EASL), Vienna, Austria.

ILC ANNUAL CONGRESS 2014

EXCEL LONDON EXHIBITION & CONVENTION CENTRE, LONDON, UK

9TH - 13TH APRIL 2014

Welcome to the *European Medical Journal* review of the International Liver Congress 2014

EMJ EUROPEAN MEDICAL JOURNAL

ILC ANNUAL CONGRESS 2014

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Welcome to the *European Medical Journal* review of the International Liver Congress 2014

LONDON, UK, a leading global city renowned not only for its spectacular beauty and superb views but also for its history and innovations was the home to this year's International Liver Congress™ – the official meeting place of the 49th European Association for the Study of the Liver (EASL) Congress.

Over 10,000 clinicians and scientists attended this truly international event, with the highest number of attendees coming from America, the UK, and China. The number of participants is a testament to the importance of this meeting. Those with an interest within the discipline of hepatology gathered to hear renowned experts present the latest research, perspectives, and treatments for liver diseases, particularly hepatitis C.

Hepatitis C was the main feature throughout the high-calibre, multi-disciplinary, scientific programme, with many presentations focusing on the new discoveries and treatments made. Prof Graham Foster, Professor of Hepatology, Queen Mary University, London, UK, remarked that the location of the Congress was very fitting as it lay on the East side of the city, near the old London docks where English men and women ventured out to explore the globe.

"So it is very fitting," Prof Foster said, "that in this historic part of town, in this place rich in history and exploration, we should begin to explore, what I think, will be a brave new world; and that is a brave new world where hepatitis C is no longer a threat and no longer a plague to mankind."

"I think this is the EASL meeting where we are going to see the end, the beginning of the end of hepatitis C."

Prof Graham Foster, Queen Mary University, London, UK

The presentation of these new, direct-acting, antiviral drugs, used in previously observed difficult-to-treat patients, are a main feature of our 'Congress Review' section. Sovaldi® (sofosbuvir) was among these new drugs presented, and we have an exclusive interview with one of the directors of medical affairs for Gilead in our 'What's New' section.

EASL and also the World Health Organization (WHO) have released guidelines to advise healthcare providers on how these drugs, or any treatment for hepatitis C, should be used to benefit the patient, which is also a feature of our 'Congress Review'.

The European Liver Patients Association (ELPA) have also expressed their concern of these new drugs, calling for better public awareness and, most of all, calling on policy-makers to tackle the virus and take inspiration from other campaigns such as breast cancer.

Prof Markus Peck-Radosavljevic said: "There are many areas [within hepatology] which we are actively promoting so that liver disease is something which policy-makers think about." As a society, EASL aims to act as an advisor to European health authorities and to help colleagues in Eastern Europe treat their patients effectively; in summary, they wish to be a lively voice throughout Europe.

These new discoveries presented not only give hope to patients but also to physicians. "I think this is the EASL meeting where we are going to see the end, the beginning of the end of hepatitis C," said Prof Foster.

Game-changing new guidelines for hepatitis C treatment

LEADING hepatology experts have unveiled their recommendations on how medical care is to be implemented following recent innovations in the treatment of hepatitis C virus (HCV).

The appearance of a new generation of safer, better tolerated, and more effective direct-acting antivirals, a group of oral medicines for use in combination therapy, and soon to be followed by further developments over the next few years, has called for a review of national treatment guidelines concerning hepatitis C.

Led by the World Health Organization (WHO) in their: 'WHO Guidelines for the screening, care, and treatment of persons with hepatitis C infection', the reviews give advice on screening for hepatitis C infection, mitigating liver damage - including calling for alcohol assessments in order to uncover and provide counsel for chronic alcohol abuse patients - treatment, and prevention.

Support will also be available to fulfil the universal goal of making the drugs affordable for all.

Up to 150 million people worldwide have chronic infection from hepatitis C and 350,000-500,000 people subsequently die each year. However, it is not uncommon for individuals to live for decades unaware of the disease, which can then progress to liver cancer, causing death.

"Many people remain unaware - sometimes for decades - that they are infected with hepatitis C," said Dr Andrew Ball, Senior Advisor for Policy, Strategy, and Equity for WHO's HIV/AIDS Department, Geneva, Switzerland. "Today's launch highlights the need for more awareness and education on hepatitis for the general public. Greater awareness on the risks associated with hepatitis C should lead to a demand for services and expansion of laboratory capacity and clinical services so that more people can be tested, treated, and cured."

> "These new EASL online recommendations...will further help physicians and other healthcare providers optimise management of patients with acute and chronic HCV."
>
> *Dr Alessio Aghemo, EASL Governing Board*

EASL too have published online their disease management recommendations, focusing on who should be treated and the treatment of special groups, among other key areas.

Dr Alessio Aghemo, Recommendation Panel Member on EASL Governing Board and Professor of Gastroenterology, University of Milan, Italy, commented: "These new EASL online recommendations on the management of HCV reflect how the treatment landscape has evolved for this disease, and will further help physicians and other healthcare providers optimise management of patients with acute and chronic HCV."

Potential Hepatitis C cure blooms in Chinese foliage

GROWN in the Far East, SBEL1 has injected fresh hope into the fight against the hepatitis C virus (HCV), blocking viral activity in cells at various points of the viral lifecycle.

Concocted from a wealth of Chinese herbal medicines, SBEL1 cuts HCV activity by around 90%. Extracted from a herb that blooms in parts of Taiwan and Southern China, the Chinese use this drug to treat sore throats and inflammations, but researchers have also unearthed a genuine virus-blocking formula.

"People infected with hepatitis C are at risk of developing severe liver damage, including liver cancer and cirrhosis. In the past, less than 20% of all HCV patients were treated because the available treatments were unsuitable due to poor efficacy and high toxicity. Recent advances means that we can now virtually cure HCV without unpleasant side-effects. However, the different virus genotypes, coupled with the complexity of the disease, means there is still a major unmet need to improve options for all populations," said Prof Markus Peck-Radosavljevic, Secretary-General of EASL, Associate Professor of Medicine, University of Vienna, Austria.

Studies have produced eye-catching conclusions. Out of two groups of liver cells, one was injected with SBEL1 prior to HCV invasion; results showed that HCV protein content was 23% lower in SBEL1 cells than control cells, while the introduced HCV internal ribosome entry site (IRES)-driven luciferase reporter suffered an activity slump of 50% in the SBEL1 cells compared to control. This points starkly at a decimating action of IRES-mediated translation by SBEL1, putting it in the spotlight for attacking the viral production process.

No one knows exactly what role the compound plays in plant of origin, but because of these results it is generating feverous anticipation around the world. For the 150-200 million people worldwide currently suffering from chronic HCV, and more than 350,000 succumbing to HCV-related infections, a discovery such as this is very promising.

"In the past, less than 20% of all HCV patients were treated because the available treatments were unsuitable due to poor efficacy and high toxicity. Recent advances means that we can now virtually cure HCV without unpleasant side-effects."

Prof Markus Peck-Radosavljevic, Secretary-General of EASL

"SBEL1 has demonstrated significant inhibition of HCV at multiple stages of the viral lifecycle, which is an exciting discovery because it allows us to gain a deeper understanding of the virus and its interactions with other compounds. Ultimately this adds to our library of knowledge that may bring us closer to improving future treatment outcomes," Prof Peck-Radosavljevic added.

Hope snowballs for oral hepatitis C cure

ORAL drug containing hepatitis C virus (HCV) inhibitors MK-5172 and MK-8742 is rising fast as the definitive treatment of chronic HCV infection.

Such hope derives from encouraging results from the C-WORTHy study, an ongoing multi-arm Phase II study that is testing the safety and effectiveness of a once-daily oral regimen combining MK-5172, an investigational HCVNS3/4A protease inhibitor, and MK-8742, an investigational HCV NS5A replication complex inhibitor in patients with chronic HCV genotype 1 (GT1) infection.

The trial, in which treatment-naïve, non-cirrhotic patients were given a 12-week MK-5172/MK-8742 regimen, with and without ribavirin (RBV), registered a sustained viral response (SVR) in 98% (N=43/44) of patients administered MK-5172/MK-8742 alone and 94% (N=80/85) in those administered MK-5172/MK-8742 plus RBV. Adding to the good news, only 2% (N=1/44) and 1% (N=1/85) actually relapsed in the non-RBV and RBV arms, respectively.

The 8-week regimen however, compared less favourably, showing lowest SVR, 83% (N=25/30). The relapse for this arm was also much higher at 17% (N=5/30), five of whom had very low MK-5172 and MK-8742 levels throughout the study.

"These Phase II results add to growing evidence for the potential efficacy of MK-5172 and MK-8742 for treatment of chronic HCV infection," said Dr Eliav Barr, Vice President of Infectious Diseases, Merck Research Laboratories, Boston, Massachusetts, USA.

Different treatment durations of MK-5172 (100 mg once daily) + MK-8742 (50 mg once daily) with and without RBV were tested. In total 471 patients with HCV GT1 RNA levels of ≥10,000 IU/mL took part across the 16 arms of the trial.

Common side-effects measured in the RBV and RBV-free groups, respectively, were fatigue (32%, 23%), headache (20%, 33%), nausea (21%, 16%), diarrhoea (13%, 9%), and insomnia (13%, 7%). However, none of the subjects dropped out of the study as a result.

"These findings are integral to advancing our research of these investigational candidates into C-EDGE, the Phase III clinical programme that will seek to more broadly evaluate the potential of MK-5172/MK-8742 in diverse patient populations," added Dr Barr.

The C-EDGE trial will analyse safety and efficacy of MK-5172/MK-8742 with and without RBV in various genotypes across a wide range of patient populations with chronic HCV.

Confronting hepatitis C: a comprehensive approach

> "Hepatitis C is still very much an emergent, unrecognised disease, and there remain aspects of it that are not fully understood."
>
> *Dr Marita van de Laar, Head of the Programme on STI, HIV/AIDs, and blood-borne viruses, European CDC, Stockholm, Sweden*

ACTIVISTS, global and national health officials, and researchers reveal the main issues of the hepatitis C virus (HCV) health challenge in a recent Economist Intelligence Unit report.

The report addressed the issue of HCV and the impact that it has on world health. At the forefront were concerns about limited knowledge on the scope of the problem of HCV; the significant barriers to addressing the disease, including a lack of scientific knowledge, poor public awareness, and delays of treatment due to great costs and side-effects; the high prevalence of people who inject drugs in developed countries – and the associated disease stigma; and, in developing countries, the healthcare system as a vector of HCV transmission.

Described as "an urgent public health issue" by Dr John Ward, Director of the Division of Viral Hepatitis at the US Centre for Disease Prevention and Control (CDC), Atlanta, USA, HCV is a world health issue, affecting equally the wealthy and those in the developing areas, although leading transmission routes, resources available, and disease genotypes vary.

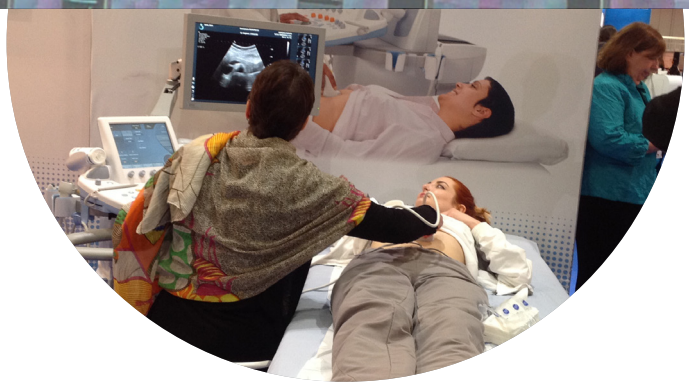
"We are living with multiple epidemics, different ones in different countries," said Mr Jack Wallace, Member of the Executive Committee, Coalition to Eradicate Viral Hepatitis in Asia Pacific, Melbourne, Australia.

It was emphasised that facing up to the challenges posed by HCV will require policymakers to produce a co-ordinated strategy covering a range of areas.

"Hepatitis C is still very much an emergent, unrecognised disease, and there remain aspects of it that are not fully understood," said Dr Marita van de Laar, Head of the Programme on STI, HIV/AIDs, and blood-borne viruses, European CDC, Stockholm, Sweden.

These areas, therefore, ought to include: countries obtaining local information on the extent of the challenge, increased public awareness to overcome stigma and increase risk prevention, healthcare systems adopting an integrated, multi-agency approach to treatment, and finding ways to reach patients most in need.

Compassion cures hepatitis C transplant patients



EXHAUSTED for options, and in appearance, 104 post-liver transplant patients with recurring hepatitis C saw new hopes realised in an expanded access sofosbuvir (SOF) + ribavarin (RBV) + pegylated interferon (PEG-IFN) trial.

The temporary authorisation for use of this treatment served pre and post-transplant hepatitis C virus (HCV) infected patients who would be at high risk of death, graft reinfection, or decompensation after 12 months with no treatment.

> "However, this new trial involving the nucleotide polymerase inhibitor SOF has demonstrated promising results, providing further evidence of its clinical potential."
>
> *Prof Patrizia Burra, Head Multivisceral Transplant Unit, Padova University Hospital, Padua, Italy*

As part of a compassionate use programme, 104 patients took a 48-week course of SOF (Gilead), a nucleotide polymerase inhibitor, added to the standard regimen (injectable PEG-IFN combined with RBV) to treat HCV genotypes 1, 4, 5, and 6.

SOF + RBV + PEG-IFN had high efficacy, potent anti-viral capacity, and was well tolerated by the 87 subjects. 62% achieved a sustained viral response to treatment regimens 12 weeks after therapy; the same number also showed improvements in clinical prognosis such as ascites and encephalopathy, and liver function tests.

Prof Patrizia Burra, Head Multivisceral Transplant Unit, Padova University Hospital, Padua, Italy, said: "There are currently no effective treatment options for this patient group. However, this new trial involving the nucleotide polymerase inhibitor SOF has demonstrated promising results, providing further evidence of its clinical potential."

The course of hepatitis C is unpredictable but commonly (in 50-90% cases) the acute form progresses into chronic hepatitis C. Without liver transplantation recurring, hepatitis C causes serious complications such as liver scarring and failure, and ultimately an increased risk of death. However in some cases, the disease can recur following liver transplant.

"For patients with advanced hepatitis C liver disease, liver transplants offer a second chance," continued Prof Burra, "and for those who continue to suffer post-surgery, it's important for us to keep following up all avenues possible to improve their quality of life."

Liver disease gets gutsy

FAECAL microbiota transplantation shows promise as a treatment for alcoholic liver disease (ALD) as animal models indicate that intestinal microbiota-associated liver injury contributes to the development of ALD.

Mouse models received grafts of human faecal matter from two groups of human patients: those with severe alcoholic hepatitis, against those with a history of alcohol abuse but no liver disease. The healthy mice were then given an alcohol diet.

The mouse transplant group who received diseased human-tissue grafts experienced more severe liver injury and greater disruption to the internal mucosa of the colon. Remarkably, researchers also discovered that two *Clostridium* bacteria were producing ethanol *in vitro*, which was consistently found to cause liver injury.

ALD is understood to occur as the result of excessive alcohol consumption, seen when an individual consumes over 50 g of alcohol at least once a week (five or more drinks) – the recommended limit is the equivalent of 8 g pure alcohol/week.

Europe consumes an astonishing rate of 12.18 L of pure alcohol per adult per year – the highest proportion in the world. More than one in five European adults reported a heavy drinking episode at least once a week. Subsequently, one in five adults in Europe is affected by a serious alcohol-related illness.

Prof Frank Lammert, EASL Scientific Committee Member, Head of the Department of Medicine, Saarland University Hospital, Homburg, Germany, commented: "Among heavy drinkers, the severity of ALD does not strictly correlate with the amount of alcohol intake, meaning that other factors must be influencing its development."

> "These findings provide first evidence for a causal role of gut microbiota in alcohol-induced inflammation, and open up new avenues for the treatment of ALD with potentially better patient outcomes."
>
> *Prof Frank Lammert, EASL Scientific Committee Member*

Gut microbiota are already known to play an important role in the body's metabolism and immune function. Where ALD has always been associated with disability caused by alcohol disease, this study has shown that microflora in the gut also play a role in whether the disease presents itself. The research gives headway towards using intestinal microbiota transplantation as a method of preventing ALD by restoring gastrointestinal function and homeostasis.

"These findings provide first evidence for a causal role of gut microbiota in alcohol-induced inflammation, and open up new avenues for the treatment of ALD with potentially better patient outcomes," Prof Lammert added.

Vitamin D deficiency is damaging to children

> "Identifying a gene that impacts or alters the disease is a step in the right direction."
>
> *Prof Jean-Francois Dufour, University Clinic, Visceral Surgery and Medicine, Bern, Switzerland*

VITAMIN D deficiency can be grave for young livers, with low levels associated with non-alcoholic fatty liver disease (NAFLD) in children, and its severity controlled by a newly identified genetic variant.

The shocking data are drawn from a study which retrospectively analysed the medical records of 120 British paediatric patients with NAFLD, carried out by the King's College Hospital Paediatric Liver Centre, and the University of Surrey's School of Biosciences and Medicine.

Patients' medical records showed low vitamin D levels all year long, and were well below national UK and US health standards. This would only be expected in winter, when vitamin D levels in the blood are normally low due to a lack of sun exposure. Such low levels might have sprung from a defective NADSYN1 gene, known to catalyse NAFLD severity.

Prof Jean-Francois Dufour, University Clinic for Visceral Surgery and Medicine, University of Bern, Switzerland, said: "The data support recent research that revealed an association between low vitamin D status and incidence of NAFLD and is an important development in helping clinicians better understand the growing rate of NAFLD in children throughout the western world."

NAFLD is an unstoppable scourge across Europe and has climbed its way up to be the number one most persistent liver condition in Western countries; it is soon expected to be top of the liver diseases worldwide. Right now, 20-30% of European adults and 10% of children are sufferers.

Changing play habits – with more kids confined to the television and video games than going outside to play in parks and streets – coupled with a growing surge in childhood obesity, and the overuse of sun creams, have all contributed to high levels of vitamin D deficiency and increasing numbers of rickets cases.

Hopes are high, however, for building on the discovery of the defective NADSYN1 gene, with scientists seeing a plethora of treatments and therapies on the horizon.

"Identifying a gene that impacts or alters the disease is a step in the right direction and could potentially lead to the development of new treatments or diagnostic techniques to address this growing issue," added Prof Dufour. "More research into this field is warranted and I look forward to seeing future developments over time."

T cell clone wars

ADOPTIVE T cell therapy could be the answer to curing liver cancer, which is exactly what the world has been waiting for.

Hepatocellular carcinoma (HCC) is the source of 7% of the world's cancers, annually killing 746,000 globally and 4,000 in the UK alone. On top of this, over three-quarters of a million new cases worldwide are diagnosed each year. If a person with liver cancer is not treated in 5 years, the likelihood of survival is 5%. Due to a late onset of symptoms and shortage of therapies, HCC is currently the second most common cause of cancer death.

70% of HCC cases involve expression of Glypican-3 (GPC3), a tumour-associated antigen which is completely absent from healthy human tissues. If GPC3-specific T cell receptors are added to a patient's T cells, they can combat HCC infection as the modified T cells will recognise and destroy GPC3-positive HCC.

MHC-multimer-positive CD8+ T cells, specific for targeted GPC3 epitopes, were cloned and multiplied in preparation for a definitive study. Scientists took the most active T cell receptor from this clutch of cells and added it to a collection of donor T cells, which immediately showed an affinity for GPC3. Armed with this weapon, the cells were able to identify, hunt down, and eliminate GPC3-positive HCC, thus giving HLA-A2+ patients the equipment to deal with the pathogen and protect the liver.

Although treatments for advanced stage HCC are constantly evolving, the current treatments, such as sorafenib, lack convincing evidence for universal treatment and do not offer a strong enough prognosis. Moreover, liver transplants are only suitable for early stage HCC, an option for just 10-15% of sufferers. T cell therapy could, therefore, plug the gap, with the results from this study providing an exciting platform for future testing.

Viral hepatitis 10 times more deadly than HIV/AIDS

DEATHS attributable to viral hepatitis are now 10 times more common across Europe than those related to HIV/AIDS.

Results from The Global Burden of Disease Study 2010 (GBD 2010), an epidemiological study which compared country and regional-level mortality attributable to hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV/AIDS between 1990 and 2010, has declared that more people are dying from viral hepatitis than HIV/AIDS across Europe.

At the time of the study, HIV/AIDS caused approximately 8,000 deaths, whereas HBV was responsible for close to 31,000, and HCV took almost double that amount of lives.

Dr Laurent Castera, Vice-Secretary at the EASL Scientific Committee, Hôpital Beaujon, Paris, France, expressed: "Although HIV/AIDS undeniably remains a key global health priority, the higher mortality from viral hepatitis than from HIV/AIDS in the EU means that HBV and HCV must clearly now be counted among the top global and local priorities for health.

"Additional resources are needed to prevent, detect, and treat HBV and HCV in order to address these imbalances in major preventable causes of human death."

There has however been a promising drop in the number of HIV-related deaths in most of the EU following 1990, with the exception of Eastern Europe.

"This goes some way to explaining why mortality from viral hepatitis does not appear to be higher than that of HIV/AIDS in other areas of Europe outside of the EU," concluded Dr Castera.

Nevertheless, on an international scale, we have seen an increase in mortality from HIV and viral hepatitis, collectively contributing to 2.76 million deaths worldwide in 2010.

Hepatic encephalopathy is not the end of the road

XIFAXAN® 550, used for treating liver disease patients with hepatic encephalopathy (HE), demonstrated a 5-year quality-adjusted life improvement, reducing the strain on healthcare systems.

Healthcare systems are impacted by increased pressure in the care of liver disease sufferers with HE. For liver disease-related illnesses, patients with HE were admitted to hospital 3-times more often than those without. For unrelated illness this was also greater at 1.5-times more admissions to hospital. It also showed that they averaged nearly 2 days longer per hospital stay. Finally, they suffered mortality as an outcome in 51.8% of cases versus 32.4% of liver disease patients without HE.

"HE 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, Newcastle, UK.

This domination of healthcare resources is expected to be reduced with XIFAXAN® 550, a new, more cost-effective treatment than the standard, lactulose.

In a Phase III trial, XIFAXAN® 550 reduced the risk of a breakthrough HE episode by 58%, reduced the risk of hospitalisation by 50%, and reduced infection rates versus placebo + lactulose over 6 months. When modelled over different time series, data suggested that cost-effectiveness ratios were positive using XIFAXAN® 550 as an option for a reduction in recurrence of HE.

Dr Hudson added: "These new data demonstrate that HE 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 HE, and the cost-effectiveness data presented today support its benefits in terms of potential cost savings versus current practice."

A cocktail to treat hepatitis C infection

KILLER of 350,000 people worldwide, hepatitis C is under renewed threat from a cocktail of drugs that scientists hope will unlock the potential to rid the globe of the disease.

This bold vision has been galvanised by a series of promising results from a range of trials that have been carried out worldwide. A combination therapy involving the NS3/4A protease inhibitor simeprevir, and Gilead Science Inc.'s nucleotide inhibitor sofosbuvir, has been the star candidate so far in combating the hepatitis C virus (HCV), reinforced by positive data from Janssen R&D Ireland's Phase II COSMOS study.

Genotype 1 (GT1) treatment-naïve and prior null-responder chronic HCV patients with advanced liver fibrosis (METAVIR F3 or F4 scores) were put on a once-daily combination therapy of simeprevir and sofosbuvir for either 12 or 24 weeks, with or without ribavirin (RBV), in cohort 2 of the study. 94% of subjects showed a sustained virologic response (SVR) 12 weeks after the conclusion of treatment (SVR12). 93% and 100% of subjects attained SVR12, respectively, on simeprevir and sofosbuvir alone after 12 weeks and 24 weeks of treatment. The application of RBV had little-to-no effect on the curative effect of the therapy; for the 12 and 24-week groups; however, RBV added to the existing therapy, produced a 93% hit rate for SVR12 in both groups.

The results were combined with equally positive data from cohort 1; 97% and 96% of HCV GT1 patients with METAVIR F0-F1 scores and F2 scores, respectively, attained SVR12 in response to treatment with simeprevir and sofosbuvir alone after 12 and 24 weeks of treatment. A 100% hit rate of SVR was produced for patients in combination with RBV after 12 and 24 weeks, respectively.

> "We look forward to bringing this regimen to patients in Europe in the near future."
>
> *Mr Gaston Picchio, Janssen Research & Development, New Jersey, USA*

Pegylated interferon (PEG-IFN) appeared to dampen the curative effects of the simeprevir treatment in the Phase III RESTORE trial; just 65% of HCV GT4 treatment-naïve and treatment-experienced patients achieved SVR12, while SVR12 was produced in 69% and 52% of patients with GT4a and GT4d HCV, respectively.

"The data presented at EASL further reinforce the benefit of simeprevir-based treatment across diverse patient populations, including European patients," explained Mr Gaston Picchio, Hepatitis Disease Area Leader, Janssen R&D, New Jersey, USA. "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."

Host of therapeutic options to improve HCC patient outcomes

HEPATOCELLULAR carcinoma (HCC) patients could benefit from advances made in screening programmes, the use of gadoxetic acid-enhanced MRI, a 3-gene signature blood test, or percutaneous radiofrequency ablation (RFA) treatment.

As Dr Helen Reeves, EASL's Scientific Committee Member, Senior Lecturer and Honorary Consultant, Gastroenterologist, Newcastle Hospitals NHS Foundation Trust, Newcastle, UK, highlighted: "Because HCC is such an extremely diverse and heterogeneous disease, improving patient outcomes has proved a difficult undertaking. A number of existing therapeutic options have been subjected to rigorous study but have not shown any patient benefit. The findings from these HCC diagnosis, staging, and treatment studies are important because they have the potential to significantly improve patient outcomes."

The geographical variation of HCC screening programmes emphasised the need for centrally coordinated screening programmes across Europe to improve patient outcomes.

In Japan, around 80% of HCC patients are detected through the use of screening, which is in stark contrast to the UK (15%), Spain (35%), and Hong Kong (<10%). Moreover, HCC survival rates were higher in Japan at 47 months, than Spain, the UK, and Hong Kong, which were 26, 20, and 7 months, respectively.

The use of a 3-gene signature - identified from analysing a blood sample - was also successful in detecting and predicting the development of HCC in high-risk individuals. It yielded a high degree of sensitivity (82%) and specificity (90.2%).

For improving treatment outcomes, the use of gadoxetic acid-enhanced MRI proved to be a valuable tool. Compared to a dynamic computed tomography (CT) or magnetic resonance imaging (MRI) scan alone, a gadoxetic acid-enhanced MRI has been demonstrated to lower the risk of HCC recurrence and overall mortality in patients with single nodular HCC.

"Using gadoxetic acid-enhanced MRI to more accurately stage HCC patients with early disease has the potential to significantly improve outcomes by ensuring each patient receives the optimum treatment," said Dr Reeves.

For the treatment of small HCC, percutaneous RFA is both effective and safe, Dr Reeves emphasised: "The development of local ablative therapy has been one of the major advances in the treatment of HCC."

Between May 2000 and May 2012 a total of 1,020 small tumour nodules in 837 patients were treated with the procedure. 98.8% of patients undertaking the treatment achieved complete ablation.

However, more research is needed in the areas of percutaneous RFA and in the use of the 3-gene signature technique to confirm the results found.

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No pain, no gain for paracetamol-related acute liver failure sufferers

INNOVATIVE new model predicts outcomes of paracetamol-related acute liver failure (ALF), giving hope of improved selection and survival to those eligible for liver transplant (LT).

500 patients with paracetamol-related ALF took part in a study allowing researchers to cultivate a model which, for patients without LT, will be able to analyse changes in ALF prognosis and quantify mortality risk as part of their suitability for LT, the standard treatment for ALF.

Predictors included age, encephalopathy, and cardiovascular failure, as well as the independent variables of arterial pH, lactate, and creatinine (measured over 3 days). Prediction of non-transplanted 15-day survival in the subjects was significantly higher than given by the standard Kings College Criteria, with the addition of quantified survival prediction provided for individual patients.

Paracetamol-related liver poisoning most commonly results in ALF - a condition that yields 15-20 LTs, and 10-times the number of deaths, each year in England and Wales.

Prof Markus Peck-Radosavljevic, Secretary-General of EASL and Associate Professor of Medicine, Medical University of Vienna, Austria, commented: "ALF is a devastating condition that triggers a cascade of events that can lead to multiple organ failure and often death."

As an over-the-counter drug, paracetamol, known as acetaminophen in the US, Canada, and parts of Asia, is both inexpensive and commonly prescribed, as well as being found as an active ingredient in other pills such as cold medicine. Due to the negligible side-effects when used as recommended, it is widely considered as a household drug.

In the UK however, there have been restrictions on the amount of paracetamol that can be bought in pharmacies and non-pharmaceutical shops for over 16 years - 32 versus 16 packets of 500 mg tablets, respectively. Even a small overdose, classified as over the recommended limit of 4,000 mg per day, and especially if taken with alcohol, can be fatal.

"This high-performance survival model for paracetamol-induced ALF will enable each individual patient to be assessed quickly and a personalised mortality risk provided. Consequently, this will allow the healthcare professional to make a very informed decision regarding a liver transplant, potentially resulting in improved patient outcomes," Prof Peck-Radosavljevic added.

Fatty livers lead to dangerous hearts

CARDIOVASCULAR disease (CVD) and diabetes mellitus (DM) are brought on by non-alcoholic fatty liver disease (NAFLD), which acts as an independent risk factor for the two conditions. However, when treatment successfully reduces the fat content of a patient's liver, the risk of contracting DM decreases.

"We now have a strong body of evidence that NAFLD may pose a CVD risk above and beyond that conferred by traditional CVD risk factors, such as dyslipidaemia, diabetes, and smoking. This means that healthcare providers managing patients with NAFLD should take this factor into account in the CVD risk stratification, although the best way to implement this remains to be defined," said Prof Jean-Francois Dufour, University Clinic for Visceral Surgery and Medicine, University of Bern, Bern, Switzerland.

Two long-term studies led by Prof Dufour have supported these claims. The first of the two studies, which involved patients at high CVD risk, found that NAFLD - a burgeoning disease with an estimated worldwide prevalence of 20-30% - catalysed the progression of early atherosclerosis independent of normal CVD risk factors.

"Whether NAFLD is incidentally or causally associated to early carotid atherosclerosis has previously been the subject of much debate," explained Prof Dufour. "While there are case-control studies that have demonstrated a significant and independent relationship between NAFLD and carotid atherosclerotic disease, up until now, long-term follow-up data have been missing."

NAFLD also plays a significant role in the progression of DM compared to non-sufferers of NAFLD, according to the second 10-year study (16.1% versus 3.1%; $p<0.001$). Those who responded well to liver fat reduction treatment also had a much reduced likelihood of developing DM than the non-treatment group (6.4% versus 17.8%).

"Evidence from previous longitudinal studies has demonstrated a clear link between NAFLD and the development of DM," said Prof Dufour. "However, this is the first study to show that DM can be prevented if NAFLD is improved," he explained.

Prof Dufour added: "A multidisciplinary approach is therefore required in the treatment of NAFLD patients, taking into account the presence of NAFLD as a critical part of diabetes prevention and care.

"New clinical trials to investigate the beneficial effects of anti-diabetes drugs on NAFLD histology, and the potential impact of anti-diabetes drugs on diabetes incidence and cardiovascular risk in non-diabetic patients with early-stage NAFLD are now underway."

New competition to tackle primary biliary cirrhosis

> "While UDCA has been the standard PBC therapy for the past 20 years, a significant percentage of patients fail to get an adequate response with this treatment, or are unable to tolerate it."
>
> *Dr Frank Lammert, Saarland University Medical Center, Homburg, Germany*

OBETICHOLIC acid (OCA) has proven effective in patients with primary biliary cirrhosis (PBC). OCA has outshone its counterpart, ursodeoxycholic acid (UDCA), producing meaningful biochemical and clinical improvements.

217 subjects with PBC who had shown either inadequate response or intolerance to UDCA were put in a Phase III study, which examined the effectiveness of OCA at different titrations; doses were applied at 10 mg and 5 mg titrated to 10 mg, respectively. Scientists managed to attain a serum alkaline phosphatase (ALP) activity of <1.67-times the upper limit of normal (ULN), coupled to a 15% drop in ALP, and a total bilirubin falling in the normal bracket.

Results were encouraging for patients reaching the primary endpoint; the 10 mg OCA group and 5-10 mg OCA groups weighed in at 47% and 46% success rates, respectively, which were a vast improvement on the 10% figure for the placebo group (both dose groups $p<0.0001$).

Dr Frank Lammert, Professor of Internal Medicine, Saarland University Medical Center, Homburg, Germany, said: "These trial results indicate that a statistically greater number of OCA-treated patients achieved the response criteria as defined by Global Primary Biliary Cirrhosis Study Group. We know that these endpoints have, in turn, been shown in previous studies to strongly correlate with clinical benefits and an improved long-term prognosis, with a reduced risk of liver transplantation and death."

OCA, a bile acid analogue, could lock into the body's defence as it is an excellent agonist of the nuclear receptor (FXR), the central human bile acid sensor. Agonism of the FXR has already yielded promising results in rats; ileal permeability increases and bacterial translocation fell in response to FXR agonism.

PBC treatment has suffered from a lack of competition for many years, OCA could provide a necessary alternative to UDCA.

"While UDCA has been the standard PBC therapy for the past 20 years, a significant percentage of patients fail to get an adequate response with this treatment, or are unable to tolerate it. We therefore need new therapies to prevent PBC from progressing to cirrhosis and liver failure, and this study suggests that OCA has the potential to be a much needed advance for these patients," explained Dr Lammert.

Three against one: the attack on hepatitis B

EXPERIMENTING with different combinations of nucleoside analogues (NUCs) and pegylated interferon (PEG-IFN) has helped scientists come closer to the most effective therapy for chronic hepatitis B (CHB) sufferers.

"Together these ground-breaking data will go a long way to influencing future CHB treatment guidelines," said Prof Cihan Yurdaydin, Educational Councillor for EASL, Department of Gastroenterology, University of Ankara, Ankara, Turkey.

In order to achieve the best results for the treatment of patients with CHB, a combination therapy involving PEG-IFN and NUCs has been put through the gears in three large trials. PEG-IFN appears to have had a positive impact in all three of the studies, strongly implying that it is now above NUCs in the order of importance for CHB therapy.

PEG-IFN seems to give NUC therapy a boost for non-respondent CHB patients to date; in the first study, more subjects achieved complete response (HBeAg loss composited with hepatitis B virus [HBV] DNA<2000 IU/mL, 60.24% versus 13.8%, and HBsAg loss, 27.7% versus 0%). PEG-IFN may also have signalled the end of NUC entecavir, causing enhanced response rates and viral decline in HBeAg-positive CHB patients with compensated liver-disease in combination.

31% in one group and 24% of patients in another who participated in the global ARES study showed HBeAg loss and HBeAg-seroconversion after 96 weeks with HBV DNA levels <200 IU/mL, compared to just 20% and 11% in the entecavir monotherapy group, respectively; this indicates that PEG-IFN add-on independently causes greater response rates.

PEG-IFNα-2a was not enhanced by the 6-week addition of a NUC in the third study, as the HBeAg seroconversion rate did not increase after 24 weeks follow-up compared to PEG-IFNα-2a treatment alone. 21-27% of subjects achieved HBeAg seroconversion at the end of treatment and 23-36% after a 24-week follow-up, very little difference from PEG-IFNα-2a therapy alone.

"In the ARES study, the NUC was prescribed for a total of 48 weeks, with the combination of PEG-IFN, and NUC prescribed for the last 24 of these 48 weeks. In the sequential combination study, the combination of PEG-IFN and NUC was prescribed for a total of 48 weeks. However, in marked contrast, in this third study, from the total treatment duration of 52 weeks, patients only received a NUC for 6 weeks. Traditionally, the NUC would be prescribed as a longer-term suppressive therapy," explained Prof Yurdaydin.

"Together these ground-breaking data will go a long way to influencing future CHB treatment guidelines."

Prof Cihan Yurdaydin, Educational Councillor for EASL

Hepatitis C: eradicated by the year 2020?

ERADICATING hepatitis is by no means a simple task, but Europe is urged to act now against chronic liver diseases before the condition in patients worsens, putting a greater strain on national health systems.

The peak for these diseases is expected to come to the forefront in the years 2020-2025; Prof Graham Foster, Professor of Hepatology, Queen Mary University, London, UK, said: "If we do not pay for treatment now we will pay a great deal more in the future."

For better public awareness of the risk factors for chronic liver disease, the European Liver Patients Association (ELPA) have recommended that EU policy makers should tackle this disease in a more proactive way; viral hepatitis should be a health policy with national strategy programmes to test and treat patients.

"This is an era of opportunity. This is the moment when hepatitis C moves from a possibly curable disease to a probably curable disease," added Prof Foster.

The costs of screening and the costs of new treatments are still obstacles to eradicate this disease, but the costs of not treating it are higher still. To overcome these barriers, healthcare providers have to improve diagnosis and access to treatment.

In the view of Mr Matthias Schuppe from the European Commission (EC), identifying, disseminating, and promoting the up-take of good practices for cost-effective prevention, diagnosis, treatment, and care, will go a long way to eradicating hepatitis C.

Cost-effectiveness analysis of screening practices, as well as an EU-wide survey on surveillance and prevention of hepatitis B and C are needed. Screening patients, however, is not without limitations; Mr Schuppe noted that it is "still an up-hill struggle."

One of the main concerns of ELPA is that the gravity of hepatitis C may be underestimated in the future because of new antiviral drugs which are appearing on the market. They have therefore suggested that the follow-up of patients receiving these drugs is crucial, as is awareness of the disease itself.

While hepatitis C may not be solved by the year 2020, the medical world will be one giant step closer. Access to new drugs, their prices, awareness, and education for patients, doctors, and the public are still challenges which need to be addressed.

"Hope is justified," said Dr Ingo van Thiel, ELPA, Germany, "but there is still a lot of work to be done."

> "This is an era of opportunity. This is the moment when hepatitis C moves from a possibly curable disease to a probably curable disease."
>
> *Prof Graham Foster*
> *Queen Mary University, London, UK*

Triangle of action against hepatitis C

> "These data show very promising results in people who are infected with either subtype of the GT1 hepatitis C virus who are either new to therapy or treatment-experienced."
>
> *Dr Stefan Zeuzem, J.W. Goethe University Hospital, Frankfurt, Germany*

THREE-PRONGED attack on the hepatitis C virus (HCV) could be the answer to eliminating one of the world's most notorious diseases.

Aiming to optimise sustained virologic response (SVR) across diverse patient populations by targeting the HCV replication process in a three-mechanism course of action, the new Abbvie regimen has been tested in two major placebo-controlled Phase III studies (SAPPHIRE-I and SAPPHIRE-II) involving non-cirrhotic chronic HCV genotype I (GT1) adult patients, yielding a succession of heartening results.

The regimen consists of the HCV NS3/4A protease inhibitor ABT-450/ritonavir (150/100 mg), co-formulated with the NS5A inhibitor, ombitasvir (ABT-267) (25 mg), taken once-daily, and the NS5B RNA polymerase inhibitor dasabuvir (ABT-333) (250 mg) with or without ribavirin (RBV), taken twice-daily; the therapy was this time administered with RBV to GT1 patients.

Encouragingly, SVR was attained in 96.2% and 96.3% of GT1 patients in the SAPPHIRE-I and SAPPHIRE-II studies, respectively.

In the SAPPHIRE-I study, GT1a and GT1b HCV subjects achieved an SVR12 of 95.3% (N=307/322) and 98.0% (N=148/151), respectively. 96.0% (N=166/173) and 96.7% (N=119/123) achieved an SVR12 in the SAPPHIRE-II study for GT1a and GT1b, respectively.

Subjects in the Sapphire-II study were divided into three groups irrespective of treatment experience: prior null-responders (49.2%), prior relapsers (29.0%), and prior partial responders (21.9%). These groups, encompassing both GT1a and GT1b infections, achieved an SVR12 with success rates of 95.2%, 95.3%, and 100%, respectively.

"Patients with chronic hepatitis C who have not responded well to treatment in the past have historically been more difficult to treat," said Dr Stefan Zeuzem, Lead Clinical Investigator on SAPPHIRE-II and Chief of the Department of Medicine, J.W. Goethe University Hospital, Frankfurt, Germany. "These data show very promising results in people who are infected with either subtype of the GT1 hepatitis C virus who are either new to therapy or treatment-experienced."

Cirrhosis next target for revolutionary therapy

FOLLOWING hot on the heels of a line of successful results from non-cirrhotic studies, an exciting new treatment has bolstered its reputation with excellent results in patients with liver cirrhosis.

TURQUOISE-II is the latest in a series of studies evaluating the safety and efficacy of Abbvie's new drug. The regimen - administered to patients over 12 weeks or 24 weeks - consists of ABT-450/ritonavir (150/100 mg) co-formulated with ombitasvir (ABT-267) (25 mg), taken once-daily, and dasabuvir (ABT-333) (250 mg) with or without ribavirin (RBV).

Results have been extremely positive; sustained viral response (SVR) was achieved in 91.8% (N=191/208) and 95.9% (N=165/172) of subjects after 12 and 24 weeks, respectively (SVR12). These high SVR rates fall in line with results from other Phase III studies, including the SAPPHIRE studies (which tested non-cirrhotic patients), showing the potential of the treatment.

In the 12-week arm, 88.6% (N=124/140) of G1a subjects achieved SVR12, with 92.2% (N=59/64) for those new to therapy. Treatment-experienced GT1a patients were split into three groups: prior null-responders, prior relapsers, and prior partial responders. 80.0% (N=40/50), 93.3% (N=14/15), and 100.0% (N=11/11) SVR12 hit rates were taken from the groups, respectively. GT1b recorded 98.5% (N=67/68) for SVR12 attainment, hitting 100.0% (N=22/22) for those new to therapy. For the GT1b treatment-experienced group: prior null-responders reached 100.0% (N=14/14), prior relapsers 100.0% (N=25/25), and prior partial responders 85.7% (N=6/7).

Better news was still to come from the 24-week arm; 94.2% (N=114/121) of GT1a patients achieved SVR12, with those new to therapy registering a 92.9% (N=52/56) hit rate. As with the 12-week arm, the GT1a treatment-experienced subjects were split into three groups. SVR12 rates for prior null-responders, prior relapsers, and prior partial responders measured 92.9% (N=39/42), 100.0% (N=13/13), and 100.0% (N=10/10), respectively. GT1b patients achieved 100.0% (N=51/51) SVR12, with equal perfection in those new to therapy (N=18/18). For those who were GT1b treatment-experienced, SVR12 was achieved in all cases in prior null-responders (N=10/10), prior relapsers (N=20/20), and prior partial responders (N=3/3).

"We designed our comprehensive HCV clinical trial programme to generate important information about treating a range of GT1 patients," said Dr Scott Brun, Vice President of Pharmaceutical Development, Abbvie, Chicago, USA. "These data will help the medical community better understand the use of our regimen for specific patient types they encounter with GT1 infection in actual practice."

Hepatitis C drug shows glittering prowess through all trials

GROUND-BREAKING drug with a three-dimensional (3D) attack formula has kept up its remarkable performance in testing, having aced tests in non-cirrhotic hepatitis C virus (HCV) genotype 1b (GT1b) sufferers.

Two major branches constitute AbbVie's 3D therapy according to dosage, consistent with the chemical makeup used in other major trials: NS3/4A protease inhibitor ABT-450 applied with ritonavir (150/100 mg), co-formulated with the NS5A inhibitor ombitasvir (ABT-267) (25 mg), taken once-daily, and the NS5B RNA polymerase inhibitor dasabuvir (ABT-333) (250 mg) with or without ribavirin (RBV), taken twice-daily.

Targeting the HCV replication cycle, the combination treatment has produced stellar results in all of the Phase III studies presented thus far, including the high-profile SAPPHIRE-I and TURQUOISE-II studies, which examined the safety and efficacy of the drug on non-cirrhotic and cirrhotic HCV genotype 1a (GT1a) sufferers, respectively.

The PEARL-III study enrolled 419 non-cirrhotic, treatment-naïve HCV GT1b subjects (the most common hepatitis subgenotype worldwide) in the study, which tested the drug's effectiveness with and without RBV. Startling results were attained; 99.0% (N=207/209) of subjects who took a placebo in place of RBV achieved sustained virologic response (SVR) after 12 weeks of treatment (SVR12), while 99.5% (N=209/210) achieved SVR12 with RBV.

> "The impressive SVR12 results seen are consistent with the results from Abbvie's Phase II studies."
>
> *Dr Alessio Aghemo, Scientific Committee Member of EASL*

In light of these results, RBV, which along with interferon is known to cause toxic side-effects, might no longer be necessary in the treatment of non-cirrhotic HCV GT1b patients.

"Using this investigational 3D regimen, with or without RBV, these studies have demonstrated consistently high cure rates across a number of patient types, including the more difficult-to-treat subtype GT1a, and HCV patients with compensated cirrhosis," said Dr Alessio Aghemo, Scientific Committee Member of EASL, Gastroenterology and Hepatology Unit, Ospedale Maggiore Policlinico, University of Milan, Italy. "The impressive SVR12 results seen are consistent with the results from Abbvie's Phase II studies."

"This collection of studies show encouraging data and further support our understanding of the efficacy and safety of this 3D regimen in a variety of patient types," added Dr Aghemo. "Such research continues to highlight the advances being made in treating complex diseases of this type."

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Quantification of liver fibrosis using collagen proportionate area (CPA) in patients with non-alcoholic fatty liver disease (NAFLD).

UCL Institute of Liver and Digestive Health, UK

William Alazawi, UK

The role of Stat2 in chronic inflammatory liver disease.

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Itziar Otano, Spain

Genetically engineering CD8+T cells to withstand the HBV infected liver milieu.

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Interferon-induced long non-coding RNAs and their role in the innate immune response to hepatitis C virus.

Hepatology Department, University of Basel, Switzerland

Simon Manuel Schultze, Switzerland

Functional characterization of long non-coding RNAs in liver regeneration under normal and pathological conditions.

Hans Popper Laboratory of Molecular Hepatology, Medical University of Vienna, Austria

Entry Level

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Effects of liver sinusoidal endothelial phenotype amelioration through COX-1 silencing in cirrhotic portal hypertension.

Barcelona Hepatic Hemodynamic Laboratory, Hospital Clinic de Barcelona, IDIBAPS, Spain

Johannie du Plessis, South Africa

The role of macrophage activation in the development of non-alcoholic steatohepatitis: an integrated study of the gut-fat-liver axis.

Lab of Hepatology KU Leuven, Belgium

Martina Gambato, Italy

Characterization and predictive role of hepatitis C quasispecies evolution in the development of fibrosing cholestatic hepatitis after liver transplantation.

Liver Unit, Hospital Clínic, IDIBAPS, CiberEHD, Barcelona, Spain

Bristol-Myers Squibb:
at the Core of HCV Research

At Bristol-Myers Squibb, our commitment to the fight against HCV involves finding innovative new therapies that attack the virus at its core, targeting the non-structural proteins responsible for forming the viral replication complex[1,2]

Our GOAL … CURE [2,3]

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Bristol-Myers Squibb

TRANSFORMING HCV MANAGEMENT

Summary of Presentations from the Gilead Sciences (Europe) Symposium, held at the 49th Annual EASL ILC, London, UK, on 9th April 2014

Chairperson

Graham Foster,[1] Patrick Marcellin[2]

Speakers

Ira Jacobson,[3] Heiner Wedemeyer,[4] Christophe Hézode,[5] Antonio Craxì[6]

1. Queen Mary University of London, London, UK
2. Université Paris-Diderot, Paris, France
3. Weil Cornell Medical College, New York, USA
4. Hannover Medical School, Hannover, Germany
5. Hôpital Henri Mondor, Créteil, France
6. University of Palermo, Palermo, Italy

Disclosure: Speakers participating in this symposium received honorarium from Gilead Sciences, Europe. Any patient cases and treatment options referred to are in the context of contemporary knowledge and medical practice in the field. For scientific completion, the educational symposium included reference to unlicensed products or unlicensed indications; where products in development are discussed, only those at the most advanced stages of development are included.

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MEETING SUMMARY

This Gilead-sponsored satellite symposium addressed the new and exciting advent of novel therapy in the field of hepatitis C by highlighting recent important clinical trials. The scientific programme covered the history of treatment of hepatitis C and the current and future treatment landscape in the management of hepatitis C virus (HCV) infection.

Historical View – Where Have We Come From?

Professor Patrick Marcellin

Prof Marcellin introduced the delegates to a history of hepatitis C by giving an overview of the discovery of the virus and the subsequent investigation into its transmission. Identification of a new virus that was not attributable to hepatitis A or B was made in the 1970s and led to a flurry of investigation, which revealed that it is a small enveloped RNA virus.[1] Further efforts to elucidate the nature of the infection resulted in a small pilot study that involved ten patients with non-A, non-B hepatitis. Administration of daily injections of recombinant human interferon-α (IFNα) led to a normalisation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and subsequent novel cloning techniques allowed the identification of this elusive virus as HCV in 1989. This discovery laid the foundation for many advances including the development of diagnostic and monitoring tools used to identify HCV, as well as an increasing number of treatment targets and regimens over the years since its discovery. Of note is the first-generation ELISA and the subsequent

development of the second-generation - more sensitive - RIBA assay, both of which are used for diagnosing non-A, non-B hepatitis.[2]

Initial treatment regimens established IFNα as a stalwart in the treatment of HCV. The addition of ribavirin (RBV) to this regimen was seen to improve viral response rates, whilst the pegylation of IFNα was observed to delay its breakdown and increase drug exposure, permitting a single dose a week and therefore representing a major treatment advancement.[3,4] However, the side-effects seen with IFNα therapy and the danger of viral resistance have led to the development of direct-acting antiviral agents (DAAs) which include first-generation protease inhibitors (PIs). Although first-generation PIs improve sustained viral response (SVR), they come with further toxicities and the continued problem of emerging resistance.

Combining IFNα therapy with other DAAs has resulted in encouraging improvements in SVR in patients with HCV.[5–9] In particular, combinations with a newly approved, once daily, oral non-structural protein 5B (NS5B) nucleotide analogue polymerase inhibitor, sofosbuvir (SOF), has been shown to achieve >90% SVR across all genotypes.[10]

Prof Marcellin concluded his presentation by proposing that these results indicate a new era in the treatment of HCV, where despite concerns that only a small portion of patients are currently being treated, huge progress has already been made. High SVR rates achievable with a shorter course of therapy and IFN-free regimens were considered to represent the future of HCV management.[11]

Investigator View – Being Part of History in the Making

Professor Ira Jacobson

Prof Ira Jacobson offered his personal perspective on the field of HCV by beginning with an introduction to IFN therapy and the use of RBV as an adjunct to IFN. Although the mechanism of action of RBV in HCV remains the subject of much debate, increased understanding of the HCV lifecycle has formed the basis of the development of a number of therapeutic agents, including protease inhibitors, NS5A inhibitors, nucleotide, and non-nucleotide inhibitors.

Dr Jacobson went on to describe his personal experiences as a young clinician, working with Dr Charles Rice at Rockefeller University, whose laboratory was pivotal in overcoming several milestones in HCV biology that have been key in the development of new therapies. These included elucidating the crystal structure of NS5A and identification of the co-receptors that mediate HCV entry into the cell as well as identifying an infectious cell culture system for the study of the mechanism of disease.

A timeline of the major therapeutic breakthroughs in HCV disease history of HCV therapy was described with major landmarks including the introduction of the first-generation PIs, boceprevir and telaprevir, in 2011 and the eventual move towards potential curability of HCV without IFN. Regulatory approval of sofosbuvir for genotypes (GTs) 1-6 followed the publication of results from a Phase III trial, which showed >91% SVR rate in patients with GT 1, 4, 5, and 6 over a shorter, 12-week treatment period.[10,11] Furthermore, Dr Jacobson highlighted key clinical trials with new DAAs of different drug classes that offer high levels of efficacy and unprecedented short treatment durations, giving new hope to patients for whom treatment with IFN is not an option.[12–14] In addition, the development of oral regimens, highlighted as a pivotal topic at the EASL meeting, was emphasised as another major culmination of recent scientific investigation.

In particular, the approval of SOF for chronic HCV by the EMA, a new drug that has no clinically significant drug interactions and has a high barrier to resistance during therapy, even in the event of a post-treatment relapse, marks the advent of a new and exciting period in the field of HCV.[14]

Virological View – Understanding the Relevance of HCV Genotype

Professor Heiner Wedemeyer

In his talk, Prof Heiner Wedemeyer addressed the diversity of GTs, demonstrating differences not only in response to treatment but also portraying differences in natural history. Prof Wedemeyer stressed that the relevance of GT was not only linked to response with IFN-based therapy but has also been shown to have importance in the context of DAAs.

Initial treatment regimens with IFN have demonstrated marked differences in response to therapy between GTs that can be explained by the divergent nature of each GT, as demonstrated in patients with GT 2 who respond better to PEG-IFN than those with GT 3 and 4.[15–21] Prof Wedemeyer explained that GT 3 is associated with rapid disease progression, including accelerated fibrosis and poor long-term survival in comparison to GT 1 and 2.[22] GTs also have varied responses to DAAs, including the first HCV protease inhibitor that rapidly decreased viral load in GT 1 patients but had little effect in patients with GT 2/3.[23,24] In contrast to previous established therapies, nearly all patients treated with SOF become HCV RNA negative within 4 weeks of therapy;[11,14] however, Prof Wedemeyer pointed out that maintenance of SVR between GTs differed and that 12 weeks of SOF + RBV therapy showed higher response rates in GT 2 versus GT 3 patients.[11,25]

Methods to overcome the lower response rates in GT 3 patients were then presented with approaches including increasing the duration of treatment, combining therapy with IFN or, finally, through the potential addition of a developmental NS5A DAA, such as daclatasvir, providing potential solutions.[10,11,14,26,28] Initial data have demonstrated that all of these approaches may provide future benefit within this group and further insights are highly anticipated.

Prof Wedemeyer concluded his presentation by reiterating that GT remains a very relevant issue in HCV therapy and one that must be taken into account when addressing the treatment needs of individual patients with different HCV GTs.

Perspectives on New Candidates for Cure

Doctor Christophe Hézode

In his talk about the treatment of difficult patients, Dr Christophe Hézode began by explaining results from the real-life CUPIC study in which cirrhotic patients were given triple therapy (boceprevir or telaprevir with PEG-IFN and RBV). Patients in this study who had platelet counts <100,000 mm^3 and albumin <35 g/L were more likely to experience complications, and less likely to achieve an SVR12, indicating that triple therapy is an inefficient way to manage patients with severe complications.[29,30] In contrast, SOF therapy in combination with RBV and PEG-IFN for 12 weeks in cirrhotic patients resulted in a promising SVR of 80%, indicating that SOF may be a new standard of care in patients with severe disease.[31]

Dr Hézode presented an important clinical case study of one of his patients, a woman with GT 4, previously taking PEG-IFN + RBV therapy. The patient had cirrhosis and encephalopathy and was awaiting transplant when she was put on a regimen of SOF with RBV. After 16 weeks of treatment the patient displayed a significant improvement in biological and clinical parameters, including a significant improvement in her Child–Pugh (C11 to B7) and MELD (15 to 12) scores to the extent that there is reconsideration of whether this patient will now indeed be in need of a liver transplant.

A more pressing question about the prevention and recurrence of HCV after liver transplant was discussed. Patients on a combination of SOF + RBV for up to 48 weeks pre-liver transplant display significantly less recurrence of HCV leading up to the transplant, with a 93% viral response rate at transplant. >30 days of undetectable viral load significantly reduced recurrence. Furthermore, 69% of patients maintained this at 12 weeks post-transplant and had very few adverse effects; however, data are not final as the trial is still ongoing.[32] Early interim Phase II trial data suggest that in patients with recurrent HCV post-liver transplant, treatment with SOF + RBV results in a high SVR after 12 weeks of treatment.[33] Dr Hézode then described a second clinical case of one of his patients, a 52-year-old male with recurrent HCV GT1 following liver transplant who failed to respond to PEG-IFN + RBV + BOC. The patient was put on a regimen of SOF + RBV, quickly reaching undetectable levels of HCV RNA, and after 24 weeks of treatment, showed improvement in his Child–Pugh (A6 to A5) and MELD (14 to 11) scores.

This presentation and the clinical cases highlighted the increasing promising data and clinical experience with the use of SOF + RBV in hard-to-treat patients undergoing liver transplant as well as those who are post-liver transplant with recurrent HCV.

Perspectives on Therapy for Challenging Patients

Professor Antonio Craxì

In his talk, Prof Antonio Craxi explored the current and future therapeutic options available to clinically challenging patients, including those who are treatment-experienced cirrhotics and those with HIV/HCV co-infection.

Results from the ATTAIN study showed little benefit in response of simeprevir over telaprevir in combination with PEG-IFN + RBV in treatment non-responders with cirrhosis, suggesting that this clinical approach is insufficient in dealing with the problem of HCV in cirrhotic patients.[34] Treatment with SOF + RBV in treatment-naïve and treatment-experienced patients with GT 3 who were further stratified into cirrhotic and non-cirrhotic groups resulted in a significant improvement in SVR after 12 weeks.[26] In treatment-experienced patients with cirrhosis SVR was 60% while in treatment-naïve patients with cirrhosis SVR was 92%.[26] The combined use of PEG-IFN with SOF + RBV further improved SVR in treatment-experienced cirrhotics to 83%, suggesting that combining PEG-IFN with currently-approved agents may give an optimal viral response.

Similarly, combining SOF + RBV + PEG-IFN in treatment-naïve patients GT 1–4 with HIV co-infection resulted in 91% SVR in this difficult-to-treat population.[10,35] Prof Craxi then went on to present data on the use of all-oral therapy (SOF + RBV for 12 or 24 weeks) in cirrhotic and non-cirrhotic patients. In patients with GT 1 and 2, viral response rates were 76–88% in treatment-naïve patients and 92–94% in treatment-experienced patients. In patients with GT 3, recognised as being a more resistant GT, viral response rate was 67% after 12 weeks; however, this was still considered promising. There was no sign of viral resistance, though HCV and HIV breakthrough was observed in two patients due to non-adherence.[36] Moreover, comparison of viral response rates in HIV/HCV co-infected and HCV mono-infected treatment-naïve patients showed that these were similar between the two groups.[11,26,36,37]

In summary, this presentation showed that patients with HIV/HCV co-infection are no longer to be considered a special patient population and can be treated with SOF, which has a high efficacy. This therapy paves the way for more investigational DAAs being considered for the treatment of more difficult patient populations.

Has the Future Arrived? – Perspectives in HCV Tomorrow

Professor Graham Foster

Prof Graham Foster introduced his presentation by highlighting the hopeful future in hepatitis C. Supporting this perspective with recent data he presented an overview of the use of SOF in GT 1–4 for up to 12 weeks in order to elicit a viral response. This is especially promising in patients for whom IFN therapy is ineffective or contraindicated, where SOF can be used for 24 weeks. Results from clinical trials have shown 90–100% SVR across GT 1–6 following 12 weeks of treatment, a result that is unprecedented with any other drug that has been previously available.[11,15]

The emergence of new drugs means that the use of IFN/RBV therapy, which carries a significant adverse effects profile, will be reduced. In addition to this, newer drugs also offer reduced pill burden and shorter treatment duration for many patients, including offering an alternative IFN-free therapy option for more difficult-to-treat patient populations.[38]

Prof Foster went on to propose early treatment of HCV-infected patients in order to reduce morbidity and mortality, but especially in the treatment of those who have progressed and are now cirrhotic, offering the possibility of removing high-risk patients from the transplant list as well as vastly improving the quality of life that is directly associated with SVR.[39,40]

Furthermore, the introduction of SOF therapy for HCV may not only be beneficial for disease burden, but is also thought to have a significant financial burden on healthcare systems. Therefore, another incentive for the development of newer, more effective treatments for HCV are burgeoning HCV-related healthcare costs.[41,42] Several new IFN-free regimens are currently at an advanced stage of development; in particular, single treatment regimen (STR) therapies, consisting of a single pill that combines two or more highly effective drugs, will provide a new portfolio of therapies for HCV patients. A combination of SOF + daclatasvir (two pills) over a 12–24-week period has

demonstrated up to 98% SVR for GT1 patients in a Phase II study, while Phase III data presented at the EASL 2014 Congress showed that combining SOF with ledipasvir as an STR (one pill) without IFN or RBV has shown up to 98% SVR over a 12 or a 24-week period in GT1 patients.[27,43–46]

Prof Foster concluded that the development of newer therapies may help to eradicate HCV as a disease, not only in patients at the front line, but also in those where the virus lies nascent.

This satellite symposium provided an insight into the dynamic, rapidly changing field of HCV. It introduced data on exciting new treatment regimens that offer new hope to those with HCV, especially for patients with severe disease for whom these new treatments offer a new lease of life. The treatment of HCV has been previously stymied in more challenging cirrhotic patients and in those with HIV/HCV co-infection; however this new paradigm shift may pave the way for a brighter future with the potential of a world without HCV.

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NEWS AT SIX: HEPATITIS C SPECIAL

Summary of Presentations from the Bristol Myers Squibb Symposium, held at the 49th Annual EASL ILC, London, UK, on 12th April 2014

Chairperson

Mark Thursz[1]

Speakers

Jean-Michel Pawlotsky,[2] Heiner Wedemeyer,[3] Ashley Brown,[1] Charles Gore,[4] Alessandra Mangia,[5] Graham Foster,[6] Rafael Esteban[7]

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4. World Hepatitis Alliance, Hepatitis C Trust
5. 'Casa Sollievo della Sofferenza' IRCCS, S. Giovanni Rotondo, Italy
6. Queen Mary, University of London, UK
7. Hospital Universitario Val d'Hebron, Barcelona, Spain

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MEETING SUMMARY

This Bristol Myers Squibb-sponsored symposium was chaired by Mark Thursz, who oversaw a novel news bulletin-themed symposium with sessions provided by a distinguished, international team of roving reporters; Charles Gore from the World Hepatitis Alliance, Jean-Michel Pawlotsky from France, Alessandra Mangia from Italy, Ashley Brown and Graham Foster from London, Heiner Wedemeyer from Germany, and Rafael Esteban from Spain.

Stop the Press! A Critical Evaluation of HCV Treatment Targets for Patients of all Genotypes

Professor Jean-Michel Pawlotsky

Prof Pawlotsky began by predicting an interferon (IFN)-free future in hepatitis C virus (HCV) infection. Within his presentation he recommended four criteria through which a highly effective IFN-free regimen could be achieved. The first was potency; achieved using combinations of direct-acting antiviral (DAA) agents with differing targets and mechanisms of action; secondly, a high barrier to resistance should be achieved through the combination of DAAs without cross-resistance. The third criterion was to ensure adequate treatment duration, and the final suggestion was the inclusion of ribavirin (RBV), which has been shown to be beneficial in some settings.

Prof Pawlotsky went on to describe key therapeutic targets in the HCV lifecycle.[1] The first target, inhibition of viral polyprotein maturation, can be achieved using NS3/4A protease inhibitors (PIs).[2] While the latest generation of PIs have shown activity against infections with all HCV genotypes (GTs), it was also noted that the effectiveness of these agents is

lower against HCV GT 3 and they possess an improved barrier to resistance compared with first-generation PIs.

Representing the second target of the lifecycle, HCV ribonucleic acid (RNA)-dependent RNA polymerase (RdRp) can be targeted using nucleoside or nucleotide analogue inhibitors to block viral RNA replication. These therapies demonstrate pan-genotypic activity and a high barrier to resistance. Non-nucleoside inhibitors (NNI) also target HCV RdRp and, despite their narrow genotypic range and low barrier to resistance, they were nevertheless considered important components of some IFN-free regimens. Finally, the HCV NS5A protein can inhibit viral RNA replication, inhibition of viral particle assembly and the release of HCV particles. First-generation NS5A inhibitors have a low barrier to resistance and while some, for example daclatasvir (DCV), are effective for all GTs, new generation non-structural 5A (NS5A) inhibitors demonstrate pan-genotypic activity and an increased barrier to resistance.

Overviewing the need for informed combination choices in order to achieve high, sustained viral response (SVR) rates, Prof Pawlotsky described three ways in which DAAs could be combined within IFN-free regimens. The first uses a nucleoside/nucleotide analogue backbone together with one or two DAA agents (PI, NS5A inhibitor, NNI). Several studies supporting this approach were presented and showed high SVR rates with this combination with or without RBV in patients infected with HCV GTs 1, 2, or 3.[3-5] The combination of sofosbuvir (SOF) and the NS5A inhibitor DCV (60 mg/day) for 24 weeks, yielded SVR rates of 100%, with and without ribavirin, in treatment-naïve patients infected with GT 1; and SVR rates of 92% and 89% in treatment-naïve patients infected with GTs 2 and 3, respectively.[3]

The second combination comprised of three DAAs; several 12-week studies were presented which investigated the PI, ABT-450 with ritonavir (ABT-450/r) + the NS5A inhibitor ombitasvir, and the NNI dasabuvir, together with RBV in treatment-naïve patients infected with HCV GT 1.[6-8] Results from one of these studies, SAPPHIRE-1 showed a high SVR rate of 96% in patients infected with HCV GT 1.[6] Similarly, PEARL-III[7,8] showed that patients infected with HCV GT 1b achieved an SVR rate of 99% with or without RBV, and in PEARL-IV,[7] patients infected with HCV GT 1a achieved an SVR rate of 97% with the addition of RBV, which fell to 90% without. A 12-week Phase III study of the NS5A inhibitor DCV with the PI, asunaprevir, and the NNI, BMS-791325,[9] in treatment-naïve patients infected with HCV GT 1 showed SVR rates of 100% and 71% for five out of seven patients with liver cirrhosis and NNI 150 mg. In patients with liver cirrhosis, SVR rates of 91% and 94% were achieved for NNI 75 mg and 150 mg, respectively. Prof Pawlotsky commented that the SVR rates achieved by these triple-therapy combinations were very high and therefore represented a very valuable treatment option.

The third treatment option combines two agents with a high barrier to resistance, specifically a second-generation PI with a second-generation NS5A inhibitor. A 12-week study of the PI MK-5172 with the NS5A MK-8742, together with RBV in treatment-naïve, non-cirrhotic patients infected with HCV GT 1, showed SVR rates of 100% and 96% with 20 mg and 50 mg of MK-8742, respectively. Without RBV, 100% SVR was achieved with 50 mg MK-8742.[10]

Prof Pawlotsky closed his presentation by stating that, currently, four classes of DAAs are available for use in IFN-free combination therapy for HCV and these achieve high SVR rates; however, the audience were cautioned against potential problems of resistance, with new therapies still required in the minority of patients for whom IFN-free DAA combinations do not lead to high rates of SVR.

The Big Debate: Has the Time Come for All-Oral Interferon/Ribavirin-Free Regimens?

Professor Heiner Wedemeyer

Before the session commenced, Prof Thursz conducted a poll of the audience, revealing that 70% would vote in favour of the motion 'Has the time come for all-oral interferon/ribavirin-free regimens?'

Prof Wedemeyer adopted the pro stance and argued the case in favour of the motion. He began by stating that overall, evidence from the US FDA has shown that a high SVR rate can be achieved irrespective of the addition of IFN or RBV.[11] When deciding between treatment options, the potential for irreversible side-effects and potential deaths from IFN treatment, as well as the safety profile

of RBV, must be taken into account.[12] Careful consideration must also be given to the financial impact of working days lost to illness, the intensive monitoring required with IFN treatment, and patient preference.

Describing the scale of the HCV disease burden across European countries and further supporting the stance for IFN-free regimens, Prof Wedemeyer reported evidence supporting this perspective from the HALT-C study, which showed that mortality in patients with advanced chronic HCV infection increases with IFN treatment.[15] In addition, serious adverse events have been reported following treatment with first-generation PIs in certain populations, particularly in patients with advanced fibrosis; cohorts which reflect those commonly seen in the clinic, demonstrating the importance of adhering to recent guidance and specifically contraindications.[13–21] Evidence was also provided from several studies which showed that high SVR rates of 95-100% could still be achieved without the addition of RBV.[3,4,10,22–29] However, the issue of increased adverse events again presented a concern in comparison with placebo.[23,30]

Prof Wedemeyer concluded by stating that 100% cure rates can be achieved for the majority of patients with HCV without the use of treatments known to result in adverse events.

Professor Ashley Brown

Opposing the motion, Prof Ashley Brown stated that while the proposal of a 100% cure rate may be achievable within an 'ideal world', in some patient populations it remained inappropriate to stop using IFN or RBV at this time. He reminded the audience that IFN is pan-genotypic, whereas the high SVR rates presented by Prof Wedemeyer were exclusively from patients infected with HCV GT 1. In addition, the studies presented have used combinations of DAAs including SOF, which has a higher barrier to resistance than that seen for the majority of DAAs.

Prof Brown suggested that the evidence cited for adverse events associated with IFN and RBV did not represent the whole picture. In the HALT-C study, the duration of IFN treatment was 3 years for patients with cirrhosis, which would predict for a high rate of adverse event reporting. He proposed a case for short-term, IFN-sparing regimens, which would reduce the number of adverse events. In particular, these regimens could include IFN-lambda; a lesser known liver-specific IFN which is associated with fewer adverse events than IFN-alpha.[31]

Reflecting his own clinical experience, Prof Brown reported that 45% of patients are infected with HCV GT 3 and represent the hard-to-treat population. Studies of SOF + RBV in these patients showed SVR rates of 56% at 12 weeks, with 85% achieved after 24 weeks.[32,33] However, this increase in efficacy comes at a high cost due to the longer treatment duration required. Studies of triple therapy with SOF or DCV + RBV and IFN showed SVR rates of 83% (with SOF) and 78% (with DCV) at 12 and 16 weeks, respectively; representing a shorter treatment duration and high SVR rates in this hard-to-treat patient population.[34–36] Prof Brown also suggested that including IFN could be useful for patients who have relapsed after initial therapy.

Evidence was provided for the use of RBV in hard-to-treat patient populations with HCV GT 3, which was shown to be effective and associated with relatively little additional cost.[37]

Utilising the evidence described, Prof Brown argued for IFN and RBV to remain in the current treatment algorithm for HCV, as a pan-genotypic option with few concerns regarding resistance. In addition to providing a much needed option within a resource constrained setting, their addition to DAA combination regimens provides a valued approach in hard-to-treat patient populations.

The Expert Angle: Physician and Patient Perspectives on Key HCV Management Challenges

Mr Charles Gore

Mr Gore provided the first of four expert perspectives into HCV management. He provided a patient's view on current treatment choices and began by asserting that in most of Western Europe only 3% of patients with hepatitis C are currently treated. Reasons for this include poor diagnosis of hepatitis C, practical considerations around accessibility to treatment centres, and patient reluctance to receive treatment - particularly due to a fear of IFN treatment. Timing considerations may also reduce treatment uptake, as patients defer treatment to have a child or to wait for their circumstances to provide more support.

Discrimination and practical difficulties were described, which could prevent treatment delivery to certain groups of patients, including those in prisons, intravenous drug users, and the homeless. Difficult-to-treat patient groups were also described, including those infected with HCV GT 3, patients with cirrhosis - especially those with decompensated cirrhosis - and patients who are coinfected with HIV. Mr Gore concluded that the challenge now is to increase awareness to ensure informed therapeutic choices are made and the proportion of patients being treated is increased.

Professor Alessandra Mangia

Prof Mangia began by stating that, despite reports of SVR rates of 67–75% being achieved in registrational studies for patients infected with HCV GT 1, the TARGET study reported SVR rates of 58–61% in 1,100 previously-treated HCV GT 1 patients.[38] This lower, real-world SVR range may be due to increased adverse events resulting in treatment discontinuation, and the development of HCV resistance-associated variants.[39] In the TARGET study, 36% of patients discontinued treatment with the study drug; in 16% of cases this was attributable to the occurrence of adverse events.[38]

EASL guidelines recommend careful monitoring of the 40% of patients who do not achieve a high SVR rate with a triple DAA combination in order to determine reasons for treatment failure and to identify patients with cirrhosis who are at increased risk of developing hepatocellular carcinoma.[21] For pretreated patients, who had not responded to telaprevir (TPV) or boceprevir + pegylated IFN alpha-RBV, a combination of DCV + SOF with or without RBV led to 95–100% SVR rates after 24 weeks treatment.[3] The safety profile of this study was encouraging, with small numerical increases in non-specific adverse events such as fatigue and headache.[3] The Phase II, LONESTAR-1 study also provided promising results for this patient population, which included those with cirrhosis,[24] SVR rates of 91% or higher were achieved after treatment with SOF and ledispavir, with 100% seen when RBV was added.[24]

Prof Mangia concluded that the 40% of patients who fail after treatment with PI can now expect promising results with DAA combination therapy, also emphasising the urgent need for treatments for patients infected with HCV GT 3, suggesting they may benefit from the combinations described above after PI treatment failure.

Professor Graham Foster [guest video]

Prof Foster joined the debate via video link from London to add his thoughts on the challenges faced by patients infected with HCV GT 3 in his practice, and shared his approach to treatment for this patient group, and how this differs from those infected with HCV GT 1.

Patients infected with HCV GT 3 have, for many years, been regarded as an easy-to-treat GT, provided they do not have advanced fibrosis. Prof Foster stated that approximately 70% of patients respond to treatment with IFN and RBV; however, once patients present with cirrhosis the treatment rates plummet dramatically, and aggressive disease is then more likely. An additional challenge is that a large proportion of patients infected with GT 3 were originally born outside the UK, very often in Pakistan, Bangladesh, and the Indian subcontinent, often being infected at birth or very close to birth as a result of poor-quality vaccinations. These patients are now presenting with advanced cirrhosis, unaware that they have HCV, and all too often there is little that can be done. Prof Foster felt that an all-oral regimen for patients infected with HCV GT 3 would transform the treatment landscape for these very unfortunate patients.

Professor Rafael Esteban

Prof Esteban also shared his experience of patients in his practice with HCV and liver cirrhosis who are waiting for liver transplants and who are in urgent need of effective treatments. He presented results from several studies, which showed that the efficacy of triple-combination therapies, including IFN or RBV, is low in patients with liver cirrhosis.[16,40–43] In a subgroup analysis of ADVANCE, a study of TPV + IFN and RBV in patients infected with HCV GT 1 for 24 weeks, SVR rates were 62% in patients with liver cirrhosis compared with 81% in patients without.[16] The tolerability of these regimens is reduced in patients with liver cirrhosis; the CUPIC study showed a high proportion of serious adverse events, which led to discontinuation of treatment in these patients.[44] Several studies of IFN-free, dual DAA regimens in this patient population have, however, shown high SVR rates together with high tolerability.[24,45–49]

Prof Esteban described the urgent need of treatments for peri-liver transplant patients. A high rate of graft reinfection is seen in patients who have detectable serum HCV RNA prior to

transplantation,[50] and with current therapies the proportion of patients who achieve undetectable HCV RNA prior to transplant is 29-59%.[51,52] Relapse rates for HCV cirrhosis is also high following liver transplantation.[50] IFN-free DAA combination therapy can lead to patients remaining HCV-RNA negative for 30 days before transplant, which maximises the chances for a high post-transplantation virologic response and reduces the HCV-cirrhosis recurrence.[53] In summary, IFN-free regimens for patients with advanced liver cirrhosis, IFN-free DAA therapy combinations, are effective and well tolerated and carry a lower risk of drug–drug interactions with transplant medications.[54–56]

Prof Esteban ended by describing his experience of a patient who developed severe cholestatic hepatitis (bilirubin 25 mg/dl) soon after receiving a liver transplant. Within 11 weeks of treatment with SOF + DCV, the patient was serum HCV RNA-negative and bilirubin levels had returned to normal.[57]

Prof Thursz brought the symposium to a close, stating that a majority of the audience and panel were in support of all-oral, IFN/RBV-free treatment regimens for all patients with HCV.

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TRANSLATION FROM MICE TO MEN: ARE DOGS A DODGY INTERMEDIATE?

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ABSTRACT

Alternatives for liver transplantation in severe liver disease are urgently needed in view of the limited availability of donor livers. The use of embryonic stem cells (ES) or hepatic progenitor cells (HPC) has been investigated in mice models of acute and chronic liver failure. To extrapolate the findings in inbred mouse strains (weighing around 20 g, with a maximal lifespan of 3 years) to the genetically more variable human beings (around 3,000-fold heavier and living 30 times longer), does seem a bit of a large step. This article describes recent developments in HPC research in dogs and compares these findings to experimental rodent studies and human pathology. Recent progress in canine liver stem cell research and canine genetics are combined to exemplify their possible role as a relevant animal model for the feasibility of stem cell transplantation in human liver failure.

Keywords: Liver, progenitor cell, cell transplantation, inherited diseases.

INTRODUCTION

The limited availability of donor livers is one of the drawbacks in liver transplantation. Regenerative medicine, not only in hepatology, is at a crossroads between fundamental research and the clinical application of either stem/progenitor cell or differentiated cell (for liver research, the hepatocytes) transplantations.[1] Where transplantation of fully differentiated hepatocytes seems obvious for parenchymal diseases, their application in biliary diseases seems of little meaning. In contrast, hepatic progenitor cells (HPCs) have the potential to differentiate into both hepatocytes and cholangiocytes, offering a potential treatment modality for parenchymal and biliary diseases. The presence of HPCs has been under dispute for about five decades, ever since the first description by Farber in 1956.[2] The necessity of these cells seems limited since fully differentiated hepatocytes and cholangiocytes can, in contrast to most other differentiated cells, proliferate.[3] This is exactly what they do in acute liver failure or after partial hepatectomy. However, in those circumstances where their replication is hampered, HPCs come into play in an effort to repopulate the affected liver. HPCs reside in the Canals of Hering, where they are in close proximity with stellate cells, Kupffer cells, together constituting the HPC-niche. Histologically, HPC activation in a diseased liver section is described as 'ductular reaction' or 'bile duct proliferation'.[3] This indicates that activation of HPC could be beneficial for the liver to recover upon injury. At the same time, there is a potential down-side of activated HPC. The two-faced (Janus-like) character of HPCs is shown by the presence of progenitor cell markers in hepatocellular carcinoma (HCC), indicative for malignancy in humans and dogs.[4,5] Since potential risks and lack of data from hepatocyte transplantations are the most common restriction for participating in a Phase I hepatocyte transplant trial, it is clear that animal models predicting long-term risk/benefits are urgently needed.[6] In this paper, the predictive potential of dogs in cell transplantation in diseased livers is addressed.

Requirements for Animal Models

Important requirements for a good animal model in liver cell transplantation studies include: firstly, phenotypic resemblance with the human clinical situation, and secondly, good experimental controllability. The phenotypic resemblance is evaluated below with emphasis on HPCs and their activation differences in liver diseases in both men and dogs. The controllable, and thus reproducible, experimental set-up is described in view of the specific population structure in different dog breeds.

THE HPCS

HPCs are present as quiescent cells in healthy adult liver tissue in small numbers in the Canals of Hering, the smallest ramifications of the intrahepatic biliary tree. Located close to the portal area they are at the interface between hepatocytes and cholangiocytes.[7] Although simply stated as HPCs, their cellular origin remains an area of debate, let alone the question of whether one or more HPC pools do exist. A number of studies state a possible biliary origin of HPCs, whereas other studies in humans describe extrahepatic peribiliary glands as the prime location for HPCs, and the haematopoietic origin of HPCs is also suggested several times.[8-17] For this review, we focus on the HPC-niche within the Canals of Hering, as described in numerous mammals.[3,18-21]

HPCs can be histologically characterised by a combination of their specific morphology upon activation (the so-called ductular reaction) and by non-cell specific marker expression.[7] The classical HPC-markers, including the cytokeratins keratin-7 (K7) and keratin-19 (K19), are expressed on cholangiocytes too, which underpins the necessity to combine marker expression with histology. The same non-HPC specificity holds true for other stem cell markers including CD133 (PROM1) and EpCAM, which are for instance expressed on other stem cells.[22] In view of the versatile character of HPCs it is no surprise that mesenchymal markers such as CD29 (integrin β1) and CD44 (hyaluronic acid receptor and co-receptor for hepatocyte growth factor) are expressed on HPCs. For an extended list including other species see Kruitwagen et al.[23] For only a subset of markers, for instance ABCG2, CD44, CD133, K7, and K19, the expression is measured in all three species.[20,24-26]

HPC Activation in Liver Diseases

Just a few publications describe the HPCs and the HPC-niche in dogs, and make a comparison with either mice and/or humans.[27,28] This comparison is mainly based on immunohistochemical analyses. In contrast to humans, in mice and dogs the availability of healthy liver samples allows for a diseased-healthy comparison. It must be taken into account that the aetiology of human liver diseases is often different from the experimental mouse models. Virus-induced hepatitis is difficult to induce experimentally, but, as outlined below, hepatic copper toxicosis can be observed in all three species. The location and characteristics of quiescent canine HPCs and portal myofibroblasts were characterised in healthy livers. HPCs were located in the space of Disse, as previously described for other species.[29] A descriptive immunohistochemical study evaluated the inflammatory infiltrate and fibrosis in samples of canine chronic hepatitis. A positive correlation was found between the stage of fibrosis and the number of myofibroblasts (alpha-SMA positivity) and bile duct proliferation.[30]

Another study reported a positive correlation between tenascin-C expression, a specific component of the extracellular matrix (ECM), and stage of fibrosis, degree of inflammation, and the number of K7 positive cells in canine chronic hepatitis.[31] These findings extend the knowledge derived from murine and human samples to the canine HPC-niche and its activation during severe liver disease. For more direct human-dog comparisons, the relation between HPCs, stellate cells, fibrosis, and disease severity in healthy and diseased livers, was described in liver samples from both species. In liver disease with fibrosis, HPC activation was most pronounced and activated stellate cells were in close proximity to the ductular reaction.[32,33] Suggestive for having a crucial role of ECM, the component laminin co-localised with activated stellate cells and HPCs and macrophages clustered at the site of injury, more specifically periportally in acute hepatitis and in the fibrotic septa in chronic hepatitis [unpublished data].

HPCs in Regenerative Medicine

Having established, as summarised from marker expression in Table 1, that HPC-activation in rodents, dogs, and men is highly similar, both at the histological and at the molecular level, the

Table 1: Markers used to investigate mouse, dog or human hepatic progenitor cells. Due to space limitations, reference to all original papers has not been possible, therefore occasionally only reviews are referred to.

Marker	Mouse	Dog	Human
A6	82, 83		
ABCG2/BCRP1	84	20	20, 34
AFP		88	34, 89, 90, 91
Albumin	85		90, 91, 92
DLK	86		
c-Kit			34, 91
CD 24	83		
CD29		88	89
CD44	36	88	34, 90, 91
CD73			89
CD90			89
CD133	36, 85, 86, 87	88	34
CLDN3			90
Chromogarnin-A			90, 93
EpCAM	83, 85, 86		94, 95
FN14/TWEAK-R	86	88	
HNF4-alpha		88	
ICAM1			90
Keratin-7	9, 85	28, 20, 88	20, 28, 34, 35, 38, 94, 95, 96
Keratin-8			90, 95
Keratin-18			90, 95
Keratin-19	79, 10, 85, 86	28, 88	28, 34, 35, 38, 91, 92, 94, 95, 96
Lgr5	36		
NCAM			34, 90, 94
OPN	10	88	
OV6			94, 95
Sca1	86		
Sox9	9, 10, 36, 86, 87	88	
Vimentin			89

For an extended list with more markers and more mammals including rats and cats see Kruitwagen et al.,[97] from which this table is adapted.

question arises of how to implement these findings into a canine model of HPC transplantation for the benefit of human clinical practice.

Autologous versus allogenic and *ex vivo* culture versus *in vivo* stimulation

In my opinion, technically it is possible to harvest autologous HPCs, expand them in culture and differentiate them into hepatocytes for transplantation purposes. This process is most likely too time-consuming for acute liver failure. In the case of inherited metabolic disease, gene correction could be applied before transplantation. Healthy dog livers contain a 'side population' enriched in progenitor cells, and canine HPCs can be cultured *in vitro* upon isolation from healthy liver tissue.[34,35]

Using a plate-and-wait method, colonies of canine HPCs grew from the non-parenchymal fraction of a digested liver sample within a few weeks. As stated above, in cases of urgent clinical need, this culture method as an autologous source for transplantation would not be feasible. In chronic cases, however, this would be an option and would circumvent rejection issues. Optimisation of culture conditions of primary HPCs is needed in addition to characterisation of cells in culture, most importantly, self-renewal and differentiation capacity and stability. A promising recent development, more specific than 'side population' or plate-and-wait, is the discovery of the Wnt-driven stem cell marker Lgr5 positive cells in injured mouse livers that can be fluorescence-activated cell sorted (FACS) or isolated as 'ducts' and form organoids upon 3D culturing.[36] These cells rapidly expand, have the capacity to differentiate into hepatocytes, and can be kept in culture for more than a year, while maintaining their genomic integrity. The existence of canine liver organoids needs to be established.

More challenging is the *in vivo* stimulation of HPCs. For this, it is of utmost importance to unravel the molecular pathways involved in HPC activation (proliferation, migration, differentiation). This has been extensively studied in rodent models, and to a lesser extent, in human and canine samples.[37-44] Amongst the activation signalling pathways are the well-known stem cell regulators such as Wnt/beta-catenin and Notch signalling. Since activating mutations in these pathways leads to various forms of cancer, it is obvious that long-term follow-up of interference in these pathways is needed before its application in the human clinical setting.

Liver Tumours

There is an obvious association between HPCs and liver tumours, both in man and dog. This association is plausible as HPCs have self-renewal capacity and migratory potential, which is required for invasion and metastasis.[45] HPCs are described as a possible cell of origin for HCC, although this lineage relationship is not directly proven.[4,46-49] Alternatively, the presence of HPC markers in HCC is in line with the possible de-differentiation of fully matured hepatocytes undergoing malignant transformation, and subsequently the expression of immature markers such as K19 in HCCs.[44,50] There is overwhelming clinical evidence that expression of HPC markers, especially K19, in human HCC is a negative prognostic indicator, as these tumours show a higher recurrence rate and shortened patient survival.[4,50] In dogs, the presence of progenitor (K19) and malignancy (glypican-3) markers was evaluated immunohistochemically; the occurrence of K19 positive HCCs was 12%, which resembles the prevalence in humans.[5] Whether, in line with the stem cell marker expression in HCC, men and dogs are similar regarding Wnt and Notch signalling in HCC remains to be answered.

HOW TO PROCEED?

With respect to HPC transplantation, metabolic diseases will probably be the first to be addressed in dogs. Transplantation of hepatocytes has been reported in a few studies of Dalmatians as a model for hyperuricosuria.[51-53] In order to standardise the experimental conditions as much as possible, a large or mid-sized animal model with a well-defined and simple inheritable disease, and a clear phenotype, is ideally suited to evaluate route of cell transplantation (e.g. portal vein versus hepatic artery), to measure short-term transplant engraftment and restoration of liver function. The lifespan allows investigation of long-term effects including the potential risk of tumour formation initiated by the transplanted cells.

In the mid-seventies, a progressive form of chronic hepatitis, accompanied by high levels of liver copper, was first described in the Bedlington Terrier in the United States.[54] It took almost three decades before the responsible gene was identified by means of positional cloning.[55]

A genomic deletion of 39.7 kb covering exon 2 of the *COMMD1* (the gene formerly known as *MURR1*) gene caused a complete absence of the protein product, leading to extreme accumulation of hepatic copper.[55,56] Gene silencing and *COMMD1* -/- mice and dogs confirmed its role in hepatic copper accumulation.[57-60] *COMMD1* is ubiquitously expressed and is involved in many cellular functions including sodium metabolism, regulation of NFκB, and HIF-1alpha-mediated transcription.[57-68] The common denominator in these processes is the fact that ubiquitylation of these proteins is mediated by *COMMD1*. Recent data indicate that *COMMD1* plays a role in the functioning and stability of the human Wilson's disease gene *ATP7B*,[1] providing a clue to how *COMMD1* absence leads to copper accumulation within hepatocytes.[69]

A *COMMD1*-deficient dog presenting with copper storage disease resulting in chronic hepatitis,

provides an excellent model for clinical HPC transplantation trials in view of the requirement for a suitable animal model. It is genetically well-defined hepatitis and fibrosis progression that have been described in detail, and this metabolic disease resembles Wilson's disease. Diseases in a more advanced stage including cirrhosis and ECM remodelling will be more challenging. These types of diseases will require a multi-modular strategy targeting hepatocyte regeneration, fibrosis resolution, and modulation of inflammation. Current developments in anti-fibrotic therapies and the co-transplantation of mesenchymal stem cells (MSCs) or macrophages to modulate inflammatory responses are promising but are currently at the *in vitro* and rodent level.[70,71]

CONCLUSION

There is much promise in the use of HPCs in regenerative therapies for human medicine. In dogs, important molecular and cellular reaction patterns in particular liver diseases are reported, and characterise HPCs and their niche. Overall, HPC marker expression in dogs is comparable to that of humans, as is response to injury and the cell types involved in modulating HPC response. This suggests that the therapeutic potential of these cells is similar in dogs when compared to man, and opens up the potential for developing new strategies for currently untreatable liver diseases, positioning dogs as potentially important animal models to progress from bench-to-bedside.

Yet, this might just be the beginning of the (re-)appreciation of dogs in regenerative and translational medicine. The discovery of the genetic background of hepatic copper accumulation in Bedlington Terriers is an example of simple Mendelian recessive inheritance. Complex human genetic disorders are much more difficult to investigate; cohorts of thousands of participants are needed here. For instance, the phenotypic variation in human Wilson's disease patients and the genetic background of Endemic Tyrolean Infantile Cirrhosis (ETIC), Indian Childhood Cirrhosis (ICC), and idiopathic copper toxicosis are unexplained, partially due to low patient numbers and small pedigrees. Dogs have an ideal population structure for exploring the genetic basis of a variety of disorders, both Mendelian and complex.[72] As a consequence of inbreeding, the genetic complexity of these diseases is reduced. Therefore, inbred dogs are a genetic magnifier, instrumental to discovering crucial and modifier genes involved in Mendelian and complex genetic diseases in humans.

Some examples of complex genetic liver disorders in dogs include copper-associated hepatitis in the Labrador Retriever, Dobermann, West Highland White Terrier, and Dalmatian.[73-77] In these breeds a complex form of copper-associated hepatitis is present, where the susceptibility for copper is genetically determined and the expression of the disease phenotype (severity and/or time of onset) relies on environmental factors like dietary copper intake. Another example is congenital portosystemic shunting, which is a very rare disease in humans, but a much more frequently observed disease in several dog breeds such as Irish Wolfhounds, Labrador Retrievers, or Cairn Terriers.[78-80] Identification of the genetic components involved in this disease will not only be useful for those patients suffering from a congenital portosystemic shunt but may have broad implications for hepatic angiogenesis in general.

The potential benefits of including dogs as an intermediate between rodent studies and human clinical practice does, in fact, close the liver transplantation cycle. It was in 1961 that Starzl and colleagues[81] reported for the first time on liver transplantation in dogs; the field of liver transplantation benefited greatly from this landmark work by Starzl and colleagues.[81] Dogs might also prove to be useful in the next 50 years, and not just a dodgy intermediate.

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OVERLAPPING CLINICAL FEATURES BETWEEN NAFLD AND METABOLIC SYNDROME IN CHILDREN

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a cluster of pathological liver conditions of emerging importance in overweight and obese children. NAFLD is associated with central obesity, insulin resistance, and dyslipidaemia, which are considered to be the main features of metabolic syndrome (MetS). Prevention of the adverse outcomes of NAFLD, as well as the risk of MetS, depends on the identification of genetic background and environmental factors that modulate susceptibility to these diseases. However, several lines of evidence highlight the strong correlation and co-currency of these two chronic diseases, both in children and in adults. In the present review, we provide an overview of the current clinical proofs on the link between NAFLD and MetS in children, with particular focus on all the possible overlapping features that connect them at paediatric age.

Keywords: NAFLD, MetS, adipocytokines, gene polymorphisms, obesity.

INTRODUCTION

The global prevalence of overweight and obesity has considerably increased in several industrialised countries over the past 20 years. In fact, based on the body mass index (BMI) definition, approximately one-third of the world's population is considered overweight (BMI 25-29.9 kg/m^2) or obese (BMI ≥30 kg/m^2). The International Obesity Taskforce (IOTF) estimates that around 1 billion adults and 150 million school-aged children are overweight, while around 475 million adults and 50 million children are classified as obese.[1] The 'obesity epidemic' is particularly relevant in some geographic areas (US, Europe, Australia) where more than 30% of children are obese. It is currently estimated that the continuation of this increasing trend will lead to an incidence of around 60% overweight/obesity in the worldwide population, with several associated early and long-term effects, including metabolic syndrome (MetS).[2] However, it is the presence of abdominal or 'central' obesity (CO), coupled or uncoupled to insulin resistance (IR), that constitutes a critical key risk factor for MetS in children.

Although there are some divergent opinions about the definition of paediatric MetS, it is widely accepted that it is characterised by a cluster of crucial metabolic components including CO, dyslipidaemia (high levels of triglycerides [TGs] and low-density lipoprotein cholesterol, and low levels of high-density lipoprotein cholesterol), hypertension, and IR.[3] In addition to the co-occurrence of these traits, the presence of a fatty liver, configuring to non-alcoholic fatty liver disease (NAFLD), identified by ultrasound, has been recently linked to MetS.[4-6] In fact, NAFLD is becoming one of the most important complications of childhood obesity, affecting approximately 3% of normal children and up to 80% of obese individuals, particularly in industrialised countries.[7]

The recent clinical implications of the long-term effects of MetS and NAFLD on liver cancer and cardiovascular disease development have highlighted the relevance of a full characterisation

of specific signs of these diseases that may appear early in life. Understanding the real role of contact-points between NAFLD and MetS development and progression may help with early identification of patients at risk and of those with some pathological traits; this could then help in the design of short and long-term personalised management programmes for child populations. The present review discusses the current evidence of the link between NAFLD and MetS in children, and addresses the overlapping features that make NAFLD and MetS two sides of the same coin.

NAFLD IN CHILDREN

NAFLD in children includes different patterns of liver diseases assessed by liver biopsy. The intra-hepatic accumulation of fat is defined alone as simple steatosis or non-alcoholic fatty liver (NAFL), whereas if it co-exists with various degrees of necrotic inflammation (lobular and portal inflammation) and ballooning degeneration, it is defined as non-alcoholic steatohepatitis (NASH).[8] In children, like in adult settings, this severe form of NAFLD may be coupled with the presence of long-standing mild-to-severe liver fibrosis.[9] Unfortunately, as published data with long-term follow-up are scarce, the natural history and prognosis of paediatric NAFLD are still uncertain. In susceptible individuals, NAFLD can evolve to cirrhosis and hepatocellular carcinoma, with the consequent need for liver transplantation even though this phenomenon is rare in children.[10] In fact, only a minority percentage of children, suffering from hepatic steatosis, progress to NASH and cirrhosis.[11]

Conventionally, the presence of steatosis in >5% of hepatocytes in the pathological section is considered to be the necessary criterion for NAFL diagnosis. However, adults and children display a different pattern of histological NAFL and NASH damage, making the paediatric form a distinct disease that requires a personalised in-depth evaluation and analysis. It is now widely accepted that the major predisposing risk factors to paediatric NAFLD, as well as for the adult form, are represented by obesity, visceral adiposity, IR, and other disorders, including glucose and lipid homeostasis deregulation, that define MetS. Therefore, to date, NAFLD is firmly considered as the hepatic manifestation of MetS, and several clinical and pathogenetic overlapping features have been found between these two diseases in child populations.

Overlap between NAFLD and MetS

Because of the many different definitions used to diagnose MetS, its prevalence in children ranges between 0-60%. However, despite this epidemiologically wide range, the definition by the International Diabetes Federation (IDF) highlights that obesity is an essential criterion, IR is a prerequisite, and dyslipidaemia is the most frequent metabolic derangement.[12] Furthermore, a simplification of the IDF definition highlights that waist circumference, considered as percentiles rather than absolute values, should represent the main component of MetS in children and adolescents.[13] Therefore, to date, paediatric MetS is differently defined by three age-groups: 6-10 years, 10-16 years, and ≥16 years (considered as adults).[14]

Of note, CO, IR, and dyslipidaemia are considered to be the most prevalent risk factors associated with NAFLD development, providing strong proof of a cross-correlation between MetS and liver damage occurring in liver disease. In paediatric NAFLD the connections with CO (defined by an apple shape), IR, and dyslipidaemia are described in several clinical studies. Despite the multifactoriality of both diseases, their strong association may be explained by a common genetic susceptibility and an analogous pattern of low-grade inflammatory circulating adipocytokines (Figure 1).

Clinical Evidence of Paediatric MetS and NAFLD Connection

One of the first lines of evidence that associates NAFLD with MetS in children is provided by a retrospective review including 43 American children with biopsy-proven NAFLD, which demonstrated that approximately 95% of patients were obese and 95% were insulin-resistant as assessed by BMI and homeostasis model assessment of IR (HOMA-IR).[15] A few years later, Manco et al.[16] performed a cross-sectional study on 197 Caucasian children with NAFLD, highlighting that 92% and 84% of these patients presented a BMI >85th percentile and waist circumference ≥90th percentile, respectively. Furthermore, these authors also demonstrated that CO measured by waist circumference was strongly associated in this cohort of children. This significant association was confirmed by a case-control study on 300 overweight/obese children (150 with biopsy-proven NAFLD and 150 without).[17] This study reported that children with MetS traits had five-times the odds of having NAFLD compared to age-matched obese children without MetS.

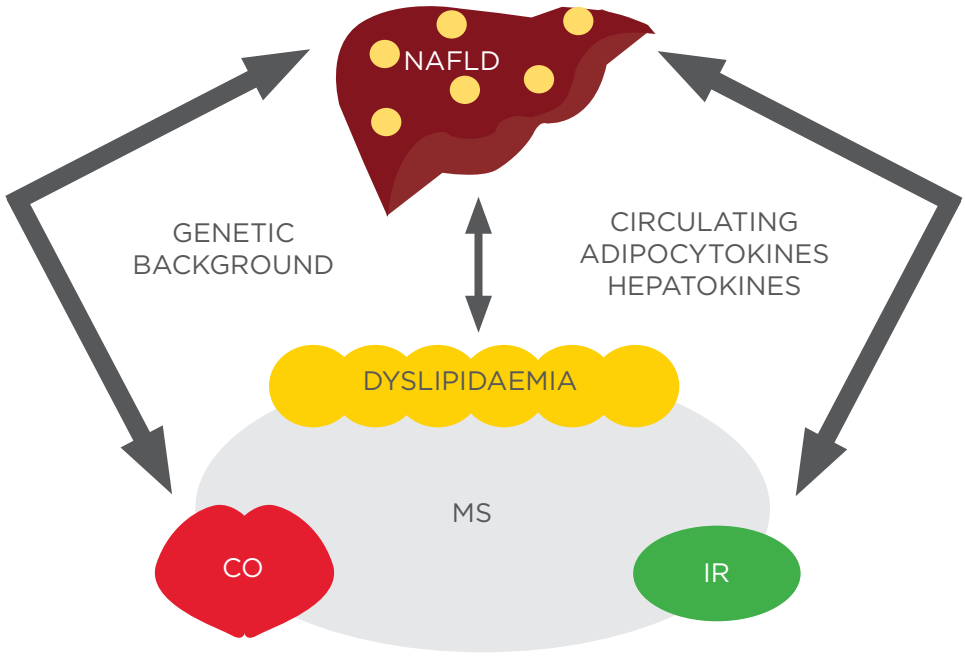

Figure 1: Schematic representation of NAFLD/MetS nexus.
NAFLD: non-alcoholic fatty liver disease; CO: central obesity; MS/MetS: metabolic syndrome; IR: insulin resistance.

Due to the escalation of NAFLD and MetS in children over the last few decades, we have witnessed an increasing amount of attention on this hot-topic from worldwide clinicians and national health care systems.

A cross-sectional study conducted on 1,107 Iranian children and adolescents (6-18 years) demonstrated that overweight or abdominal obesity was the most sensitive predictor of paediatric NAFLD assessed by surrogate markers (i.e. alanine aminotransferase, ALT) and ultrasound.[18] More recently, 254 children enrolled in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) were included in a retrospective study that confirmed not only a prevalence of MetS in NAFLD patients with respect to general population, but also a significant association of CO and IR with the histological severity of liver damage (i.e. fibrosis).[19] Accordingly, a UK cross-sectional descriptive study reported that 34 out of 216 obese children presented increased levels of ALT and other traits of MetS, including elevated BMI and alterations of glucose metabolism.[20] Furthermore, Fu et al.[21] demonstrated that, among 861 Chinese obese children, 68.18% had NAFLD and 25.67% had MetS, and an overlap between features of the two diseases was found in 84.61% of subjects. Therefore, NAFLD should be considered as an early mediator that mirrors MetS status, which could be screened by liver ultrasound.

Finally, a recent study by Silveira et al.[22] has well documented that intra-abdominal fat positively correlates with NAFLD and MetS in children. They showed that intra-abdominal fat was positively correlated with NAFLD (p=0.005), MetS (p=0.013), dyslipidaemia (p=0.001), and HOMA-IR (p=0.007) in 180 subjects aged between 6 and 16 years. Further evidence of the NAFLD-MetS connection highlights that other MetS-related features, including type 2 diabetes, dyslipidaemia, and albuminuria could be at the cross-road between metabolic homeostasis imbalance and liver damage in children.[23-25]

Genetic Polymorphisms in Paediatric NAFLD and Susceptibility to MetS

The escalation of paediatric NAFLD and MetS worldwide prevalence is partly due to over-nutrition and a sedentary lifestyle, which characterises urban adolescents and children.[26] However, the aetiology of MetS and its contribution to NAFLD is complex and is closely related both to lifestyle and genetic predisposing factors.[27] Several potential single nucleotide polymorphisms (SNPs) in genes have been studied in children with NAFLD. These SNPs include: the polymorphism of a gene coding

for the Kruppel-like factor 6 (KLF6), which is associated with fibrosis; the polymorphism of a gene coding for insulin receptor substrate-1 (IRS-1), which is associated with fibrosis; the polymorphism of a gene coding for adiponutrin/patatin-like phospholipase domain-containing 3 (PNPLA3), which is associated with the severity of different histopathological features of NASH (i.e. steatosis, ballooning, inflammation, and fibrosis); the polymorphism of a gene coding for manganese-dependent superoxide dismutase (SOD2), which is associated with liver fibrosis; and the polymorphisms on LPIN1 gene (coding for Lipin-1), which displays an inverse association with disease severity.[28-32] Recent studies demonstrated that the presence of one or more of these SNPs may predispose children to the more severe forms of NAFLD (e.g. NASH and fibrosis), and interestingly, homozygosity for the 148M PNPLA3 allele is associated with a lower response to therapy with docosahexaenoic acid.[33,34]

It is now widely accepted that the components of MetS are also strongly inherited.[35] In fact, data from numerous studies provided challenging evidence suggesting that gene-environment interactions (i.e. the modulation by a genetic polymorphism of a dietary component effect on a specific phenotype) could interact in a way that increases susceptibility to MetS.[36] During the last 5 years numerous genome-wide association studies identified in children many SNPs associated with a large number of conditions related to obesity and traits of MetS per se.[37-39] Although all of these SNPs potentially affect the metabolic function of encoded proteins that may also predispose to more severe NAFLD, until now, there has been no evidence of their association with hepatic damage in children.

Few concerted efforts have been made to investigate SNPs that may recognise subjects with a simultaneous high risk for MetS and NAFLD in children. A recent study, conducted on 250 NAFLD and 200 healthy Chinese children aged between 6 and 16 years, demonstrated that the rs1800849 variant of uncoupling protein 3 (UCP3) gene is associated either with MetS traits (elevated BMI and waist circumference) or increased risk of NAFLD.[40] Furthermore, Nobili et al.[41] recently demonstrated that the severity of obstructive sleep apnoea (OSAS) was associated with the presence of NASH and with the severity of histological necroinflammation and fibrosis, independently of CO, IR, and MetS. However, as OSAS is associated with increased risk of MetS and higher plasma levels of fatty acid binding protein 4 (FABP4), the presence of selective SNPs in the gene encoding for this protein could explain the OSAS common trait in children with MetS and NAFLD.[42] In order to prevent the adverse outcomes of NAFLD, as well as the risk of MetS, the identification of genetic susceptibility profiles for these diseases and their severe patterns (e.g. hepatic fibrosis and cardiovascular disease) will be crucial in designing and testing multi-panels of SNPs as non-invasive markers.

Low-Grade Inflammatory Circulating Adipocytokines

The link between NAFLD and IR in children is now a widely recognised fact, even though the causal/effect relationship between them is still a matter of debate.[6,22,25,43] However, accumulating evidence has demonstrated that NAFLD and IR are strongly associated with low-grade inflammation characterised by the release of circulating adipocytokines.[44]

Adipocytokines such as tumour necrosis factor-α (TNF-α), interleukin 6 (IL-6), adiponectin, leptin, and resistin, which are synthesised and secreted by adipose tissue to regulate energy balance, glucose homeostasis, and insulin sensitivity, seem to be critical mediators of the pattern of low-grade inflammation that often characterise subjects with IR and NAFLD.[45] Therefore, it is not surprising that circulating levels of adipocytokines have also been considered as overlapping features in children. In fact, Nobili et al.[46] demonstrated that values of fasting serum leptin increased concomitantly to steatosis, inflammation, ballooning, and fibrosis worsening, suggesting that hyperleptinaemic status observed early in NAFLD children could be a precondition for promoting IR, overweight, and obesity. Interestingly, 3 years later Lebensztejn et al.[47] found that adiponectin and resistin negatively correlated with grade of liver steatosis at ultrasound, suggesting a protective anti-inflammatory role of these two circulating molecules. However, the same authors demonstrated that only hypoadiponectinaemia was significantly contemporaneously connected with a reduced NAFLD and IR. As adiponectin and leptin control the expression and secretion of TNF-α and IL-6, it is not surprising that these adipocytokines may also be associated with IR and NAFLD.[43]

Although the real role of these two adipocytokines in paediatric NAFLD is still under investigation, a recent study demonstrated that a meal high in saturated fat induced postprandial dyslipaemia, hyperinsulinaemia, and altered lipoprotein expression and low-grade inflammatory profile in obese children with and without NAFLD.[48] Finally, the potential role of adipocytokines as biomarkers for both paediatric NAFLD and IR has been confirmed by three more recent studies, even though discrepancies among the specificity and sensibility of the single mediators as tags for the severity of disease have emerged.[49-52] In addition to the most studied adipocytokines, the circulating levels of retinol-binding protein 4 (RBP4), which is associated with IR pathogenesis, also present an inverse correlation with the degree of liver damage in children with NAFLD.[53] On the contrary, very recently Boyraz et al.[54] demonstrated that RBP4 levels positively correlated with ALT and NAFLD at ultrasound in 63 obese children.

It is plausible that the apparent divergences among the adipocytokines profile and NAFLD/IR association could be ascribable to a different ethnic-dependent pattern distribution of polymorphisms in genes encoding for these molecules. Further multicentre studies that evaluate profiles of circulating adipocytokines and exome-sequencing of the related SNPs to define their nexus with NAFLD and IR co-occurrence in children are needed.

Dual Role of the Hepatokines

In addition to an inflammatory response, the steatotic liver may also contribute to an altered pattern of release of other circulating factors known as hepatokines, directly affecting metabolism and contributing to MetS.[55] Among the hepatokines, two - including fetuin-A, fibroblast growth factor 21 (FGF21), and insulin-like growth factors (IGFs) I and II - could be important as potential non-invasive biomarkers and have been suggested as promising therapeutic targets for MetS and/or NAFLD in children. In fact, Reinehr et al.[56] demonstrated that circulating levels of fetuin-A, which inhibits tyrosine kinase activity of hepatocellular insulin receptor, were higher in NAFLD children and (in these subjects) were also related to MetS features, including IR. Furthermore, Reinehr et al.[57] detected higher values of circulating levels of FGF21 in obese children than in normal-weight children. Despite this, the study demonstrated no association between FGF21 levels and NAFLD, while recently an inverse correlation of this hepatokine with hepatic damage in obese children with NAFLD was reported, suggesting its potential dual role in metabolic and hepatocellular damage.[57,58]

It has become apparent that IGFs may influence not only growth, but also protein, carbohydrate, and lipid metabolism, thus protecting individuals from several MetS features.[55] Interestingly, Cianfarani et al.[59] very recently linked the decreased levels of IGF I and II not only with IR but also with more severe degrees of steatosis, inflammation, and ballooning in paediatric patients with NAFLD. All these studies suggest that although the mechanisms that could explain a potential dual role of hepatokines in paediatric NAFLD and MetS remain fully elucidated, these circulating molecules could represent novel markers of liver and metabolic damage progression.

CONCLUSION

Clinicians need to be aware that to contain the evolution of MetS and NAFLD in children, it would be necessary to diagnose the disease as soon as possible, and particularly before the occurrence of related organ damage (i.e. liver fibrosis and cardiovascular disease). Furthermore, it has recently emerged that NAFLD co-occurs with MetS signs (including elevated BMI and increased triglyceride levels) after the first 5 years of liver transplantation MetS, which probably affects post-transplant survival of patients.[60] Finally, it is interesting that in a recent case-report a non-obese child with acute lymphoblastic leukaemia, preconditioned with total body irradiation before bone marrow transplantation, developed early hepatic steatosis, mild hypertriglyceridaemia, and IR, suggesting that a risk of MetS and NAFLD combination should also be monitored after cancer-related short and long-term treatments that alter MetS-related features.[61]

Childhood cancer survival is now excellent for certain malignancies in which total body irradiation treatment is a mainstay treatment. Therefore, the oncologists should consider a follow-up that includes evaluation of all possible *de novo* metabolic effects that could exacerbate MetS and NAFLD phenotypes. In this context it becomes very relevant to understand the pathogenic connections between MetS features and NAFLD development in the paediatric population,

either for establishing the primary determinant, and/or for extrapolating possible background predisposing conditions that, in the presence of other aetiological environmental factors (i.e. lifestyle), may promote severe liver damage and cardiovascular disease in adulthood.

Therefore, there is a need for new and sensitive early screening methods that are able to provide a large-scale of information about subjects at risk or who are presenting early signs of MetS and NAFLD. These methods could include: the analysis of polymorphism patterns on certain genes encoding for pathway regulatory molecules involved in IR and for circulating mediators associated with a low-grade inflammatory state; and/or the quantitative assessment of plasma circulating mediators that pathogenetically link NAFLD to MetS. In the near future, for patients whose SNPs and circulating profiles are known, it would be possible to draw-up a personalised prevention programme. In fact, the plethora of novel clinical/biological information extrapolated by this type of study could also have a strong impact on the evaluation of the therapeutic properties of drugs currently used and those being tested.

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ADIPOSE TISSUE, METABOLIC SYNDROME, AND NON-ALCOHOLIC FATTY LIVER DISEASE - A SHORT REVIEW

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease globally, and it is expected to rise even further as a result of the increase in obesity and related risk factors. This short review summarises current evidence on the role of adipose tissue and insulin resistance in NAFLD and the interrelationship between NAFLD and the metabolic syndrome (MetS), considering central adiposity is a major feature of both the MetS and NAFLD, and that NAFLD has been previously described as the hepatic manifestation of the MetS. In addition, genetic studies of NAFLD with relation to adiposity and insulin resistance are reviewed, and up-to-date diagnostic and therapeutic tools are also discussed.

Keywords: NAFLD, adipose tissue, insulin resistance, metabolic syndrome, genetics of NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered the most common chronic liver disease worldwide, covering a wide range of diseases from simple steatosis to non-alcoholic steatohepatitis (NASH), with and without fibrosis.[1,2] The liver cell damage that is observed in patients with NASH can lead to cirrhosis or even end-stage liver disease.[3,4] However, estimates of the magnitude of progression to cirrhosis and subsequent transplantations for NASH-related cirrhosis have not been well established.[5,6] In the US, NASH accounted for around 5.5% of the total liver transplants over the period 2001-2009, as either the primary or the secondary indication, with the latter including mostly cryptogenic cirrhosis cases.[6]

NAFLD has been linked to obesity, dyslipidaemia, diabetes mellitus, and hypertension-associated cardiovascular disease, while it also contributes to the overall morbidity and mortality of the latter.[1,2,7] As indicated by its name, the disease is diagnosed in patients who do not have a history of alcohol abuse and have an excessive accumulation of fat in their liver parenchyma.[7] The histopathological diagnosis of NAFLD is actually very similar to alcoholic liver disease, making the diagnosis of NAFLD challenging since differentiation is based on self-reported alcohol consumption.[8]

Normally, only a small amount of fat from dietary fat and carbohydrates is stored in the liver. Lipolysis (in adipose tissue) and *de novo* hepatic lipogenesis also contribute to the stored mass of hepatic fat but counteracting fat removal processes keep this mass low. However in cases of prolonged excess energy supply, where adipose tissue cannot store more free fatty acids, the liver becomes the site of storage, thus resulting in higher fat accumulation.[9] Additionally, changes in the rate of fat synthesis and fat removal by oxidation of fatty acids or by secretion of triglyceride-rich lipoproteins from the liver also contribute to this imbalance.[2,7] In cases of over-nutrition, obesity, and insulin resistance, the balance between fat accumulation and fat disposal is disturbed, resulting in a fatty liver.[2] This excess accumulation of fat in adipose tissue and in the liver induces inflammation and metabolic stress,[9] highlighting

the central role of adiposity in the pathology of NAFLD.

ADIPOSE TISSUE AND ITS ROLE IN NAFLD

Adipose tissue consists of brown and white adipose tissue.[10] Brown tissue (BAT) has a metabolic and a thermogenic role and is more prominent in neonates and infants; however, evidence suggests that it also persists in adulthood, controls energy expenditure, thus protecting against adiposity,[11-12] and exhibits an inverse relationship with Body Mass Index (BMI).[13] Yilmaz et al.[14] have also reported that a reduction in BAT is also associated with an increased risk of NAFLD. The suggested explanations for this finding concentrate on the ability of BAT to reduce hyperlipidaemia[15] and on its thermogenic role, which is mediated by the actions of uncoupling protein 1 (UCP1).[16] On the other hand, white adipose tissue (WAT), which makes up most of the adipose tissue in adulthood, has been better studied and has been shown to be the major site of energy surplus storage in the form of triglycerides that contain intracellular droplets.[17]

In the last two decades, especially after the discovery of the first adipokine - leptin - a lot of attention has been focused towards the dual role of adipose tissue as an endocrine organ, in addition to energy storage. WAT is involved in metabolic activities by releasing adipokines, which are hormone-like proteins, thus promoting adipocyte differentiation and contributing to energy homeostasis and insulin sensitivity. In addition, it releases inflammatory cytokines and anti-inflammatory factors which contribute to the inflammatory processes.[10]

Adiponectin is the most important adipokine, secreted from adipose tissue, found to be associated with insulin resistance. More specifically, decreased levels of adiponectin activity have been associated with obesity or complications of obesity, including insulin resistance and diabetes, as well as with NAFLD.[10,18] It acts through binding to one of its three receptors (AdipoR1, AdipoR2 and T-cadherin), resulting in the activation of a cascade that eventually leads to glucose uptake, decreased entry of free fatty acids into the liver, increased free fatty acid oxidation, and prevention of insulin resistance and inflammation. Expression of adiponectin is inhibited by inflammatory factors secreted by adipose tissue, including tumour necrosis factor alpha (TNF-α) and interleukins IL-6 and IL-18, as well as by oxidative stress and a high fat diet, that act through different signalling pathways.[18] Adiponectin exhibits its effect on insulin resistance mainly through the sensitisation of insulin. Clinical evidence suggests that in people with type 2 diabetes, adiponectin levels are lower than normal,[19] while in patients with NASH, adiponectin was found to be significantly lower compared to a group of patients with simple steatosis.[20]

In addition to adiponectin's central role in insulin sensitivity, leptin and resistin have also been linked to insulin resistance.[21] Leptin controls the pancreatic islet cells, and its levels are usually proportional to insulin levels. However, when there is a decreased signalling of leptin, hyperinsulinaemia may be observed.[10] Resistin has also been linked to insulin resistance (owing its name to that), however, despite experimental evidence in mice where resistin has been shown to be involved in the pathogenesis of diabetes, its role in humans remains unclear. Other adipokines that are proportionally linked to insulin resistance include: apelin, which is raised when insulin levels are elevated, and also in the presence of obesity, in order to regulate insulin resistance by altering the concentration of adiponectin; and visfatin, which is secreted by lymphocytes in adipose tissue and lowers resistance to insulin.[10]

INSULIN RESISTANCE AND MITOCHONDRIAL DYSFUNCTION IN THE LIVER AND MICROBIOTA

Evidence suggests that the liver becomes insulin resistant after steatosis (fatty liver).[21-23] In a fatty liver, insulin can no longer inhibit the production of glucose, thus a high concentration of glucose is produced and released in the plasma (hyperglycaemia) triggering hyperinsulinaemia. At the same time, there is a higher production of very low-density lipoprotein (VLDL) cholesterol, followed by a decreased high-density lipoprotein (HDL) concentration.[21,23] If hyperinsulinaemia persists, then the pancreatic beta cells could be damaged, eventually leading to the development of type 2 diabetes.[24] Contrary to this, it has also been argued that steatosis and subsequent NAFLD are a consequence of insulin resistance. Insulin resistance enhances lipolysis in adipose tissue and stimulates the uptake of free fatty acids by hepatocytes and their accumulation in the liver.[2,7] Moreover, insulin

resistance is accompanied by hyperinsulinaemia, which in turn activates the formation of fatty acids in the hepatocytes by *de novo* lipogenesis and the deposition of triglycerides inside these cells, in a vicious cycle.

Mitochondrial dysfunction is another important characteristic observed in NAFLD.[7] More specifically, the over-production of reactive oxygen species (ROS) by adipocytes, due to hyperglycaemia or excessive energy intake, exerts an overload on the mitochondria, resulting in insulin resistance accompanied by cell damage and mutations in the mitochondrial DNA (mtDNA), as well as a reduction in adipocyte's oxygen consumption. This in turn prevents the oxidation of free fatty acids, thereby enhancing lipid accumulation.[17] Lipid peroxidation, cytokine induction, chemoattraction of inflammatory cells, activation of hepatic stellate cells, and fibrinogenesis are also outcomes of the mitochondrial ROS overload.[21]

In addition to insulin resistance and mitochondrial dysfunction, in the last few years there has been an emerging interest regarding the role of intestinal microbiota in the metabolism of the host,[23] especially with regards to obesity and the metabolic syndrome (MetS). Experimental evidence from mice has shown a change in the gut microbiota when fed a high-fat diet; these alterations triggered the release of pathogen or damage-associated molecular patterns, which increased the intestinal permeability and activated innate immune responses causing inflammation and severe fibrosis.[1,22] The causal association between microbiota composition and development of obesity was established experimentally in mice, by transplanting faecal microbiota from lean and obese mice in germ-free mice.[25] Mice which received the microbiota from the obese mice had a greater fat mass increase. Similar studies have been extended to humans. In a double-blinded, randomised, controlled trial by Vrieze et al.[26] patients with MetS who had received faecal material from lean donors showed a decrease in their fasting triglycerides and improvement in peripheral and hepatic insulin sensitivity.[27] Additional studies have linked gut microbiota with intestinal permeability, and have also linked NASH[15] and microbiota contribution to the lipid metabolism in the liver and development of NAFLD, independent of obesity.[28] Although evidence from human studies is promising, it is inconclusive and clinical trials on the use of probiotics are still needed.

THE METABOLIC SYNDROME AND NAFLD

Development of NAFLD results from the interaction between environmental factors - including obesity, a high calorie diet, and sedentary lifestyle - and genetic predisposition through liver crosstalk with gut, adipose tissue, and the pancreas.[1] Despite being the most common liver disease, the underlying mechanisms of its pathogenesis are not well understood, however it has been suggested that there is an association with insulin resistance and mitochondrial dysfunction.[7] The temporal pathway is still unclear so it is not known whether insulin resistance precedes NAFLD or is just an epiphenomenon. Since NAFLD has previously been associated with several features of MetS including obesity, type 2 diabetes, atherogenic dyslipidaemia, and hypertension, and is characterised by insulin resistance, it has been suggested that this disease may actually be the hepatic manifestation of MetS.[29] Epidemiological data from European populations show that the prevalence of NAFLD among people with type 2 diabetes ranges between 42.6-69.5%, underlining the association between the two diseases.[30]

MetS describes a spectrum of disorders that may contribute to visceral obesity, insulin resistance, hyperglycaemia, dyslipidaemia, and hypertension.[1,10] There has been some evidence that NAFLD promotes the development of MetS and of type 2 diabetes in predisposed individuals[22] - although whether one precedes the other is not yet quite clear - as well as an increased prevalence of MetS in patients with NAFLD, varying with their obesity levels (18-67%).[21,31] Despite its association with MetS and type 2 diabetes, NAFLD can also be observed in non-obese and non-diabetics, thus, it should be referred to as a manifestation of MetS, independent of obesity and plasma glucose.[32]

In obese people, adipocytes, activated macrophages, and Kupffer cells secrete TNF-α, which interacts with its receptors causing systemic and hepatic inflammation.[33] This proinflammatory cytokine (TNF-α), in turn, targets the adipocytes and causes a reduction in adiponectin and an increase in leptin levels. Furthermore, the increase in TNF-α and IL-6, and the subsequent decrease in adiponectin levels, is responsible for insulin resistance in the muscles and the liver.[10,33] However, inflammation and insulin resistance do not always develop in obese people, as every individual has a different capability of

expanding its adipose tissue, thus these effects will only be experienced by the ones that cannot store the excess fat in their adipose tissue.[17]

Regardless of the effects of adipose tissue and MetS on liver histology, several prescribed drugs used for treatment or prevention of other disorders have also been associated with increased risk of NAFLD, especially in people with MetS. More specifically, Tamoxifen, used for the treatment of oestrogen-receptor positive breast cancer, has been associated with increased risk of NAFLD in women MetS,[34] as well as with increased hepatotoxicity in women with pre-existing liver steatosis and breast cancer, regardless of their BMI.[35] Other listed medications that exhibit a similar hepatotoxic effect include antidepressants[36] and corticosteroids.[37] All of the above may do so by exacerbating MetS components like insulin resistance, central obesity, and hypertriglyceridemia.[37] Polypharmacy and/or drug accumulation may also contribute, and careful observation after drug administration is needed to prevent serious liver injury.

GENETICS AND NAFLD

Nutrition and lifestyle choices have been known to affect an individual's probability of developing NAFLD. However, since the first small family studies indicated the potential of familial clustering of NAFLD cases,[38,39] a significant amount of evidence has accumulated, highlighting the importance of the genetic component of the disease as well. Variations of NAFLD in different ethnic populations have also suggested the probability of genetic susceptibility;[40] however, some of those differences could also be explained by the difficulty of actually diagnosing NAFLD without a conclusive liver biopsy. While this may be true, in the controlled setting of the US SCALE study, African Americans had a lower risk of presenting with NAFLD compared to Hispanics, who appeared to be the most susceptible.[40] Subsequent reports by the same group also focused on the increased risk for siblings (59%) and for parents (78%) of probands compared to siblings and parents of non-probands (17% and 37%, respectively).[41] The genetics of NAFLD are therefore quite complex, presenting with a polygenic pattern of susceptibility and with genetic factors participating in many metabolic pathways, including lipid and glucose metabolism, oxidative stress, immune response, and apoptosis.[42] Here we have focused on genetic studies and methods that have looked at the association between adiposity/MetS and NAFLD.

Older studies had shown an association between simple genetic variants with NAFLD, but findings about a common genetic ground between visceral adiposity and liver fat have not been consistent.[43-45] More recently, Speliotes et al.[46] confirmed a previously described genetic association with the PNPLA3 gene, and have identified additional genetic variants (single-nucleotide polymorphisms [SNPs]) in genes associated with both computed tomography (CT) hepatic steatosis and histologically proven NAFLD (rs2228603-NCAN, rs12137855-LYPLAL1, rs780094-GCKR). Among them only the NCAN and GCKR genes were associated with metabolic traits like serum and liver lipids and also glucose traits. Adding to previous reports,[45] these findings suggest that the pathophysiology of NAFLD may result from distinct abnormalities, visceral adiposity and glucose resistance being considered as two of them.

Another important pathway that has been implicated with progression of the disease is the combination of variants of ENPP1/PC-1 membrane protein (ectoenzyme nucleotide pyrophosphate phosphodiesterase 1/plasma cell antigen) and variants of the IRS-1 (insulin receptor substrate-1), which affects insulin receptor signalling in the liver. The Lys121Gln polymorphism in the ENPP1/PC1 combined with the Gly972Arg variant in the IRS-1 genes have been recently and significantly associated both with the features of MetS and with a more progressive state of NAFLD, independently of confounding factors.[47]

Another set of evidence that supports the presence of a common genetic ground between adiposity/MetS and NAFLD comes from studies that had focused on adiponectin, secreted by adipose tissue. Via its receptor, AdipoR2, which is expressed primarily in the liver, adiponectin increases free fatty acid oxidation and inhibits insulin resistance, while it has also been reported to suppress fibrosis and inflammation.[18,48] Genetic variants in the adiponectin gene have been found to be more prevalent in cases of severe fibrosis compared to milder manifestations of NAFLD (+45T>G) and also in patients versus controls (+276G>C and -11377G>C).[49,50] Moreover, genetic polymorphisms that affect adiponectin levels have already been hypothesised to be implicated in the variability seen in the NAFLD phenotype.[51]

Some examples include the UCP1 -3826 A>G polymorphism, which has been associated with severe hepatic steatosis even in the absence of MetS,[52] and the 161C>T polymorphism in the peroxisome proliferator-activated receptor-gamma (PPAR-γ) gene, which has been reported to be associated with NAFLD status in a case control study in a Chinese population.[53] More recently, additional SNPs that have been previously implicated in the adiponectin pathway have been examined for association with NAFLD as well, with reports that a combination of the APPL1-C/APPL2-A alleles significantly increased the risk of NAFLD (OR=2.50; 95% CI:1.45 to 4.32) as well as the probability of severe steatosis compared to the major allele combination (OR=3.88; 95% CI:1.582 to 9.531).[54] A meta-analysis of eight case-control studies, however, failed to confirm an association between the PPAR-γ2 Pro12Ala polymorphism and NAFLD.[55]

DIAGNOSTIC TOOLS AND TREATMENT OPTIONS FOR NAFLD

An important first step in the diagnosis of NAFLD is the exclusion of significant consumption of alcohol, with a consensus threshold of <21 drinks per week for men and <14 drinks per week for women (≈2 years before the examination) being used.[56] Along with absence of other causes of chronic liver disease like the use of steatogenic medication, hereditary disorders, Wilson disease, severe malnutrition, and Hepatitis C, NAFLD can be diagnosed using histology or imaging for the quantification of hepatic steatosis.[57] Histology assessment following invasive, expensive, and sometimes serious complication-inducing liver biopsy is considered the only definite test, but several non-invasive diagnostic methods have also been developed for NAFLD. These include plasma liver aminotransferase measurements, ultrasound imaging (US), CT, magnetic resonance imaging (MRI), and transient elastography, as well as plasma cytokeratin-18 fragment levels.[58]

In a recent review, Festi et al.[59] highlighted the strengths and weaknesses of the diagnostic methods that are currently in practice and proposed an algorithm of non-invasive tests that could facilitate physicians and improve diagnostic accuracy while eliminating the need for liver biopsy. US was confirmed as the most appropriate screening method, and although already widely adopted,[60] it is still being evolved; the most recent example is the development of the Controlled Attenuation Parameter (CAP) application, which is based on ultrasound attenuation by liver fat measured by FibroScan® (Echosens, France).[61] Early reports regarding the validity of this method compared to the 'gold standard' liver biopsy, highlight its ability to assess steatosis in a simple fashion and in an operator-independent manner.[62,63] In a similar fashion, liver stiffness measurement (LSM) has been used to evaluate the stage of fibrosis[64] but, mainly due to its high negative predictive value, LSM has been proposed as a tool that could cost-effectively exclude fibrosis and cirrhosis of the liver.[65]

Imaging techniques can always be coupled with biochemical markers, while approaches like cytokeratin-18 plasma levels, CT, and MRI can be utilised in case of conflicting results.[59] However, the challenge lies in the quantification of fibrosis, and a number of biomarker combinations have been examined towards this goal, including the officially recommended[57] NAFLD fibrosis score (NFS),[66] the FibroTest,[67] the Original European Liver Fibrosis (OELF) panel,[68] and the FibroMeter.[69] Each of them has proven to be quite useful;[59,70] however, comparisons between them are not easy since their results are based on heterogeneous populations.[70]

Some novel non-invasive approaches include the use of an oral chlorine tolerance test and the utilisation of the terminal peptide of procollagen III (PIIINP). Using the oral chlorine tolerance test, it was shown that fasting plasma free choline (fCh) levels with a threshold value of 0.16 mg/dL could lead to the early detection of NASH patients with a sensitivity of 80.1% and a specificity of 82.6%,[71] while the terminal peptide of procollagen III was able to distinguish patients with advanced fibrosis.[72] Furthermore, a recent report on cell death biomarkers used in NAFLD diagnostics stated that both caspase-cleaved and uncleaved cytokeratin-18 performed better in diagnosing lower levels of fibrosis compared to only caspase-cleaved cytokeratin-18 fragments,[73] while a separate group in Germany has recently developed and validated a cost-effective fibrosis scoring system called Koeln-Essen-index (NIKEI). This alone, or especially when combined in a stepwise mode with the FIB-4 test, could exclude advanced fibrosis in NAFLD patients.[74]

Up-to-date clinical practice for treating NAFLD involves both dietary and physical activity

interventions. Although randomised controlled trials (RCTs) that had assessed the effect of lifestyle interventions on NAFLD were subject to limitations, evidence suggests an overall positive effect for the patients, with such interventions as weight loss being reported to lead to a significant reduction (10-51%) in the percentage of liver fat.[58] In addition, strong evidence suggests that physical activity coupled with diet modification has a significant effect on several parameters of NAFLD, including reduction of aminotransferase and intra-hepatic fat levels, as well as improved insulin sensitivity.[75] A number of recent reviews and meta-analyses have compared the differential effect of the low-carbohydrate diet versus the most widely adopted low-calorie diet on several NAFLD metrics; these reported that waist circumference, which is a proxy of abdominal fat, was significantly reduced by the low carbohydrate diet as opposed to the low-calorie diet.[76-79] The importance of such findings is further highlighted by independent studies reporting a significant relationship of visceral adipose tissue (VAT) with increased risk of fatty liver disease[51,80] as opposed to subcutaneous adipose tissue.[81] Furthermore, of special interest for clinicians and nutritionists worldwide is the effect of the amount of alcohol consumed on the risk of developing NAFLD. Although high alcohol consumption is definitely associated with liver toxicity, a recent meta-analysis reported that moderate alcohol consumption (<40 g/day) is associated with a protective effect, especially in women, and with a 50% reduction in the risk of the disease progressing to more advanced stage (NASH) compared to abstainers.[82]

Overall, a successful lifestyle intervention should not only target VAT and evaluate the amount of alcohol consumed, but should also include a diet with appropriate macronutrients, as recent studies have identified discrepancies between diet composition and improvements in fatty liver disease.[76,83] Long-term clinical trials on the dietary recommendations for NAFLD are still needed.[83]

In patients for which lifestyle intervention has not resulted in long term improvement or stabilisation of the disease, bariatric surgery may be suggested, which would result in a relevant improvement in steatosis, inflammation, and fibrosis.[84] Although there is no pharmaceutical compound specifically administered for NAFLD or NASH, several products are being used or investigated for their effects on several pathways or manifestations of the disease. Insulin sensitisers (like pioglitazone) are well studied, and evidence supports that a significant reduction of insulin resistance, liver inflammation, and fibrosis can be achieved with their use.[85-87] Statins have also been found to reduce the risk of hepatic steatosis,[88] while antioxidants like vitamin E, a relatively cheap choice, can also be recommended and have been shown to be comparable to pioglitazone in a comparative study.[89] Low levels of such antioxidants have been related to the development of the disease,[90] however a well-designed study failed to show a statistically significant effect.[91] Future studies should concentrate on revealing the true nature of antioxidants on NAFLD progression and also evaluate the potential health risks from increased doses of such synthetic compounds, as recent evidence, although weak, suggests the possibility of vitamin E being positively associated with oncogenesis.[92]

On the other hand, obeticholic acid, a semi-synthetic farnesoid X receptor (FXR) agonist, recently under investigation by Intercept Pharmaceuticals (double-blind, placebo-controlled FLINT trial) for its potential effect on NAFLD, appears to be a promising candidate for the treatment of nonalcoholic steatohepatitis. Although relevant publications on the FLINT trial are not yet available, the company has officially announced that the trial has been stopped as interim results have confirmed the efficacy of the compound (significant reduction in the NAFLD Activity Score of at least two points in the treated group compared to the placebo group). A number of animal studies have also demonstrated the positive effect of obeticholic acid on liver histology, with reported reductions in profibrotic growth factors, liver inflammation, and oxidative stress.[93] The only other human study looking at obeticholic acid also reported significant improvements in insulin sensitivity and reduction in markers of liver fibrosis.[94] An antihyperglycaemic agent, metformin, is another well studied candidate for NAFLD pharmaceutical intervention as animal[95,96] and human studies[97-99] indicated its safety and effectiveness in treating NAFLD. However, small sample sizes and conflicting results from other studies[100] highlight the need for larger and well planned human studies.

Central adiposity is a major feature of both MetS and NAFLD, and NAFLD has been described as

the hepatic manifestation of MetS, while in patients with both NAFLD and type 2 diabetes, rapid progression of both diseases, accompanied by an increased number of complications, are the end result. Current research on pharmaceutical agents for NAFLD seems promising, with new compounds expected to be available soon. However, given the worldwide rise in obesity prevalence, an ensuing rise in the prevalence of MetS and NAFLD is to be expected, with a relevant increase in related morbidity and mortality. This further highlights the importance of both primary and secondary lifestyle modifications in reducing the incidence of NAFLD and managing its symptoms.

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COST-EFFECTIVE INTERVENTIONS IN THE CONTROL OF CHRONIC HEPATITIS B (CHB) INFECTION

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ABSTRACT

The hepatitis B virus (HBV) causes infection in the liver that can lead to cirrhosis, liver cancer, and premature death. The disease is not widely recognised as a serious public health problem, and as a result, inadequate resources are being allocated to hepatitis B prevention and control. Vaccination against HBV has been a great success and has resulted in a reduction in the rate of chronic infection; however, the vaccine is of no help for those already infected. The big challenge is how to deliver effective and affordable care to those who are carriers and who are eligible for treatment, and affordable diagnostics to detect those who are not yet aware of their infection, to prevent the spread to susceptible individuals. This review intends to give the reader a brief overview of the types of control strategies that have been examined in recent cost-effectiveness studies on the control of chronic hepatitis B.

Keywords: Chronic hepatitis B, cost-effectiveness, vaccination, treatment, screening.

COST-EFFECTIVE INTERVENTIONS FOR CHB

Cost-effectiveness analysis (CEA) is a method used to evaluate the outcomes and costs of interventions designed to improve health.[1] The purpose of a CEA in healthcare is to help the decision-maker determine how to allocate resources across a defined number of competing needs in order to maximise health outcomes from a limited budget.[2] The quality-adjusted life year (QALY) is a measure of effectiveness - more time spent in good health. The incremental cost-effectiveness ratio (ICER) is the net increase in cost of the intervention compared to standard care/no treatment to gain 1 QALY. The ICER is the incremental costs of implementing an intervention over another intervention (or no intervention), divided by the incremental effectiveness (QALYs) from another intervention (or no intervention). The World Health Organization (WHO)[3] defines the threshold value for intervention cost-effectiveness as one-to-three times the gross domestic product per capita (GDP) of a country. An intervention is considered cost-saving if it is more effective and less costly than the comparator. Chronic hepatitis B (CHB) is a serious public health problem; an estimated 1 million people annually die of hepatitis B virus (HBV) related chronic active hepatitis, cirrhosis, and liver cancer. Therefore, the cost of this disease to public healthcare systems is considerable. For the control of this infectious and chronic disease, vaccination, screening, and treatment strategies have been studied in various settings and countries. Table 1 gives an overview of the recent cost-effectiveness studies.

VACCINATION

HBV vaccination created the first breakthrough in HBV prevention, which is the most effective measure to prevent new HBV infections and its consequences. Studies on cost-effectiveness from the UK and Ireland (low endemic countries) were carried out to estimate the impact of a universal infant vaccination programme;[4,5] both of these countries have a policy to selectively vaccinate individuals at high risk of HBV infection, but neither

have, as yet, introduced universal HBV vaccination policies. Siddiqui et al.[4] concluded that in order for universal infant vaccination to be considered cost-effective, the average cost of vaccinating should be reduced to £4.09, which is the average cost for vaccine and administration costs of all three doses. In Ireland, universal infant vaccination will be cost-effective with an ICER of €37,018, which the authors concluded compares favourably with other preventive programmes in Ireland. A study from Germany[6] concluded that the use of a vaccination strategy to reduce transfusion transmission of HBV would represent a potential cost reduction of €200 million over a 20-year period when compared with current mandatory testing in Germany, while also offering the near-elimination of transfusion infections with HBV.

Rein and Weinbaum[7] were interested in the cost-effectiveness of using hepatitis A/B combined vaccine versus HBV vaccine alone for high-risk heterosexuals in the US. They found the use of combination A/B vaccine to be substantially less cost-effective than other vaccination strategies against viral hepatitis. An ICER of administering combination vaccine to all high-risk heterosexuals aged 15-44 was $120,000/QALY gained, equal to almost three-times the GDP per capita. The authors concluded that the cost-effectiveness of this intervention appears to be at the outer reaches of acceptability by WHO standards.

Kuan et al.[8] compared the cost-effectiveness of HBV vaccination using heplisav - which uses fewer doses over a shorter time than currently licensed vaccines - in selected adult populations in the US compared to Engerix-B vaccine. The authors concluded that the results from this CEA demonstrate that Heplisav is cost-saving in patients with chronic kidney disease and end-stage renal disease, and is cost-effective ($25,000/QALY) in the diabetic population, healthcare workers, and for travellers. Hoerger et al.[9] examined the cost-effectiveness of a HBV vaccination programme for unvaccinated adults diagnosed with diabetes in the US. They concluded that HBV vaccination for diabetic adults aged 20-59 was modestly cost-effective ($75,094/QALY), while vaccination for adults 60 years and older was cost-ineffective ($2.7 million/QALY).

Kim et al.[10] assessed the cost-effectiveness of four strategies for vaccinating potentially high-risk adults attending two major types of publicly funded HIV counselling and testing sites: freestanding counselling and testing sites, and sexually transmitted disease clinics in the US. Results of this study implied that integrating routine HBV vaccination programs into existing HIV counselling and testing sites may be a cost-effective ($3,500-$4,400) public health intervention. Looking at various willingness-to-pay thresholds, Chen et al.[11] concluded that intramuscular hepatitis B immunoglobulin (HBIG) treatment for neonates of hepatitis B surface antigen (HBsAg) carrier mothers is likely to be cost-effective in addition to universal vaccination, particularly in settings with adequate healthcare infrastructure; however, in very resource-limited settings, universal vaccination alone is optimal. Two other studies from high endemic areas, China and Taiwan,[12,13] estimated that universal vaccination compared to no vaccination is cost-saving and even avoids loss of productivity. According to the study by Hutton et al.[14] catch-up vaccination among children and adolescents is a cost-saving strategy in China, where the endemicity is the highest in the world.

TREATMENT

Vaccination against hepatitis B has resulted in a reduction in the rate of chronic infection;[15] however, vaccine is of no help for those already infected. Antiviral therapy is the only option to control and prevent progression of disease in patients with active CHB.[16] The goal of therapy for CHB is to improve quality of life and survival by preventing progression of the disease to cirrhosis, decompensated cirrhosis, end-stage liver disease, hepatocellular carcinoma, and death.[17] A review I recently compiled[18] gives an overview of cost-effectiveness studies on CHB treatment, where most of these studies primarily focused on entecavir and tenofovir monotherapy, followed by rescue therapy for patients who developed resistance.

SCREENING

Testing for CHB meets established public health screening criteria as formulated originally by Wilson and Junger.[1,19] It is a serious health disorder that can be diagnosed before symptoms develop,[2] and it can be detected by reliable, inexpensive, and minimally invasive screening tests.[4] Chronically infected patients have years of life to gain if medical evaluation, monitoring, or treatment is initiated early; also,[5] the costs of screening are reasonable in relation to the anticipated benefits.

Table 1: Summary of recently published cost-effectiveness studies.

Intervention	Country/Target Group	Summary of Study Strategy	Outcomes
Vaccination			
Siddiqui et al.[4]	UK/infants	Universal infant vaccination	Not cost-effective (£263,000/QALY) - if vaccine cost is reduced strategy becomes cost-effective
Tilson et al.[5]	Ireland/infants	Universal infant vaccination	Cost-effective (€37,018)
Fischinger et al.[6]	Germany/blood donors	Vaccination to reduce transfusion transmission	Cost-saving if tested for anti-HBs and receives a time-dependent booster vaccination
Rein and Weinbaum[7]	USA/high-risk heterosexuals	A/B combined vaccine versus B vaccine alone	$120,000/QALY
Kuan et al.[8]	USA/selected high-risk groups	Fewer doses over a shorter time versus currently licensed vaccines	Cost-effective ($25,000/QALY) in the diabetic population, healthcare workers, and travellers
Hoerger et al.[9]	USA/diabetic adults	Vaccination for diabetic adults	Cost-effective ages 20-59 ($75,094/QALY) not cost-effective age 60+ ($2.7 million/QALY)
Kim et al.[10]	USA/adults attending STD clinics	Vaccinating high-risk adults attending HIV counselling and testing sites	Cost-effective ($3,500-4,400)
Chen et al.[11]	Taiwan/neonates	Immunoglobulin for neonates in addition to universal vaccination	Cost-effective ($1,400-4,000)
Hung et al.[12]	Taiwan/infants	Universal infant vaccination versus no vaccination	Cost-saving
Lu et al.[13]	China/infants	Universal infant vaccination long-term outcomes	Cost-saving
Hutton et al.[14]	China/children and adolescents	Catch-up vaccination	Cost-saving
Treatment			
Toy[18]	Systematic review, Global	Overview of recent cost-effectiveness studies on treatment of CHB	CEA studies for CHB focused on entecavir and tenofovir monotherapy followed by rescue therapy for patients that develop resistance
Screening			
Hutton et al.[22]	USA/Asian and Pacific Islanders	Screening and vaccination	Cost-effective ($36,088-39,903)
Robotin et al.[23]	Australia/Asian-born adults	HCC surveillance versus HCC prevention (including CHB treatment)	HCC prevention strategy cost-effective (AUD $12,956/QALY)
Wong et al.[24]	Canada/foreign-born adults	Screen and treat versus no screening	Cost-effective (CAD $69,209/QALY)
Rossi et al.[25]	Canada/migrants and refugees	Screen and treat versus no screening	Cost-effective (CAD $40,880/QALY)
Veldhuijzen et al.[26]	Netherlands/migrants	Screen and treat versus no screening	Cost-effective (€8,966/QALY)

Intervention	Country/Target Group	Summary of Study Strategy	Outcomes
Screening (Continued)			
Eckman et al.[27]	USA/ asymptomatic outpatients	Screen and treat versus no screening	Cost-effective ($29,230/QALY)
Ruggeri et al.[28]	Italy/high-risk groups	Screen and treat versus no screening	Cost-effective (€17,179/QALY)
Davidson et al.[29]	Sweden/blood donors	Nucleic acid testing among blood donors	Not cost-effective ($2.7million/ QALY)
Zurawska et al.[30]	USA/patients with lymphoma	Screening for HBV before chemotherapy versus high-risk groups or no screening	Cost-effective ($32,589/QALY)
Adibi et al.[31]	Iran/adults prior to marriage	Screening versus no screening	Cost-effective ($197-202 per infection averted)
Other			
Guo et al.[32]	China/pregnant women	HBIG injection versus no HBIG	Cost-effective ($118)
Nayeri et al.[33]	USA/pregnant women	Lamivudine treatment at third trimester versus no treatment	Cost-saving
Unal et al.[34]	USA/pregnant women	Lamivudine or HBIG treatment at third trimester versus no treatment	Cost-saving
Toy et al.[35]	China/general population	Monitor (inactive) and treat (active) strategy CHB versus current practice (no monitoring)	Cost-effective ($2,996/QALY)

CHB: chronic hepatitis B; HBIG: hepatitis B immunoglobulin; HCC: hepatocellular carcinoma; STD: sexually transmitted disease.

One-time HBV screening may identify most individuals and will give the opportunity to vaccinate those who are susceptible, and to initiate effective antiviral therapy before the development of advanced liver disease.

In many high-risk areas, particularly those in Asia, HBV is transmitted from mother to newborn (vertical transmission); as many as 90% of infected babies develop chronic infection.[20] Hepatitis B screening during pregnancy, and postpartum immunoglobulin and HBV vaccination in neonates born to HBV-infected mothers is far from being universally implemented.[21] In low endemic countries, CEA studies are mainly focused on analysing whether various screening programmes that are specifically targeting migrants from endemic countries - those considered high-risk - are cost-effective. Hutton et al.[22] chose to target the Asian and Pacific Islander population in the US, since the incidence of liver cancer is more than three-times higher among this population, and around 60-80% of liver cancer cases are attributable to HBV infection. Hutton et al.[22] concluded that screening the Asian and Pacific Islander adult population is likely to be cost-effective ($36,088-$39,903/QALY gained). Robotin et al.[23] targeted the Asian-born adults in Australia as their study population for the different management strategies for the control of CHB. They concluded that the liver cancer prevention strategy coupled with antiviral treatment is cost-effective (AUD $12,956/QALY gained).

Wong et al.[24] were interested in the screening strategies for 20-65 year-old individuals who were

born abroad but are currently living in Canada. Their analysis suggested that the screening and treatment of all migrants is moderately cost-effective ($69,209/QALY gained). Another CEA study[25] from Canada concluded that HBV screening and treatment for newly arrived adult Canadian immigrants and refugees is reasonably cost-effective ($40,880 QALY gained); however, if they were to combine screening, treatment, and vaccination, this strategy would not be considered cost-effective ($437,335/QALY gained). According to Veldhuijzen et al.[26] systematic screening and early treatment of migrants in the Netherlands is a cost-effective strategy (€8,966/QALY gained).

Eckman et al.[27] examined screening, followed by treatment of those who were eligible, in asymptomatic outpatients living in a region in the US with an HBV infection prevalence of 2%, which was cost-effective ($29,230/QALY). Ruggeri et al.[28] examined a test strategy in Italy, where they define the high-risk group as: immigrants from high endemic countries, intravenous drug users, prisoners, individuals with other infections, patients undergoing dialysis, pregnant women, and subjects with high transaminase; it involved the administration of a screening test to patients at high risk, and the treatment of the infected, and it yielded an ICER of €17,179/QALY compared to no testing. The cost-effectiveness of introducing nucleic acid testing among blood donors in Sweden was studied by Davidson et al.,[29] where they concluded that the cost-effectiveness ratios for this intervention are far beyond what is considered cost-effective, with a cost of $12.7 million per avoided viral transmission, and $2.7 million/QALY gained. Zurawska et al.[30] were interested in whether HBV screening before chemotherapy for lymphoma was considered cost-effective, and concluded from their finding that, in patients receiving chemotherapy for lymphoma, screening all patients for HBV reduces the rate of HBV reactivation (10-fold) and is less costly ($32,589/QALY) than screening only high-risk patients or screening no patients.

A CEA approach on whether testing adults for HBV prior to marriage has an effect on the impact of transmission prevention and whether it is cost-effective in Iran or countries with similar cultural backgrounds was examined by Adibi et al.,[31] in which they concluded that it costs between $197-202 for each infection averted.

Three studies have examined the cost-effectiveness of maternal treatment to prevent perinatal HBV transmission.[32-34] Two of these studies, both from the US, aimed to estimate the cost-effectiveness of maternal lamivudine, or HBIG treatment, in addition to standard neonatal immunoprophylaxis; they concluded that both of these interventions, compared to doing nothing, were cost-saving across a wide range of assumptions. The third, also from the US, concluded that lamivudine administration in the third trimester of pregnancy is a cost-effective ($1,073/QALY) and, frequently, a cost-saving intervention. The group from China concluded that injecting immune globulin to infants after birth is more cost-effective ($118) compared to injecting immune globulin during pregnancy.

A study done by our group,[35] compared the current strategy - not monitoring inactive chronic HBV patients - to a monitor and treat (M&T) strategy in Shanghai, China. The M&T strategy would include twice-yearly assessment of HBV and alanine transaminase (ALT) levels in patients with chronic HBV. Our findings suggested that lifelong monitoring of inactive chronic HBV patients is cost-effective ($2,996/QALY), but relies on identifying more cases of HBV infection and also on increasing treatment, monitoring, and antiviral adherence to achieve health gains.

CONCLUSION

Governments around the world face budget constraints that compel them to make tough decisions about how to best invest funds for public health.[36] CEA is an essential evaluation tool that allows policymakers and health planners to compare the health gains that various interventions can achieve with a given level of input.[36] An example of a real-life impact of a CEA on health policy is the screening study where Hutton et al.[37] convinced the Centers for Disease Control (CDC) to update their recommendations; the CDC's most recent hepatitis B screening guidelines recommend screening all adult Asian and Pacific Islanders for hepatitis B as well as all adults born in areas of intermediate (2-7%) HBV prevalence. Also, Hutton et al.[37] were successful in influencing the China CDC with their analysis of the cost-effectiveness of the catch-up vaccination program: in April 2009, China decided to include free HBV catch-up vaccinations for all children under the age of 15. In most countries, estimating what it

would cost to expand the coverage of existing interventions or to add new interventions relies on assumptions. CEA will almost always include a series of assumptions as it is generally not possible to measure everything necessary for a comprehensive analysis.[38] CHB needs to be widely recognised as a serious public health problem, and as a result, resources need to be allocated to HBV prevention and control. The big challenge is how to deliver the cost-effective interventions to control the disease.

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USE OF HBSAG QUANTIFICATION TO GUIDE HBIG PROPHYLAXIS AFTER LIVER TRANSPLANTATION

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ABSTRACT

Hepatitis B surface antigen (HBsAg) quantification has recently been introduced to guide treatment in chronic hepatitis B virus (HBV) patients. No information is currently available on use of HBsAg levels to guide HBV immune globulin (HBIG) administration after liver transplantation (LT). We performed a retrospective analysis of a prospectively collected database. Patients were included if: adults (≥18 years); recipients of a primary liver graft; HBsAg-positive and HBV DNA-negative at transplantation; hepatitis C and/or HIV-negative; not transplanted for fulminant hepatic failure; on nucleoside analogues. All patients were administered 30,000 IU HBIG, perioperatively, and hepatitis B surface antibody (HBsAb) was tested at day 7, 14, 28, and monthly thereafter. A further 30,000 HBIG were administered if HBsAb <100 mIU/mL and/or HBsAg >100 IU/mL on day 7. The primary endpoint was the efficacy of HBIG as a percentage of patients achieving HBsAg <100 IU/mL and HBsAb ≥100 mIU/mL at day 7. Secondary endpoints were performance of HBsAg levels in predicting HBsAg loss at day 7, HBV recurrence, graft, and patient survival at last follow-up. 41 LT recipients - transplanted between January 2011 and June 30, 2013 - were included (median age 54 years; male 78%). Hepatocellular carcinoma was present in 24 (58.5%) and hepatitis delta in 19 patients (46.4%); 7 (17.1%) patients did not achieve efficacy at day 7 and were boosted with additional 30,000 HBIG. A pre-transplant HBsAg level ≥1,000 IU/mL was associated with 60-fold odds for failure at day 7 (p=0.0002). At a median follow-up of 14 months after LT, graft and patient survival were 100% and no case of HBV recurrence had been observed. Based on our results, we advocate the use of HBsAg titre to guide HBIG prophylaxis after LT.

Keywords: Liver transplantation, hepatitis B virus, HBsAg, hepatitis B immune globulin, quantification.

INTRODUCTION

Hepatitis B virus (HBV)-related liver disease is one of the major indications of liver transplantation (LT) worldwide[1] and the leading indication in Asia.[2,3] When used in combination with oral nucleos(t)ide analogues (NA), hepatitis B immunoglobulin (HBIG) allows prevention of reinfection of the liver graft with HBV recurrence rates at <10% of recipients.[4,5] Although not entirely elucidated, the mechanisms accounting for the efficacy of HBIG prophylaxis are a reduced production of hepatitis B surface antigen (HBsAg) and a decreased rate of escape mutations in the presurface/surface gene and polymerase regions.[3] Due to recent introduction of more potent NA and to costs associated with use of HBIG,[6] there has been impetus to explore reduced-dose and/or short-term HBIG schedules, while maintaining low HBV recurrence rates.[3,7-11] As a result, several prevention strategies have been reported in the literature in the past decade, producing a shift in current practice

from high-dose intravenous (i.v.) HBIG - administered indefinitely from the intraoperative phase[8,9] - to life-long, low-dose intramuscular (i.m.) HBIG - without intraoperative administration[3] - to tailored HBIG schedules based on recipients' recurrence risk,[9,10] or selective and planned HBIG withdrawal.[10,11]

Quantification of HBsAg is increasingly used to determine the treatment response in patients with chronic hepatitis B (CHB).[12] Previous evidence suggests that in hepatitis B envelope antigen (HBeAg)-negative patients, HBV DNA <2,000 IU/mL and HBsAg <1,000 IU/mL can predict inactive carrier status, low risk of hepatocellular carcinoma (HCC), and the probability of HBsAg clearance.[12] When used in combination with HBV DNA, HBsAg decline can be used to predict response to interferon therapy, and a level <100 IU/mL during 6 months may be a marker of sustained response after treatment cessation.[13] There are limited data about the clinical implications of HBsAg quantification for LT recipients undergoing post-transplant prophylaxis with HBIG and on use of pre-transplant HBsAg titres to predict HBsAg clearance after transplantation. We investigated the clinical correlation between pre-transplant HBsAg levels and the probability of HBsAg loss in adult recipients of a liver graft-administered HBV prophylaxis with a combination regimen of NA and HBIG.

MATERIALS AND METHODS

Patients

This was a retrospective analysis of a prospectively collected database on adult (≥18 years) LT recipients transplanted at our institution for HBV-related disease. In January 2011 we implemented a quantitative measurement of HBsAg level for patients on the LT waiting list and transplant patients during their follow-up period. Patients were included in the current analysis if: 1) male or female recipients of a primary, whole-size, ABO-compatible liver graft from a deceased donor; 2) ≥18 years at transplantation; 3) HBsAg-positive (±hepatitis delta [HDV]); 4) HBV DNA-negative; 5) on NA therapy for ≥30 days before surgery. Patients were excluded from analysis if: 1) transplanted for HBV-related fulminant hepatic failure; 2) co-infected with hepatitis C virus and/or HIV; 3) enrolled in concurrent clinical trials on post-transplant HBV prophylaxis or immunosuppressants; 4) the liver was transplanted in combination with other organs; 5) deceased donors were HBsAg-positive.

Treatment Schedule

Patients underwent administration of 30,000 HBIG i.v. (NeoHepatect™, Biotest, Dreiech, Germany) over 5 days, starting from the day of transplant (6,000 daily) and in combination with NA. HBsAg titres were measured at transplantation (baseline) and at day 7, 14, 28, and monthly thereafter. Titres of the antibody to HBsAg (HBsAb) and HBV DNA were obtained at transplantation and at day 7, 14, 28, and monthly thereafter. If patients failed to achieve HBsAg titres <100 IU/mL and/or HBsAb ≥100 mIU/mL at day 7, a further 30,000 IU HBIG were administered i.v. over 5 days (6,000 daily). Once HBsAg was <100 IU/mL and HBsAb was ≥100 mIU/mL, patients were switched to i.m. (Igantibe™, Grifols, Barcelona, Spain) or subcutaneous (s.c.) (Zutectra™, Biotest, Dreieich, Germany) HBIG within 14 days of last i.v. administration, and dosing was adjusted as per HBsAb ≥100 mIU/mL through the entire follow-up period. With regard to NA, patients on lamivudine (LAM) were switched to entecavir (ETV, Baraclude™, Bristol-Myers-Squibb Italy, Rome, Italy) 0.5 mg/day starting from transplantation, while patients on ETV or tenofovir disoproxil (TDF, VireadTM, Gilead Italy, Milan, Italy) were kept on their pre-transplant treatment. Patients on combination of LAM and adefovir dipivoxil pre-transplantation (Hepsera™, Gilead Italy, Milan, Italy) were switched to TDF after surgery.

Immunosuppression

Patients were administered quadruple immunosuppression with 20 mg basiliximab i.v. (Simulect™, Novartis Italy, Origgio [VA]) at transplantation and on day 4, in association with tacrolimus (TAC, Prograf™, Astellas Pharma SpA, Assago [MI], Italy), steroids, and mycophenolate mofetil (MMF). TAC was initiated within 5 days after surgery according to renal function, and trough levels were 6-10 ng/mL for the first year and 3-8 ng/mL thereafter. MMF was initiated immediately after surgery at 1 g/day and maintained for 4 months, unless otherwise indicated by renal function. Steroids were started intraoperatively at 10 mg/kg and tapered within 3 months after surgery. Introduction of everolimus (EVR, target range 3-8 ng/mL) (Certican™ Novartis Italy, Origgio [VA]) starting at month 1 was evaluated on an individual basis when TAC minimisation was sought (3-5 ng/mL), such as in the presence of TAC-related adverse effects (i.e. renal function deterioration, neurotoxicity, diabetes mellitus, and cardiovascular

complications) or in patients with HCC and unfavourable prognosticators on explant histology (microvascular invasion, perineural infiltration, low grading).

Liver Histology

At donor's surgery, liver histology was obtained if clinically indicated. Post-transplantation, liver biopsy was performed in cases of suspicion of acute cellular rejection, HBV recurrence, or whenever clinically indicated.

Enrolment Period and Setting

Patient enrolment into the present treatment schedule started in January 2011. Current analysis includes patients transplanted until 30 June 2013. Setting: academic hospital. In Italy, the compulsory National Health System (NHS) covers for post-transplant prophylaxis with HBIG and pre and post-transplant treatment with NA.

Variables

The variables included in the current analysis were: 1) patients' demographics (gender, ethnicity, age at transplantation, body weight, height, body mass index); 2) donors' demographics (gender, ethnicity, age, body weight, height, body mass index, liver histology if available); 3) clinical (with focus on HDV co-infection and presence of HCC); 4) transplant data (date, model for end-stage liver disease [MELD] at transplant); 5) immunosuppression (drugs, doses, blood levels, and duration of treatment); 6) non-immunosuppressive treatment (with focus on HBIG dosing); 7) laboratory tests (liver function tests, serum creatinine, HBsAg (IU/mL), HBsAb (mIU/mL), and HBV DNA qPCR (IU/mL); 8) histology when clinically indicated.

Laboratory

HBsAg quantification and HBsAb titres were obtained with a chemiluminescence immunoassay (Architect™, Abbott, Chicago, Illinois, USA) with an inferior sensitivity threshold of 0.05 IU/mL for HBsAg and 10 mIU/mL for HBsAb. For the purposes of the current analysis, HBsAb values >1,000 mIU/mL were capped at 1,000.

Endpoints

The primary endpoint of the proposed study was efficacy of HBIG as the percentage of patients achieving HBsAg <100 IU/mL and HBsAb ≥100

Table 1: Demographic and clinical characteristics of the overall population (#41 patients).

Variable	
Age (years, median: IQR)	54, 9
Male (n, %)	32, 78
Ethnicity (Caucasian n, %)	41, 100
NA (LAM n, %)	20, 48.8
Duration of NA, months (median, IQR)	15, 12
HDV (n, %)	19, 46.4
HCC (n, %)	24, 58.5
MELD (median, IQR)	18.5, 8
Baseline HBsAg titre, IU/mL (median, IQR)	345.6, 419.8
Donor age, (years, median: IQR)	73, 11
Donor HBcAb (positive n, %)	11, 26.8
Brain dead donors (n, %)	41, 100
Whole-size graft (n, %)	41, 100
Time from listing to transplantation, months (median, IQR)	4, 12

HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma; HDV: hepatitis delta; IQR: interquartile range; LAM: lamivudine; MELD: model for end-stage liver disease; NA: nucleos(t)ide analogues; HBcAb: antibody to hepatitis B core antigen.

mIU/mL at day 7. The secondary endpoints were: evaluation of the performance of a pre-transplant HBsAg titre ≥1,000 IU/mL for prediction of the risk of failure at day 7;[13] identification of the pre-transplant HBsAg cut-off value for prediction of the risk of failure at day 7; incidence of HBV recurrence, defined as HBsAg and/or HBV DNA positivity after negativity or consistent liver histology; graft and patient survival at last follow-up. Graft survival was censored at the time of re-listing at the transplant centre(s) or at latest follow-up. Patient survival was censored at time of death or latest follow-up. HBV recurrence was censored at the time of HBV DNA and/or HBsAg positivity, histology, or latest follow-up.

Data Collection and Management

The study was approved by our Internal Review Board (IRB) and was carried out in compliance with the principles set forth in the Helsinki Declaration and in the Italian Medicinal Agency (Agenzia Italiana del Farmaco [AIFA]) code set for observational drug studies in human populations (www.aifa.gov.it). Data were imputed into an electronic database at our institution. Data anonymity and management were in agreement with the Italian data protection code law.

Statistical Analysis

According to type and level of distribution, data are reported as medians, interquartile ranges (IQR), percentiles, means, standard deviations (SD), ranges, extremes, and frequencies, as appropriate. The chi-square and the Fisher's tests were used for categorical variables, while the t-test or the Mann-Whitney tests were used for variables with continuous distribution according to their level of variance. A receiver operating characteristic (ROC) analysis was carried out to select the pre-transplant HBsAg cut-off value for prediction of HBsAg loss at day 7. Graft and patient survivals were obtained with the Kaplan-Meier curves. The level of statistical significance was set at 5% and the confidence interval at 95%.

RESULTS

Pre-Transplant

Out of 51 HBsAg-positive patients transplanted between January 2011 and June 30 2013, a total of 41 matched the eligibility criteria and were included in the current analysis (Table 1, Figure 1). The median (IQR) age was 54 years (9) (range 30-66), and 32 patients (78%) were male. Ethnicity was Caucasian in all patients.

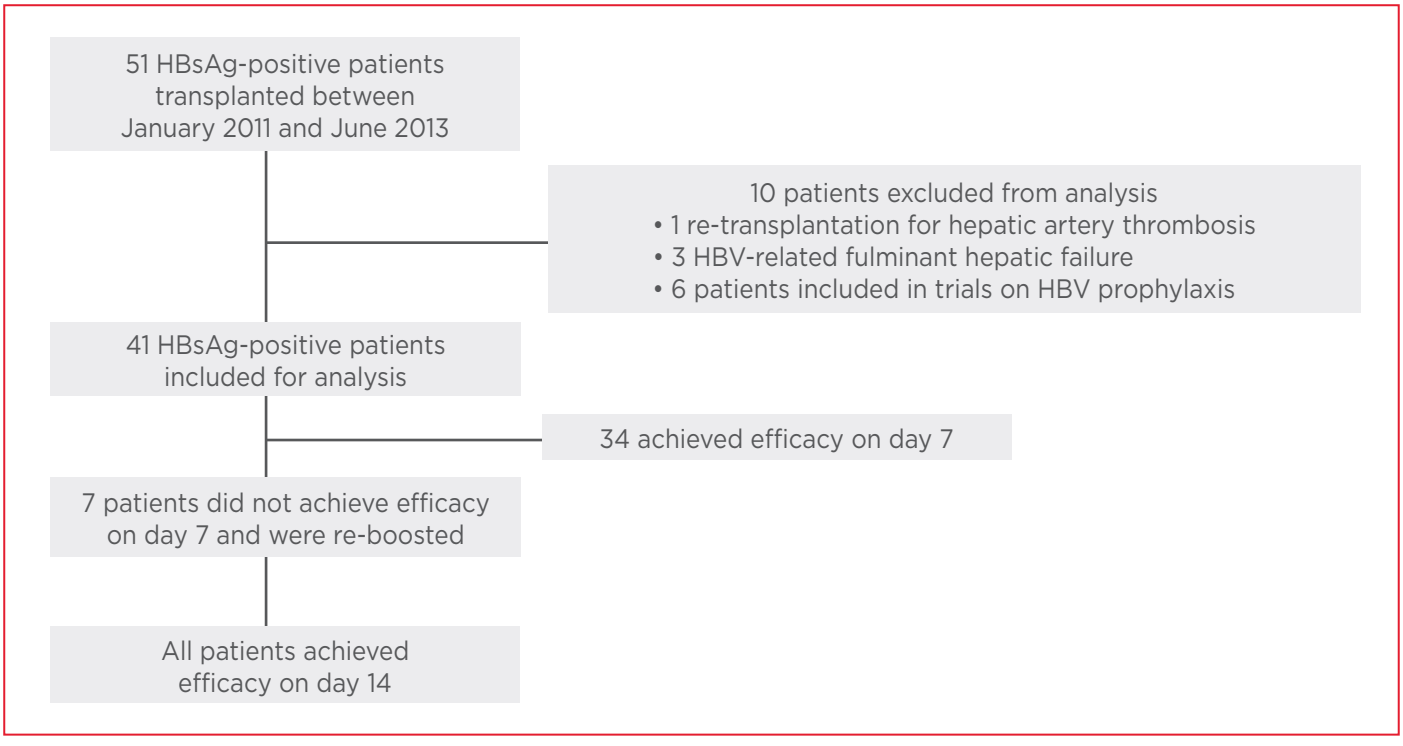

Figure 1: Patients' disposition algorithm.
HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus.

HCC was present in 24 (58.5%) and HDV infection in 19 (46.4%). Pre-transplantation, 20 (48.8%) patients were on LAM, 18 (43.9%) on ETV, 2 (4.9%) on LAM + ADF, and 1 (2.4%) on TDF. The median (IQR) duration of pre-transplant treatment with NA was 15 (12) months (range 3-43) in the overall population, whilst it was significantly longer (p<0.0001) for patients on LAM (median [IQR], 22.5 (16.2) months) versus those on ETV (median [IQR], 11.4 (6) months) (*data not shown*). Four (5.9%) patients had been administered interferon at any time point pre-transplantation. The median (IQR) time from wait listing to transplantation was 4 (12) months (range 1-14 months) and the median (IQR) MELD score at transplantation was 18.5 (8) (range 11-22). All donors were brain dead and graft was whole-size in all cases. The median (IQR) donor age was 73 (11) years (range 43-89) (Table 1).

Post-Transplant

On day 7 post-transplantation, the current schedule achieved efficacy in 34 (82.9%) patients (Table 2, Figure 1); 7 patients (17.1%) required additional administration of 30,000 IU HBIG, and all achieved efficacy on day 14 (Table 2, Figure 1). The HBsAg titre decreased from a median (IQR) of 345.6 (419.8) IU/mL (range 54.6-16,523) at transplantation to a median (IQR) of 0 (0) IU/mL (range 0-3,673.2) on day 7 (p=0.017), and the 7-day median (IQR) HBsAb level was 1,000 (342.2) mIU/mL (range 6.5-1,000). On day 14, HBsAg was lost in all patients and the median (IQR) HBsAb titre was 806.7 (345.7) mIU/mL (range 432.8-1,000) (p=0.45 between day 7 and 14). At a median (IQR) follow-up of 14 (6) months after LT (range 7-35) graft and patient survival were 100% and no cases of HBV recurrence had been observed.

Table 3 illustrates the univariate comparison between efficacy patients (#34) and failures (#7). Patients not achieving efficacy at day 7 had higher pre-transplant HBsAg levels than efficacy patients (median (IQR) 3,352.05 (2885.4) versus 345.2 (243.5) IU/mL; p<0.0001). An HBsAg titre ≥1,000 IU/mL was associated with 62.2 (95% CI 6.0-1993.2) odds for failure at day 7 (p=0.0002). The ROC curve analysis revealed that the cut-off value for prediction of HBsAg clearance at day 7 was 876.5 IU/mL (area under ROC curve =0.98) and was associated with 100% sensitivity (95% CI, 54.1-100%), 94.3% specificity (95% CI, 80.8-99.3%), 75% positive predictive, and 100% negative predictive values (Figure 2).

Table 2: Results.

Variable	Overall (#41)	Efficacy (#34)	Failures (#7)	p*
HBsAg titre at day 7, IU/mL (median, IQR)	0 (0)	0 (0)	2,178 (916)	<0.0001
HBsAg titre at day 14, IU/mL (median, IQR)	0 (0)	0 (0)	0 (0)	-
HBsAb titre at Day 7, mIU/mL (median, IQR)	1,000 (342.2)	1,000 (231.2)	12.9 (14)	<0.0001
HBsAb titre at Day 14, mIU/mL (median, IQR)	806.7 (345.7)	786.4 (345.7)	837.7 (434.7)	0.85
HBV recurrence** (%)	0 (0)	0 (0)	0 (0)	-
Graft survival** (%)	100	100	100	-
Patient survival** (%)	100	100	100	-

HBsAb: antibody to hepatitis b surface antigen; HBsAg: hepatitis B surface antigen; IQR: interquartile range; HBV: hepatitis B virus.
*p is between efficacy and failure groups.
**median (IQR) follow-up is 14 (6) months (range 7-35).

Table 3: Univariate comparison between efficacy patients (#34) and failures (#7).

Variable	Efficacy (#34)	Failures (#7)	p
Age, years (median, IQR)	54 (13)	53 (7)	0.65
Males (n, %)	25, 73.5	5, 71.4	0.65
NA (LAM n, %)	19, 55.9	3, 42.8	0.56
Duration of NA, months (median, IQR)	15 (13)	17 (19)	0.61
HDV (n, %)	15, 57.1	4, 44.1	0.68
HCC (n, %)	21, 61.7	3, 42.8	0.42
MELD (median, IQR)	22 (8)	19.5 (8)	0.88
Donor age, years (median, IQR)	73 (12)	70.5 (10)	0.91
Donor HBcAb (positive n, %)	9, 26.5	2, 28.6	0.88
HBsAg titre, IU/mL (median, IQR)	345.2 (243.5)	3,352.05 (2,885.4)	<0.0001
HBsAg titre ≥1,000 (n, %)	1, 3.0	5, 71.4	0.0002

HBsAg: hepatitis B surface antigen; HCC: hepatocellular carcinoma; HDV: hepatitis delta; IQR: interquartile range; MELD: model for end-stage liver disease; NA: nucleos(t)ide analogues; HBcAb: antibody to hepatitis B core antigen.

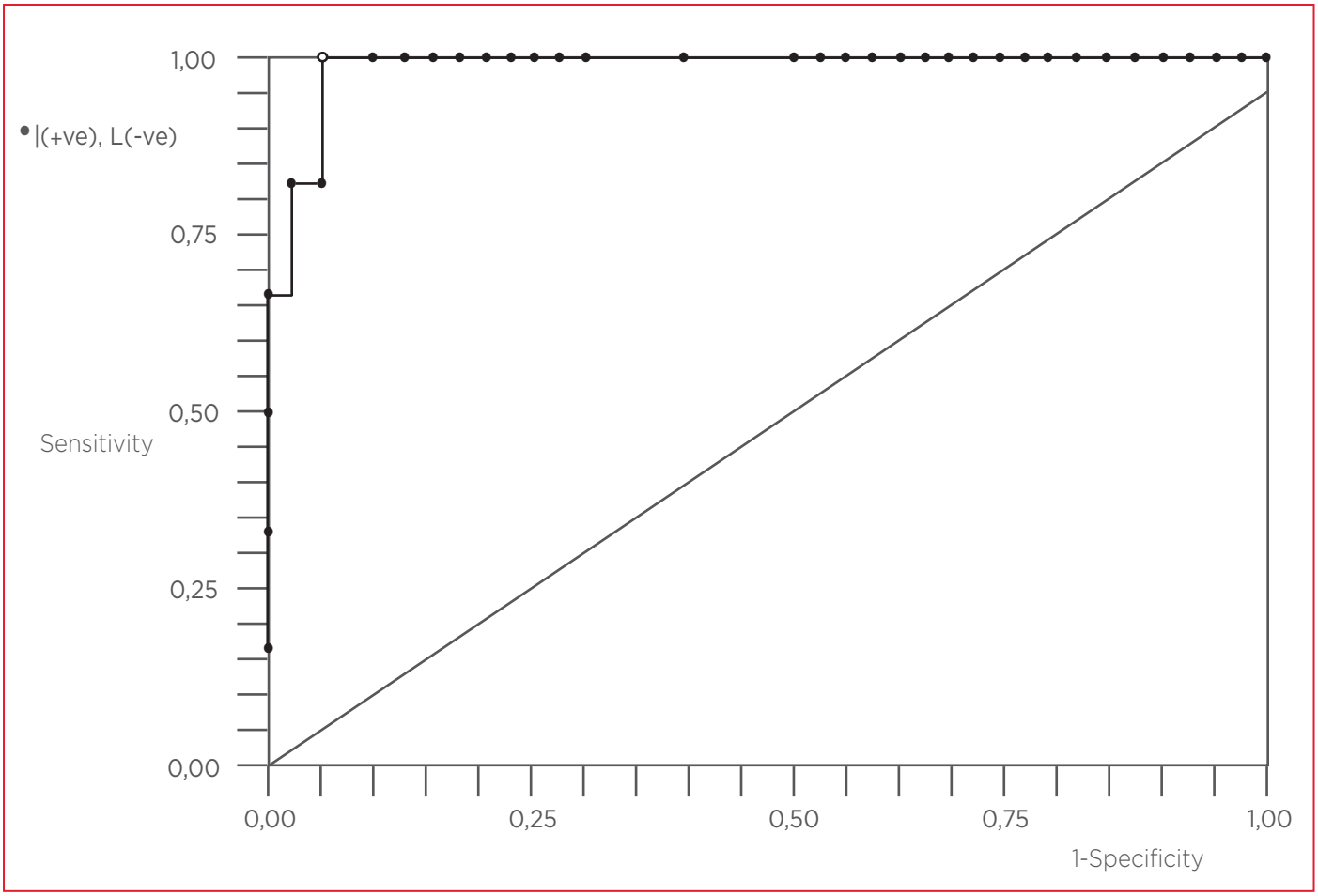

Figure 2: ROC curve analysis for pre-transplant HBsAg levels (IU/mL) in predicting probability of failure at day 7.

HBsAg: hepatitis B surface antigen; ROC: receiver operating characteristics.

DISCUSSION

In the setting of LT for HBsAg-related disease, HBsAg clearance is sought to reduce the risk of disease recurrence and its negative impact on graft and patient survival. To achieve this, the policy most frequently adopted post-transplantation is combination treatment with NA and HBIG.[2,5] Recent experimental data support the evidence that HBsAb is necessary for HBsAg neutralisation and clearance during HBV infection.[14] HBsAb exerts antiviral activities by blocking viral entry into cells, interfering with virion release, accelerating HBV clearance from the circulation, and leading to a decreased rate of escape mutations in the pre-surface gene/surface gene and polymerase regions.[15] Previous *in vitro* and *in vivo* studies have demonstrated that HBIG suppresses functional maturation of cytokines by human blood-derived dendritic cells and inhibits proliferation of peripheral T cells, thus reducing the incidence of rejection in the post-transplant period.[16] After LT, combination prophylaxis with HBIG and NA provides better control of HBV reinfection (<10% up to 3 years after LT)[17-20] versus either HBIG (15-25%)[21,22] or LAM mono-prophylaxis (20-40%),[23,24] and administration of HBIG is usually initiated in the intraoperative, anhepatic phase[8] or immediately after surgery.[3] The recent introduction of novel NA with better resistance profiles and the costs associated with immune globulins are challenging the paradigm of long-term HBIG administration and raising interest in low-dose HBIG and/or HBIG-sparing protocols.[11] Replacement of long-term HBIG with active immunisation has resulted in conflicting results, with some authors reporting achievement of protective HBsAb titres,[25,26] and others only a 7.1% rate (1/14 patients) of seroconversion after vaccination.[27]

One further strategy to reduce the economic burden associated with HBIG is aiming at lower HBsAb protective titres. While some authors still comply with titres emerging from early studies on HBIG monotherapy (<6 months post-LT =500 mIU/mL; 6-12 months post-LT =251-500 mIU/mL; and >1 year post-LT =150-250 mIU/mL),[22] the majority have lowered the threshold to ≥100 mIU/mL throughout the post-transplant period[3] and a few are suggesting even lower thresholds (≥50 mIU/mL) with favourable results.[5] Several reports have documented the feasibility and efficacy of a low-dose HBIG schedule, which currently seems the strategy most frequently adopted in Asia and Southern Europe.[28-30] Other investigators have attempted to discontinue HBIG after the initial post-transplant period and continue with antiviral monotherapy alone, especially in low-risk patients (usually HBeAg negative with undetectable HBV DNA at transplant), with recurrence rates varying from 0-16%.[11,31-33] However, HBIG withdrawal may be associated with a variable risk for HBsAg re-emergence and HBV recurrence, according to a patient's risk profile and viral characteristics. In one of the series with the longest patient follow-ups, the probability of HBV recurrence 4 years after HBIG discontinuation was 9%,[34] and eventual studies have yielded similar results (6.3% recurrence at a median follow-up of 24 months after HBIG withdrawal).[11]

Limited information is currently available on the factors contributing to HBsAg clearance after LT. To better understand the role of pre-transplant HBsAg titres, we set up the current study using recent evidence on HBsAg quantification in CHB. Introduction of commercial quantitative assays has improved our understanding of the fate of HBsAg in CHB patients, and a combination of HBsAg <1,000 IU/mL and HBV DNA <2,000 IU/mL can identify a 3-year inactive state in genotype D HBeAg-negative carrier populations.[13] In Asian populations, where genotype B and C are dominant, HBsAg levels between 10 and 100 IU/mL can predict loss over time.[35] In low-viraemic carriers, HBsAg levels <1,000 IU/mL have been associated with reduced risk for HBeAg-negative hepatitis, cirrhosis, and HCC.[35] In CHB patients treated with interferon, HBsAg quantification is being introduced to guide response to treatment,[13] whilst HBsAg decline is slow for CHB patients on NA. However, a rapid decline in HBsAg may help to identify patients with higher probability of HBsAg clearance in this latter category.[35]

Our study hypothesised that pre-transplant HBsAg levels might influence the rate and velocity of HBsAg loss after LT with a schedule of fixed-dose HBIG administration. This was in view of tailoring HBIG administration on the individual risk profile and improving cost-effectiveness of HBV prophylaxis. Our preliminary data suggest that patients with pre-transplant HBsAg ≥1,000 IU/mL require higher HBIG doses to achieve HBsAg clearance and protective HBsAb titres (≥100 mIU/mL). These patients presented a 60-fold higher risk for HBsAg persistence at day 7 after a course of

30,000 IU HBIG (p=0.0002) and underwent further boosting with 30,000 IU HBIG, as per the current protocol. However, future studies should focus on tailoring HBIG dosing until HBsAg clearance has been achieved by means of on-demand HBIG schedules guided by daily/weekly HBsAg and/or HBsAb testing. The cut-off value of 876.5 IU/mL we found in the present trial needs to be validated in larger series before being used in clinical practice to guide HBIG dosing in the early post-transplant phase.

CONCLUSION

The major limitation of the current study is that we did not fully investigate all viral and disease characteristics that might determine the fate of HBsAg clearance after LT, such as viral genotype and resistance, previous anti-viral treatments (NA versus INF), liver disease history, and severity. All of these factors should be evaluated and integrated to provide optimal care to HBsAg-positive LT recipients. However, to the best of our knowledge, this is the very first time that HBsAg quantification has been explored in LT population as a marker of HBsAg loss and seroconversion with a fixed-dose HBIG schedule. We advocate larger series to introduce such a marker in clinical practice and guide, among other clinical parameters, HBIG dosing in the early post-transplant course.

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LIVER TRANSPLANTATION FOR ALCOHOLIC LIVER DISEASE – TIME FOR A PARADIGM SHIFT?

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ABSTRACT

Liver transplantation for alcoholic liver disease (ALD) is controversially discussed. Although overall survival after liver transplantation is similar to other indications, long-term survival is significantly reduced in patients with recurrence of excessive alcohol consumption. Criteria of transplant eligibility and prediction of risk of alcohol relapse after transplantation are the core concerns in this setting. Most transplant centres therefore require an abstention period of 6 months prior to listing. However, data on the '6-month rule' as a surrogate parameter for prediction of relapse are conflicting, and first reports on liver transplantation in highly selected patients with acute alcoholic hepatitis without response to medical treatment are promising. Therefore, a thorough pre-transplant evaluation by an experienced addiction specialist in addition to regular counselling and a highly supportive social surrounding after transplantation seem to be the key factors for long-term survival in ALD patients.

Keywords: Liver transplantation, alcoholic liver disease, alcohol markers, abstention period, relapse.

INTRODUCTION

Excessive alcohol consumption is responsible for 4% of mortality and presents the third leading risk factor for disease and disability worldwide.[1] Alcoholic liver disease (ALD) is the most prevalent liver disease in Europe and is the second most common indication for liver transplantation (LT) in Europe and the United States.[2,3] It comprises of a wide range of hepatic manifestations including alcoholic fatty liver disease, alcoholic steatohepatitis (ASH), and liver cirrhosis complicated by portal hypertension. LT has been well established as a life-saving treatment for end-stage ALD. However, as determined by the shortage of donor livers, alcoholic cirrhosis as an indication for LT is discussed controversially. Since alcoholism is a life-long disease and is not cured by LT, optimal selection of patients with a low risk of alcohol relapse, as well as continuous monitoring and support after LT, are essential.

DIAGNOSIS OF ALCOHOL ADDICTION AND ALD

Alcohol dependence has to be differentiated from alcohol abuse (DSM-IV), or hazardous and harmful drinking (WHO),[1] as well as from sporadic drinking episodes.[4,5] This classification is of particular importance within the transplant setting, since patients may be denied LT in the case of suspected alcohol abuse. Moreover, alcohol relapse in transplant recipients has to be detected at an early stage to provide psychomedical support and preserve long-term graft function. Clear diagnosis of alcohol consumption and ALD is complicated by the lack of definite cut-off values of ethanol, identified as harmful in certain populations.

Hepatic steatosis was found in 60% of patients with regular alcohol intake >60 g/day.[6,7] Liver cirrhosis was confirmed by liver biopsy in 29% of a large series of patients with alcoholism.[8] In a meta-analysis the daily consumption of >25 g ethanol has been associated with an increased risk of liver cirrhosis and its complications.[9]

Recently, increased risk of mortality due to liver cirrhosis was even found with consumption <25 g of ethanol per day (12-24 g/day).[10] Thus, patients might be put at risk even if ethanol levels are below the current public recommendations for alcohol consumption. Differential diagnosis to non-alcoholic fatty liver (NAFL) and steatohepatitis (ethanol cut-off: 20 g/day for women, 30 g/day for men), and the assessment of alcohol as an additional hit to the liver in other liver diseases are difficult.

RISK FOR ALCOHOL RELAPSE AND IMPACT ON OUTCOME

Outcome of LT for ALD in Europe is similar to other indications with a 5 and 10-year survival of 73%, and 59%, respectively.[11] Relapse of alcohol consumption occurs in 10-50% of patients undergoing LT for end-stage ALD.[4,12-14] Of these, 10-36% of patients resume drinking heavily.[5,14,15] Graft dysfunction can be found in up to 17% of patients.[16]

Cuadrado et al.[17] reported significantly reduced 10-year survival rates of 45% versus 86% in transplant recipients returning to alcohol use. Similarly, a recently published study - investigating alcohol relapse rates in living donor LT (LDLT) in Japan - described 10-year survival rates of 22% (versus 74%) in patients with alcohol relapse.[18] Of note, in contrast to patients with recurrence of heavy drinking, who suffer from subsequent organ dysfunction due to recurrent ALD and have mainly liver-related mortality, the majority of patients with low-to-moderate alcohol consumption (in the absence of other liver diseases), or those who are long-term abstainers, die from *de novo* malignancies, cardiovascular disease, or infections.[13]

Reports studying non-adherence to immunosuppression in patients with alcohol relapse show a wide range of 3-47%.[19-21] However, non-adherence is not directly associated with alcohol relapse, but rather with each patient's personality.[22] Unexpectedly, patients with LT due to ALD have, in general, a lower rejection risk than patients transplanted due to other indications, suggesting an immune-inhibitory effect of alcohol.[3,23,24]

Lower social support, psychobehavioural comorbidities, family history of alcoholism, diagnosis of alcohol dependence, repeated attempts at rehabilitation, non-compliance with clinic visits after LT, and smoking were all identified as risk factors for alcohol relapse after LT, along with pre-transplant abstinence period;[4,25,26] protective factors include patient insight and perception of the negative consequences of alcohol.[24,26] Therefore, thorough evaluation of psychosocial influencing factors and psychobehavioural disorders should be considered the core of the risk assessment prior to transplantation.

ELIGIBILITY FOR LT

The '6-Month Rule'

Originating from the fact that alcohol abstention can lead to dramatic improvement in liver function to a point where LT is no longer necessary, most transplant centres require a 6-month abstention period before patients become listed for LT. Above all, plausible abstention ≥6 months has been used as a surrogate parameter for long-term sobriety after LT to identify patients who will most benefit from LT. However, data on the '6-month rule' are controversial and a clear rationale is lacking.[27] Although shorter sobriety periods prior to LT are predictive of future relapses,[4,13,28] sobriety becomes robust only after 5 years of alcohol abstention.[29,30] On the one hand, this may be a result of inconsistent definition of alcohol use and alcohol dependence used in these studies, on the other hand, this may be due to the difficulty in evaluation and detection of alcohol abuse and relapse. Therefore, the UK Liver Transplant Group, rather than using a specified period of abstinence, agreed on certain contraindications for listing, including: alcoholic hepatitis, repetitive episodes of non-compliance with medical care, returning to drinking following full professional assessment, and concurrent illicit drug use.[31-33]

LT for Alcoholic Hepatitis

The discussion concerning selection criteria of patients with ALD becomes even more controversial in the context of alcoholic hepatitis. Historically, patients suffering from acute ASH have been denied LT due to active alcohol consumption.[34] However, mortality in patients failing to respond to corticosteroids in comparison to responders is veritably high (28-day survival 53% versus 91%, 6-months survival 30%).[35] Particularly, recent reports on favourable outcomes after LT for severe ASH have led to a change in therapeutic algorithms, and according to the

European Association for the Study of the Liver (EASL) guidelines, LT could be a treatment option for highly selected patients.[36] Singal et al.[37] demonstrated similar 5-year patient survival rates in patients transplanted for ASH compared to patients transplanted for alcoholic liver cirrhosis (73% versus 78%). Furthermore, a case-control study by Mathurin et al.[38] showed a dramatically improved survival at 6-month follow-up for patients who had received LT in comparison to patients who had received medical treatment, but who only partially responded or were non-responders (77% versus 23%). Only patients without prior episodes of ASH, as well as patients with good family support, lack of relevant comorbidities, and commitment to alcohol abstention, were included in the study. Of note, only 3 of 26 patients relapsed to alcohol consumption after 2 years.

On the other hand, in the context of organ shortage, major ethical concerns may be raised. Frequently, alcoholism is seen as a self-inflicted disease not only by the public, but also among medical personnel, and LT for ALD has led to sustained controversies. Unselected organ distribution can thus result in decreasing donor numbers. Therefore, transparent selection criteria for LT patients with ALD, and particularly ASH, are mandatory.[39]

MONITORING CONSUMPTION AND MANAGING RELAPSE

Identification of patients at risk of alcohol relapse displays a challenge for multidisciplinary transplant teams. Alcohol relapse, in contrast to temporary slips (which are recognised by the patient as potentially harmful and may foster a new abstinence), is defined by abusive consumption (at least four drinks per day or one drink in ≥4 consecutive days).[40,41] In a patient population where optimal selection is difficult, the treating physicians in particular must be aware of signs of recidivism. Whereas alcohol relapse prior to LT may preclude patients from the waiting list,[42,43] good psychosocial and medical support of patients may be decisive to prevent or to detect the signs of alcohol relapse earlier after LT in order to support long-term graft survival.[44] As such, most centres have implemented regular follow-up visits by addiction specialists after LT,[42,45] thereby achieving a significant reduction of recidivism;[20,46] intervention trials were only of limited success.[47,48]

To guarantee optimal patient selection for LT and best psychological and medical support, in addition to assessment by experienced addiction professionals, objective tools for alcohol detection are mandatory. In fear of negative socioeconomic consequences or of being denied LT, patients frequently do not admit alcohol consumption, adapt their drinking patterns to scheduled hospital visits (in order to be able to provide negative alcohol tests), or do not indicate actual amounts of alcohol intake.[43,49] To face these challenges some centres have implemented random alcohol testing without prior notice for patients on the waiting list.[50,51]

The National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health (Bethesda, MD) recommend a combination of carbohydrate deficient transferrin (CDT), mean corpuscular volume (MCV), and gamma glutamyl transferase (GGT) for alcohol screening,[52] reaching a sensitivity and specificity of 88% and 95%, respectively.[53-55] However, MCV and GGT, as well as the commonly used liver enzymes such as alanine-amino transferase (ALT) and aspartate-amino transferase (AST), have only low specificity in patients with end-stage liver disease or LT recipients.[43,56] Besides self-reporting questionnaires, such as Alcohol Use Disorders Identification Test (AUDIT-C),[57,58] and interviews by addiction specialists, a combination of alcohol markers in the blood (CDT), urine (urinary ethylglucuronide [uEtG]), and hair (hEtG) have been reported to be of high value in this patient cohort.[43,50] In particular, EtG in hair - a metabolite of ethanol - has the advantage of differentiating between excessive drinking (<60 g ethanol/day), moderate alcohol consumption (10-40 g ethanol/day), and teetotallers or very moderate drinkers (<10 g ethanol/day) for up to 6 months before, independent of the severity of liver disease.

Importantly, since a recent study proved the negative effect of excessive alcohol consumption on long-term patient survival regardless of the indication for LT, screening for alcohol consumption also in non-ALD transplant recipients should be included.[59] Due to the potentiated negative effect of alcohol in hepatitis C, we should be especially aware of alcohol consumption in this patient population.[2]

CONCLUSION

LT for alcoholic cirrhosis is a matter of continuous controversy since Starzl et al.[60] first drew attention to successful outcomes of LT for ALD. The most relevant concerns within the context of ALD and LT are the reliable pre and post-transplant perceptions of alcohol dependence and relapse. Universally applicable criteria for the evaluation of patient eligibility for LT border their limits when it comes to the individual patient, and the frequently applied '6-month rule' should be reconsidered. Moreover, in highly selected patients where spontaneous recovery of liver function cannot be expected, such as acute alcoholic hepatitis not responding to medical treatment, the '6-month rule' is not applicable. To develop an individual risk profile based on psychosocial factors in combination with the analysis of drinking patterns seems to be more decisive. In addition to routine visits by a multidisciplinary transplant team, including an addiction specialist prior to and after LT, screening for alcohol consumption and relapse by the use of biomarkers in blood, urine, and/or hair should be performed on a regular basis. Early detection of recurrence of harmful drinking and alcohol dependence is mandatory to preserve long-term graft function.

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ACUTE LIVER FAILURE: A DANGEROUS AND CHALLENGING SYNDROME

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ABSTRACT

Despite progress in understanding the underlying mechanisms, acute liver failure (ALF) is still one of the major clinical challenges in hepatology. A wide variety of aetiologies, and similarly, variable courses of the disease, make it crucial to identify the cause of ALF in each individual patient. Conservative therapy is only available for some patients; for many others, liver transplantation remains the only curative option for ALF. Thus, early evaluation and prognostication of the ALF syndrome is warranted for a timely decision to list a patient for transplantation or even as high urgency. This review aims to compose our current knowledge on epidemiology, mechanisms, and prognosis in ALF, and to give a perspective for future studies in this field.

Keywords: Acute liver failure, drug-induced liver injury (DILI), clinical management, cell death, prognosis.

DEFINITION

Sudden severe liver dysfunction in previously apparently healthy individuals is referred to as acute liver failure (ALF). ALF is characterised by rapid and massive hepatocellular death[1] and in many cases takes a life-threatening course, with liver transplantation (LTx) the only therapeutic option. In 1970, Trey and Davidson[2] defined fulminant hepatitis as a potentially reversible condition with occurrence of hepatic encephalopathy within 8 weeks of symptom onset.

Based on this first definition, the clinical hallmark of ALF is coagulopathy (International Normalised Ratio, INR ≥1.5) and hepatic encephalopathy in obviously healthy subjects.[3] The exclusion of pre-existing liver diseases is important for the current definition of ALF; however, this cannot be achieved in all cases. More than 50% of Europeans are overweight, which is often associated with fatty liver, a serious liver disease.[4] As such, it would be challenging to diagnose ALF in these individuals, who may have the underlying metabolic syndrome or fatty liver disease. A more appropriate definition than ALF for patients with underlying chronic liver diseases (alcoholic hepatitis, chronic hepatitis B and C virus [HBV/HCV], autoimmune hepatitis, non-alcoholic fatty liver disease [NAFLD]), but without liver cirrhosis, would be acute-on-chronic liver failure (AOC-LF). The third main group of liver failure patients are those with acute-on-cirrhosis liver failure (AOCi-LF). These separated definitions may be more appropriate as the management and the outcomes differ in each group (Figure 1). Some patients with ALF have the possibility to recover without LTx. However, in patients with AOC or AOCi liver failure, the recovery after acute injury is unlikely and LTx is warranted.

With progressive loss of hepatic function, ALF leads to hepatic encephalopathy, coagulopathy, and multi-organ failure within a short period of time. Established specific therapy regimens and the introduction of LTx have improved the prognosis for some aetiologies; however, the overall mortality rate remains high.[5] ALF accounts for approximately 6-8% of LTx procedures in the US and Europe.[6] The accurate and timely diagnosis of

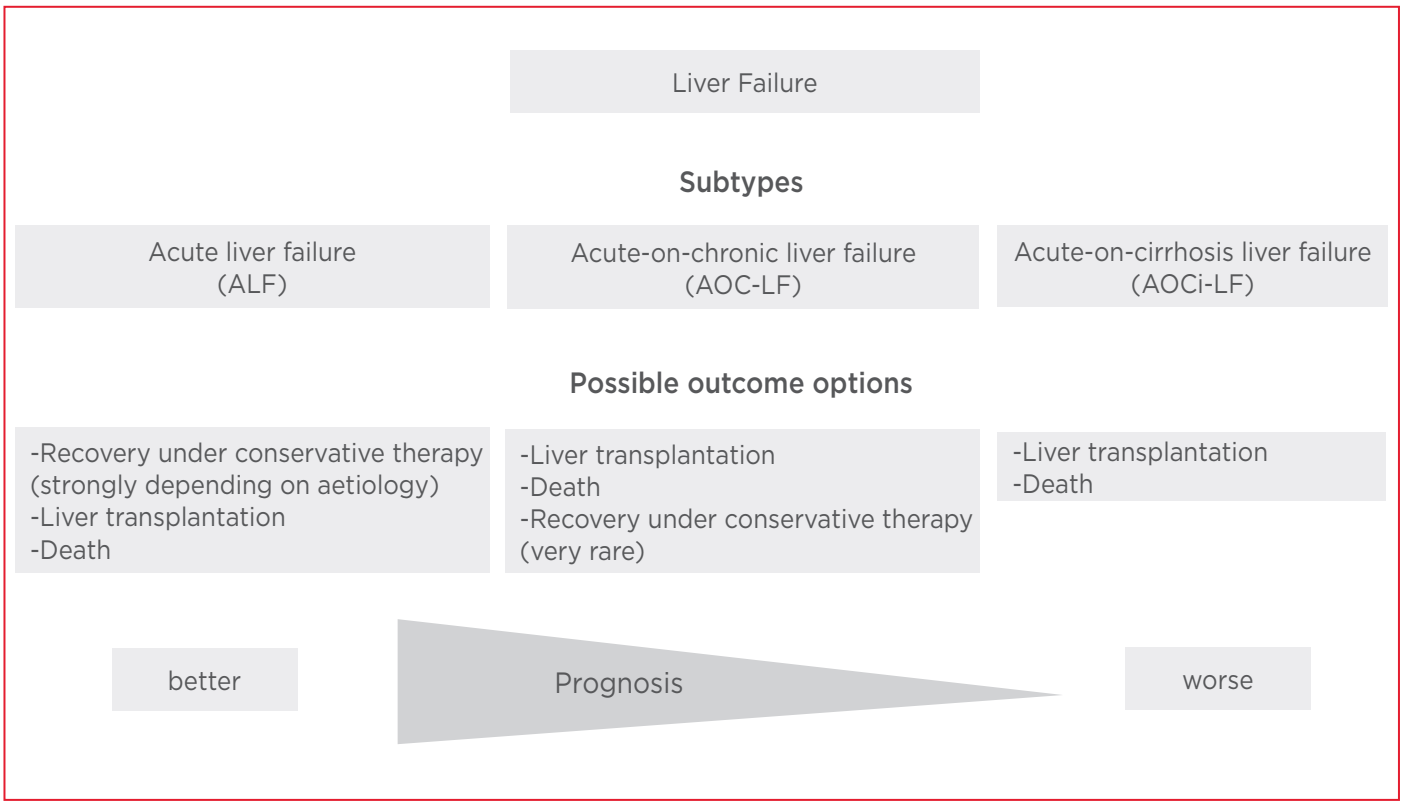

Figure 1: Diagram of liver failure subtypes, possible outcome, and clinical course.

ALF, rapid identification of the underlying cause, transfer of the patient to a specialised transplant centre and, if applicable, initiation of a specific therapy and evaluation for LTx are crucial in current ALF management. Therefore, we focus here on epidemiology and molecular mechanisms, as well as novel tools, to predict the outcome in ALF.

EPIDEMIOLOGY AND AETIOLOGIES

ALF is a rare condition with multiple causes and varying clinical courses, and the exact epidemiologic data is scarce. The overall incidence of ALF is 1-6 cases per million people each year.[5] Recent data from the US,[7] the UK,[8] Sweden,[9] and Germany[10,11] reveal drug toxicity as the main cause of ALF, followed by viral hepatitis and seronegative hepatitis (i.e. unknown or uncertain aetiology). In contrast, in the Mediterranean, Asia, and Africa, viral hepatitis is the leading cause of ALF.[12-15] In addition to HBV, recent data indicate that hepatitis E virus (HEV) is more common than previously considered. Indeed, the incidence of HEV-induced liver injury in Europe appears to be increasing.[16-18] An estimate of worldwide distribution and a comprehensive list of possible aetiologies of ALF are listed in Figure 2 and Table 1, respectively.

Despite being the leading cause of ALF in Western societies, the actual incidence of drug-induced liver injury (DILI) varies significantly among individual countries. For example, DILI in the general population was estimated at 1-2 cases per 100,000 person years,[19] but this is much higher in Germany, where DILI accounts for approximately 40% of ALF.[11] As a structured medical history may be difficult in some cases, a standardised clinical management to identify the cause of DILI and optimise specific treatment has been proposed.[20] This includes assessment of clinical and laboratory features, determining the type of liver injury (hepatocellular versus cholestatic). Furthermore, to identify a cause, one must distinguish between a dose-dependent and an idiosyncratic (immune-mediated hypersensitivity) type of liver injury.[2]

Acetaminophen intoxication, as discussed in detail below, is the classic example of a direct, dose-dependent intoxication with acute hepatocellular necrosis.[21] However, the vast majority of DILI are idiosyncratic reactions with a latency period of up to 1 year after initiation of treatment. Drugs that induce idiosyncratic DILI include antibiotics (amoxicillin/clavulanate, macrolides, nitrofurantoin, isoniazid), antihypertensive drugs (methyldopa),

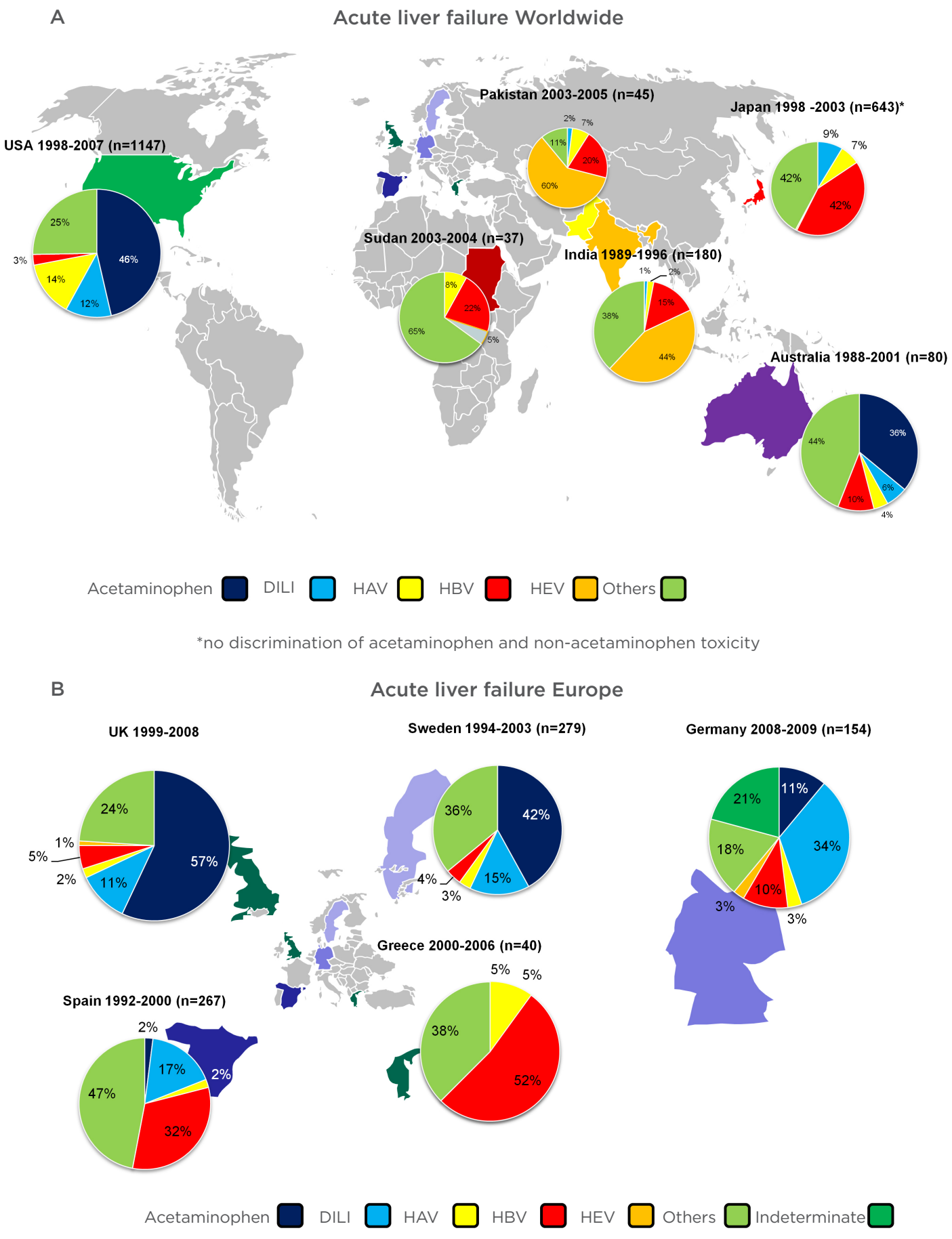

Figure 2: Distribution of main aetiologies for acute liver failure (ALF).
A) Worldwide overview of data on aetiologies for ALF.
B) Overview of European countries with studies on aetiology distribution for ALF.

Table 1: Aetiologies of acute liver failure (ALF).

Intoxication	Direct, idiosyncratic, paracetamol, ecstasy, amanita, phenprocoumon, tetracycline, halothane, isoniazid, anabolic drugs
Viral hepatitis	HBV, HAV, HEV, HBV+HDV, CMV, EBV, HSV
Immunological	Autoimmune, graft-versus-host disease (GVHD)
Metabolic	Wilson's disease, alpha-1 antitrypsin deficiency, haemochromatosis
Vascular	Budd-Chiari syndrome, ischaemic, veno-occlusive disease
Pregnancy-induced	HELLP syndrome, acute fatty liver in pregnancy

HBV: hepatitis B virus; HAV: hepatitis A virus; HEV: hepatitis E virus; HDV: hepatitis D virus; CMV: cytomegalovirus; EBV: Epstein-Barr virus; HSV: herpes simplex virus.

anticonvulsants, antipsychotic drugs (valproic acid, chlorpromazine), and many others, including herbal medicine. Demonstrating the need for new algorithms and biomarkers of liver injury, Hy Zimmerman's observation - elevation of transaminase levels above three-times the upper limit of normal indicates early DILI - has been in use to assess the risk of DILI in drugs in development since the 1970s.[22]

In a recent study, >70% of the patients with acetaminophen-induced ALF were associated with suicidal intents, while the remaining cases were a result of accidental over-dose.[10] It is recognised that specific risk factors, such as obesity and excess alcohol consumption, increase the risk of acetaminophen-associated drug toxicity and DILI.[23-25] Thus, for individuals with any cryptic liver disease or injury, current recommended dose ranges of acetaminophen might be too high. Acetaminophen serum concentration above 300 μg/mL, 4 hours after ingestion is a predictor for severe hepatic necrosis. With high doses of acetaminophen, the metabolite N-acetyl-p-benzoquinone imine (NAPQI) accumulates in hepatocytes and induces hepatocellular necrosis.[21,26] In the presence of glutathione, NAPQI is rapidly metabolised to non-toxic products and excreted via the bile.[27] In acetaminophen intoxication, the glutathione pool is rapidly diminished, but could easily be restored by N-acetylcysteine therapy.

MOLECULAR MECHANISMS

ALF occurs as a result of acute hepatocellular injury from various aetiologies (toxic, viral or metabolic stress, or hypotension). However, regardless of the initial aetiology, ALF triggers a series of events inducing hepatocellular necrosis and apoptosis, reducing the regenerative capacity of the liver. Massive loss of hepatocytes consequently reduces the functional capacity of the liver for glucose, lipid, and protein metabolism, as well as biotransformation and the synthesis of coagulation factors. This then leads to encephalopathy, coagulopathy, hypoglycaemia, infections, and renal and multi-organ failure. In fact, even the pattern of hepatic cell death might be of clinical importance, as necrosis, apoptosis or necro-apoptosis seem to be specific for different causes and are associated with clinical outcome.[28,29]

Apoptosis, programmed cell death, is a process in which ATP-dependent mechanisms lead to the activation of caspases that finally lead to the breakdown of the nucleus into chromatin bodies and disintegration of the cell into small vesicles, called apoptotic bodies, which can be cleared up by macrophages.[30] Upon massive cell injury, ATP depletion leads to necrosis with swelling of the cytoplasm, disruption of the cell membrane, imbalance of electrolyte homeostasis, and karyolysis. Necrosis leads to local inflammation, induction of cytokine expression, and migration

of inflammatory cells.[31] However, apoptosis itself might induce mechanisms that lead to necrosis, and the ratio of apoptosis versus necrosis seems to play a role in liver injury.[30] This hypothesis is supported by observations that a death receptor agonist triggers massive necrosis secondary to the induction of apoptosis.[32] It has also been shown that this severe liver injury is paralleled by fibrosis and the activation of hepatic stellate cells, even in patients without prior liver damage.[33] This type of liver fibrosis in ALF might be part of the regenerative response ('regenerative fibrosis').

The rates of apoptosis or necrosis in ongoing ALF processes seem to be different according to the underlying aetiologies.[26,34] Apoptosis seems to be the predominant type of cell death in HBV and amanita-related ALF versus necrosis in acetaminophen and congestive heart failure.[35] Furthermore, antiviral treatment of fulminant HBV significantly reduced serum cell death markers and improved clinical outcome.[36]

The regenerative capacity of the liver depends on the patient's gender, age, weight, and previous history of liver diseases. Important mediators of liver regeneration include cytokines, growth factors, and metabolic pathways for energy supply. In the adult liver, most hepatocytes are in the G_0 phase of the cell cycle and non-proliferating. Upon stimulation with the proinflammatory cytokines tumour necrosis factor α (TNFα) and interleukin-6 (IL-6), growth factors like transforming-growth factor α (TGFα), epidermal growth factor (EGF), and hepatocyte growth factor (HGF) are able to induce hepatocyte proliferation. TNF and IL-6 also induce downstream pathways related to NFκB and STAT3 signalling. Both transcription factors are crucial for coordination of the inflammatory response to liver injury and hepatocyte proliferation.[37] Emerging data support an important role for hepatic progenitor and oval cells, as well as vascular endothelial growth factor (VEGF)-mediated angiogenesis in liver regeneration.[38-40]

CLINICAL PRESENTATION

Renal failure, hepatic encephalopathy, and brain oedema are the consequences of the pathophysiologic changes in ALF. Hyperammonaemia correlates with brain oedema and survival.[41] Decreased hepatic urea synthesis, renal insufficiency, the catabolic state of the musculoskeletal system, and an impaired blood-brain barrier all lead to ammonia accumulation and alterations in local perfusion, which result in brain oedema. After acute and massive hepatic cell death, the release of proinflammatory cytokines and intracellular material results in low systemic blood pressure leading to the impairment of splanchnic circulation. Renal failure in ALF patients is common in up to 70% of patients.[42] Reduced number and function of platelets as well as inadequate synthesis of prothrombotic factors are the causes of coagulopathy. Leukopenia and impaired synthesis of complement factors in ALF patients increase the risk of infections, which might result in sepsis. Infections increase the duration of intensive care unit (ICU) stays and the mortality rate in ALF dramatically. With the impairment of hepatic gluconeogenesis, hypoglycaemia is also a frequent feature of patients with ALF.[43] For a more detailed discussion of the clinical presentation in ALF, the reader is referred to the recent, excellent overview by Bernal and Wendon.[44]

PROGNOSIS

Accurate prediction of the clinical course is crucial for management and decision-making in ALF. Identification of the underlying aetiology improves prognosis and opens the pathway for specific treatment. The degree of hepatic encephalopathy is considered an important indicator of prognosis.[1] Cerebral oedema and renal failure worsen the prognosis dramatically. In some studies, the INR was determined as the strongest single parameter in predicting the outcome of ALF. Another interesting point is that the presence of hepatic encephalopathy implies a poor prognosis for acetaminophen-induced ALF, which, in contrast, has little meaning for amanita mushroom poisoning. LTx is the last therapeutic option in patients with ALF, when conservative treatment fails and a lethal outcome is imminent. Therefore, individual assessment if a patient will undergo a fatal course is important for timely listing. Standardised prognosis scores based on reproducible criteria are crucial tools in times of donor organ shortage and to avoid LTx in patients that might fully recover without LTx.[43] An overview of currently available scores to assess the severity of ALF is given in Table 2.

King's College Criteria (KCC) were established in the 1990s based on findings from a cohort of

Table 2: Scoring systems in patients with acute liver failure for emergency liver transplant.

Scoring System		*Prognostic factors*
King's College Criteria (KCC)[45]	Paracetamol intoxication	Arterial pH <7.3 or INR >6.5 and creatinine >300 μmol/L and hepatic encephalopathy Grade 3-4
	Non-paracetamol	INR >6.5 and hepatic encephalopathy or INR >3.5 and any of these three: bilirubin >300 μmol/L, age >40 years, unfavourable aetiology (undetermined or drug-induced)
Clichy Criteria[48]	HBV	Hepatic encephalopathy Grade 3-4 and Factor V <20% (for <30 years old); <30% (for >30 years old)
MELD[49,50]		10 x [0.957 x ln(serum creatinine) + 0.378 x ln(total bilirubin) +1.12 x ln(INR+0.643)]
CK-18 modified MELD[26]		10 x [0.957 x ln(serum creatinine) + 0.378 x ln(CK18/M65) + 1.12 x ln(INR + 0.643)]
Bilirubin-lactate-aetiology score (BILE score)[51]		Bilirubin (μmol/)/100 + Lactate (mmol/L) +4 (for cryptogenic ALF, Budd-Chiari or Phenprocoumon induced) -2 (for acetaminophen-induced) +0 (for other causes)
ALFSG Index[52]		Coma grade, bilirubin, INR, phosphorus, log10 M30
ALFED Model[53]		Dynamic of variables over 3 days: HE 0-2 points; INR 0-1 point; arterial ammonia 0-2 points; serum bilirubin 0-1 point

Adapted from Canbay et al.[43]
INR: International Normalized Ratio; MELD: model of end stage liver disease.

588 patients with ALF.[45] The authors also introduced a classification based on the onset of encephalopathy after an initial rise in bilirubin levels into hyperacute (<7 days), acute (8-28 days), and sub-acute (5-12 weeks) liver failure.[46] KCC includes assessment of encephalopathy, coagulopathy (INR), acid homeostasis (pH), bilirubin, and age. For patients with acetaminophen-induced ALF, a separate KCC formula was suggested. A recent meta-analysis of 17 studies for the performance of KCC in predicting outcome in non paracetamol-induced ALF revealed a good specificity with more limited sensitivity. Moreover, the best performance of KCC was reached in groups with high grades of encephalopathy and in earlier studies.[47] Clichy criteria were introduced for patients with fulminant HBV infection and include the degree of encephalopathy and Factor V fraction as a measure for hepatic synthesis.[48]

The model for end-stage liver disease (MELD) was designed to predict the likelihood of survival after transjugular intrahepatic portacaval shunt (TIPS) in cirrhotic patients. However, it has been established for some time as an allocation tool for LTx in patients with cirrhosis in the US and Europe. The MELD has been tested for prediction of ALF and was found superior to KCC and Clichy criteria in independent studies.[49,50] All three models are still in use but are based on clinical and laboratory data, while new options for diagnosis (molecular laboratory diagnostics) have been developed.

Novel approaches that include mechanistic characteristics of ALF, like the CK-18 modified MELD, which includes markers for hepatocellular death or lactate, are promising, but need validation in larger prospective cohorts.[26,51,52] In a recent, large, prospective study, a prognostic model was developed using dynamic changes of four independent variables (atrial ammonia, INR, serum bilirubin, hepatic encephalopathy) over 3 days to predict mortality.[53] Still, the possibilities

of novel markers and diagnostic methods should be tapped to their potential to provide more accurate and reliable prognosis. A major limitation for this type of study is the relative rarity of ALF, further complicated by a variety of different regionally predominant aetiologies. To generate a reliable, widely applicable scoring system, it would be essential to establish large transnational cohorts to produce more powerful studies.

LIVER TRANSPLANT IN ALF

Recent data from the European Liver Transplant Registry (ELTR) showed that ALF accounted for 8% of indications for LTx in Europe during 1988-2009, and the survival rates after LTx have improved significantly.[54] The study by Germani et al.[54] showed 1, 5, and 10-year patient survival rates of 74%, 68%, and 63%, respectively. The 1, 5, and 10-year graft survival rates were 63%, 57%, and 50%, respectively. Similar results were observed in a US database. The authors concluded that these improvements are due to optimised pre, peri, and post-operative treatment. Another important finding of this study was the identification of combined recipient and donor age as major risk factors for early mortality after LTx. Graft recipients older than 50 years, paired with donors older than 60 years, had a very high mortality/graft loss within the first year.[54] This suggests that better allocation algorithms for organs are needed, taking current knowledge into account.

SUMMARY

ALF continues to pose a challenge to clinicians. The identification of the underlying cause of disease remains a critical first step to allow conservative treatment, if available. Many diagnostic tools are available to help in this process, though for some patients the causes remain undetermined. As a second step, disease severity and progression will need to be evaluated to determine if LTx is necessary, and if so, the speed of organ allocation. Several scores assist this decision, though all have limitations and especially lack a positive prognostic value for patients, who can survive without LTx. Further insight into the mechanisms of individual aetiologies will probably enhance management of ALF and hopefully lead to more patients surviving without the need for transplant organs.

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ACUTE LIVER FAILURE: PATHOPHYSIOLOGIC BASIS, AND THE CURRENT AND EMERGING THERAPIES

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ABSTRACT

Acute liver failure (ALF) is a devastating condition that occurs in patients who previously had a normal liver. Although the outcome of patients with ALF has improved, without liver transplantation (LT) mortality rates remain in the range of 35-50% in different geographical areas and therefore, its treatment remains an unmet need. In the Western world toxic liver injury from acetaminophen remains one of the common causes but, in the East, hepatitis of unknown aetiology remains the most common cause. Treatment options are limited to meticulous attention to multi-organ support, use of N-acetyl cysteine, judicious use of antibiotics, and timely LT. This review describes the state-of-the-art techniques in the issues related to prognosis, outcome, and treatment of this devastating syndrome.

Keywords: Acute liver failure, intracranial hypertension, multi-organ failure, liver transplantation, N-acetylcysteine, acetaminophen.

INTRODUCTION

Acute liver failure (ALF) is a life-threatening clinical syndrome resulting from a rapid *de novo* hepatocellular necrosis in the absence of previous liver disease, and occurring within hours to up to 26 weeks of initial injury.[1] Acute hepatic insult leads to a rapid loss of liver function and is characterised by severe coagulopathy and hepatic encephalopathy (HE), the two defining features of ALF. In clinical terms, any mental alteration in conjunction with a prolonged prothrombin time (PT >20 seconds) or International Normalised Ratio (INR >1.5), constitutes a diagnosis of ALF. Notable exceptions to this definition are some cases of Wilson's disease (WD), autoimmune hepatitis (AIH), and reactivation of hepatitis B virus (HBV), where cirrhosis or chronic liver impairment may already be present but the clinical phenotype is one of ALF, which is indistinguishable from acute viral hepatitis.

Fulminant WD accounts for 6-12% of all patients with ALF referred for emergency transplantation. An early diagnosis of WD, ALF is critical for prognostication and subsequent need for liver transplantation (LT); the mortality rate in WD patients with ALF and HE approaches 100%. Typically, the combination of Kayser-Fleischer rings and a low serum caeruloplasmin (<0.1 g/l) level is usually sufficient to establish a diagnosis as other sophisticated biochemical tests of deranged copper metabolism are often not rapidly available. The Nazer score (based on serum bilirubin, aspartate aminotransferase [AST], and prolongation of PT), modified Nazer score (PT replaced by INR), and the Wilson Index (serum bilirubin, AST, INR, white cell count [WCC], and serum albumin) by Dhawan et al.[2] are useful tools to identify patients with poor prognosis. Similarly, in 30-40% of cases the onset of AIH is acute, mimicking that of acute hepatitis due to other causes, constituting 2-16% of all causes of ALF.[3] Viral hepatitis B is one of the most common causes of ALF worldwide. The presentation could be acute in the absence of previous liver impairment or a consequence of the reactivation of HBV replication in hepatitis B carriers undergoing immunosuppressive or cancer chemotherapy.

ALF is a rare condition, with a reported annual incidence in the developed world of 1-6 new patients per 1 million people.[4] The aetiology of ALF varies widely around the world, with infections from hepatitis A, B, and E viruses accounting for most cases in the developing world.[5] However, in the USA and much of Western Europe, viral hepatitis is on the wane, and drug-induced liver injury - such as acetaminophen toxicity - is by far the most common cause of ALF.[6,7] Although identifying the underlying cause of the liver failure is important in order to modify the approach to management and to provide prognostic information, in 15-20% of cases, aetiology remains unclear even after an extensive clinical and laboratory assessment. The differential diagnosis of patients presenting abnormal liver function tests and decreased level of consciousness could be difficult; a patient's history, risk factors, clinical presentation, and general examination are fundamental to identify the underlying aetiology. Drugs, alcohol toxicity, WD, decompensated cirrhosis, and severe liver involvement of systemic infections (malaria, typhoid, leptospirosis, rickettsial infection, etc) are a few possible causes to take into consideration in this setting. Unabated hepatocellular necrosis leads to extra-hepatic end-organ damage, characterised by vasoplegia and haemodynamic alterations, renal, pulmonary, and cerebral dysfunction representing a constellation of features resembling sepsis phenotype.

The outcomes of ALF in developed countries have improved dramatically in the last 25 years, owing to better understanding of its aetiopathogenesis, and the advent and availability of emergency LT for those who progress to severe liver failure. Parallel advancements and evolving standards of care in evidence-based practices in organ-system support in the liver units and the intensive care units (ICU) have played a vital complimentary role in managing these complex patients. This has culminated in a significantly improved outcome for patients managed with medical treatment alone and those who received LT for the treatment of ALF, the latter showing a vast improvement in 1-year post LT survival rates from 36% in 1984 to approximately 80% currently in Europe.[8]

PATHOPHYSIOLOGIC BASIS OF ALF

Liver Injury

Acute liver injury is caused by both direct injury to the hepatocytes, and an innate immune-mediated response, mediated through activation of monocytes, macrophages, dendritic cells, leukocytes, natural killer (NK) cells, and natural killer T (NKT) cells. These cells express receptors that are able to recognise pathogen-associated molecular patterns (PAMPs) in viral hepatitis and damage-associated molecular patterns (DAMPs) in toxin-mediated liver injury, leading to the activation of signal transduction pathways which determine the pattern of cytokines released, initially locally within the liver itself, spilling over to the systemic circulation eventually. The principle mode of hepatocyte death is through necrosis; particularly in the acetaminophen-induced liver injury.[9] Recent studies also suggest a significant role of apoptosis in cell death of ALF, which is mediated through activation of caspases. Serum M30 antigen, a marker of apoptotic hepatocyte cell death, is significantly elevated in patients who die of ALF, thus making serum M30 antigen a potential biomarker for monitoring progression of liver injury, and the use of caspase or other apoptotic inhibitors as an important therapeutic target in preventing further cell death.[10]

Extra Hepatic Injury

Irrespective of the aetiology, a large majority of the patients eventually go on to progress to various degrees of extra-hepatic organ involvement with some developing frank multiple organ failure. While intense systemic inflammatory response syndrome (SIRS), which develops in ALF - mediated by the pro-inflammatory cytokines released from the damaged liver and subsequent activation of endothelial, coagulation, and immunological systems and organ cross talk - seems to be largely responsible for distant organ damage, direct toxic injury may also occasionally contribute, as is seen in patients with acetaminophen toxicity who develop toxin-related acute renal tubular injury. The existence of a parallel compensatory anti-inflammatory response syndrome (CARS) - mediated by the anti-inflammatory cytokines, IL-4, IL-10, and transforming growth factor-β - is not only insufficient in counter-modulating this response, but acts to predispose to superimposed bacterial and fungal sepsis during the latter stages of the disease and late mortality.[11]

Brain dysfunction in ALF deserves special mention as the pathophysiology of HE in ALF goes beyond the inflammatory response associated with the condition. The exact mechanisms are not fully understood, but alterations in cerebral

blood flow (CBF) and cerebral autoregulation, circulating neurotoxins such as ammonia, systemic inflammation, and hypo-osmolality seem to play a major role. The consequences of HE can range from subtle neuropsychiatric alterations to full-blown cerebral oedema and intracranial hypertension (ICH). Increased CBF,[12] in conjunction with the loss of auto-regulation, results in cerebral hyperaemia leading to intracerebral hypertension.[13] Ammonia is the most commonly studied neurotoxin in this context, with serum levels >124 μmol/L shown to be associated with a high incidence of cerebral oedema and raised intracranial pressure (ICP),[14] and levels >150 μmol/L with cerebral herniation.[15] It is produced from glutamine in the gut to then be metabolised chiefly in the liver into urea, which is excreted in the urine. Failure of this system and urea synthesis leads to elevated serum levels in liver failure.

Recent clinical and experimental data, however, suggest a much more complex inter-organ traffic involved in the production and metabolism of ammonia, opening up new possibilities for therapeutic interventions, which can be targeted at various stages of its metabolism, thus achieving reduction in ammonia production and enhanced elimination. Skeletal muscles provide a minor alternative site for ammonia metabolism, by converting ammonia through glutamine synthetase activity to glutamine. Glutamine that is produced can undergo reconversion into ammonia and glutamate by the enzyme glutaminase, present in the kidneys and gut. Ammonia that reaches the brain is converted to glutamine by the astrocytes, which in high concentrations exert an osmotic effect leading to oedema and causing oxidative stress from reactive oxygen species.[16] Systemic inflammation, particularly in the presence of sepsis, further impairs central nervous system (CNS) function by causing oxidative injury, endothelial damage with increased permeability of cerebral vasculature, and alterations in blood flow, leading to further brain swelling and raised ICP.[17]

PRINCIPLES OF MANAGEMENT

The key management principles of ALF are complex and require a team of highly trained hepatologists, intensivists, and transplant surgeons. Establishing a diagnosis of the underlying insult is crucial in determining specific therapy options for certain aetiologies, which could result in the cessation of the damage process and may even reverse liver failure. For example, in the case of acute HBV infection, more than 95% of patients may seroconvert spontaneously, while the remaining 5% with severe acute or protracted hepatitis (defined as prolonged PT and deep jaundice persisting for >4 weeks) may benefit from nucleoside/tide analogue treatment.[18] The Budd-Chiari syndrome (acute hepatic vein thrombosis), inherited or acquired, is an uncommon cause of ALF characterised by a pro-coagulant state, where therapeutic options include: anticoagulation, performance of transjugular intrahepatic portocaval shunting, or LT.

HELLP syndrome and acute fatty liver degeneration constitute two severe complications occurring during pregnancy. Early recognition of these syndromes and prompt delivery of the foetus are critical in achieving good outcomes. The overall prognosis and the spontaneous recovery of liver function in pregnancy-associated ALF is excellent with only a minority of patients requiring LT. Interventions are primarily aimed at optimising organ perfusion to promote spontaneous liver regeneration and recovery, while efforts are directed towards reduction and amelioration of liver injury through liver-specific therapy wherever applicable, along with early diagnosis and treatment of new complications, and timely identification of those who are unlikely to improve with medical management alone and would need emergency LT.

MONITORING AND GENERAL MEASURES

ALF patients should ideally be nursed in dedicated liver units, with clear escalation plans for early admission to ICU, particularly when they develop higher grades of HE (≥Grade 3) requiring airway protection, or are in need of advanced cardiovascular, pulmonary, renal, or cerebral support. However, simple protocol-based management, focused on maintaining metabolic homoeostasis with an attention to detail on nutrition, glucose levels, oxygenation, blood pressure control, and maintenance of serum electrolytes, is crucial. Most patients in ICU require insertion of central venous lines and arterial accesses for closer haemodynamic monitoring, and provision of therapy such as vasopressors and renal support. Elevated PT in ALF does not necessarily predict bleeding risk and should not be routinely corrected for insertion of vascular catheters, thus allowing it to be used to monitor synthetic liver function.[19]

Controversy continues to rage about the use of ICP transducers to monitor ICP but the current recommendations state they should be used with caution, only inserted by experts after correction of coagulation. Fatal intra-cerebral haemorrhage has been reported in up to 10% of patients with ICP monitoring. Epidural or sub-dural transducers are preferred as they are associated with lower risks of haemorrhage and infective complications. While invasive CNS monitoring and cerebral optimisation have failed to translate to survival benefit, Keays et al.[20] have shown delayed cerebral death in Grade 4 HE patients managed with invasive monitoring with survival time improving 6-fold from 10 hours to 60 hours, thus arguing for an extra amount of time for a graft to become available.

It is likely that arterial ammonia levels (>150 μmol/L) and/or jugular venous oxygen tension (<65 mmHg or >85 mmHg) may help to identify patients at particular risk of developing intracranial hypertension (ICH).[21] The decision about invasive ICP monitoring should be on a case-by-case basis, assessing the risks and benefits of the procedure. At our institution all patients with Grade 3 HE have reverse jugular catheters, while ICP bolt is reserved for hyperacute presentation, persistent hyperammonaemia >150 μmol/L, pupillary changes, fluctuations in reverse jugular saturations, and features of hyperexcitability such as sustained clonus (Figure 1). The patient's neck should be maintained in a neutral position with the head of the bed elevated to 30° to facilitate venous drainage.

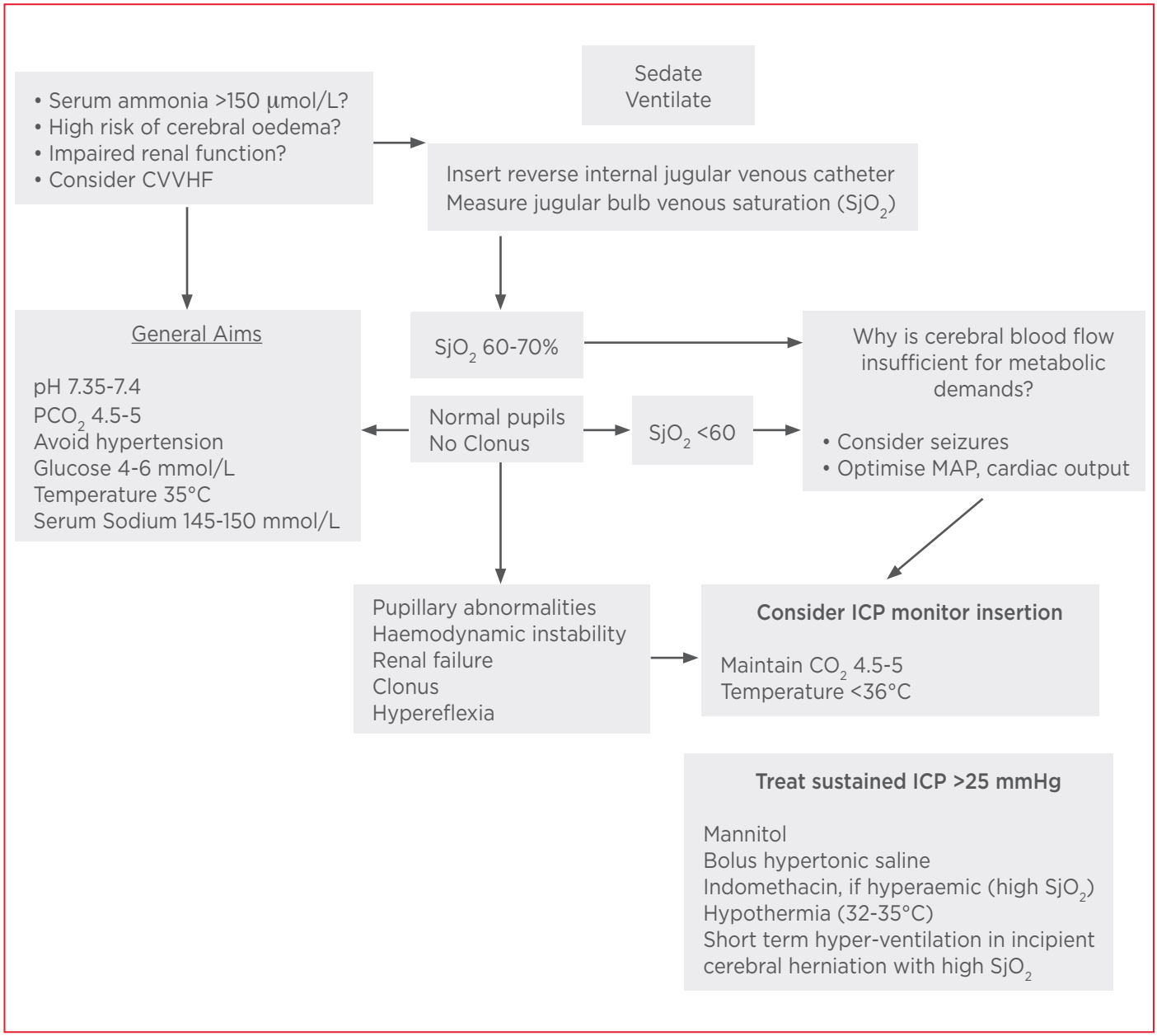

Figure 1: Management of Grade 3/4 hepatic encephalopathy in acute liver failure.

Infections and Sepsis in ALF

Immune paralysis, neutrophil dysfunction, and bone marrow suppression seen in ALF predispose patients to secondary bacterial infection and sepsis, with evidence of culture-positive bacterial and fungal infections in up to 70% and 30% of patients, respectively.[22] Pneumonias, urinary tract infections, intravenous and other catheter-related blood stream infections, and spontaneous gut bacterial translocation constitute the most common sources of sepsis driven by organisms such as enteric Gram-negative bacilli, Gram-positive cocci, and *Candida* species. Superimposed sepsis (infection with SIRS) leads to further deterioration of HE and cerebral oedema, and cardiovascular and renal failure precluding transplantation occasionally, and is associated with a poorer outcome. A rigorous surveillance process to detect and treat infections early is imperative in the management of these patients. Prophylactic antibiotic therapy, though routinely used, has not shown survival benefit, but is associated with a significant reduction in the infection rates and the intensity of SIRS thereof, with improved cerebral oxygenation and circulatory stability.[23] Prophylactic antifungal therapy is usually reserved for Grades 3/4 HE. Broad spectrum antimicrobial therapy is commenced in all patients at the time of admission and is modified periodically according to the organism identified and the sensitivity reports.

Modulating Liver Injury

N-acetylcysteine (NAC) is the most commonly used liver-specific therapy in acetaminophen toxicity, helping to replenish exhausted glutathione stores in the liver and promoting metabolism of N-acetyl p-benzoquinoneimine (NAPQI), the toxic metabolite of acetaminophen responsible for liver toxicity. While the best evidence of its effectiveness is reserved for early commencement following acetaminophen toxicity (within 24 hours), even delayed therapy (after 24 hours) produces improvement in the haemodynamic and cerebral oxygenation parameters.[24,25] In addition, in the early stages of HE (Grade 1-2) NAC has also been shown to exert beneficial effects in non-acetaminophen aetiologies of ALF.[26] Other liver-specific therapies, such as lamivudine and -intravenous immunoglobulin (IVIG) in hepatitis B, or acyclovir in herpes simplex virus-related liver failure, and corticosteroids in AIH, although routinely used, have failed to demonstrate substantial beneficial effect, partly because of the rarity of occurrence of these diseases and the associated wide variations in the severity of illness, which makes conducting randomised trials to establish best practise and the evidence base difficult.

Circulatory Dysfunction

Circulatory failure is universal in severe disease and is characterised by systemic vasodilatation, vasoplegia and hypotension (despite increased cardiac output), and not infrequent subclinical myocardial dysfunction. Aggressive fluid therapy to maintain circulatory volume and tissue perfusion is achieved by rapid infusion of crystalloid or colloid solutions. The role of human albumin solution (HAS) as a plasma expander, and as a drug with potential extra-oncotic effects such as stabilisation of endothelial function, drug handling, and anti-inflammatory and anti-oxidant effects, is yet to be studied in ALF. However, in the wake of recently published studies demonstrating the ill effects of starch solutions in patients with sepsis, HAS could be considered for resuscitative purposes in hypovolaemic patients not responsive to crystalloids alone.[27,28] Caution must be exercised to avoid hypervolaemia with albumin as this can lead to a deleterious effect on ICP.

Vasopressors are required for the volume-unresponsive shock, norepinephrine being the vasopressor of choice. Vasopressin, and its analogue terlipressin, can be used as a second-line agent in combination with norepinephrine but can be associated with increases in ICP.[29,30] The target of therapy is difficult to define and at present is arbitrarily based upon maintaining adequate cerebral perfusion. Most units would aim for a mean arterial pressure of 65-70 mmHg. In refractory shock, corticosteroids may have a role especially when administered early in the course of illness. This is to treat relative adrenal insufficiency associated with ALF.[31]

Respiratory Support

Oro-tracheal intubation for airway protection and invasive mechanical ventilation to manoeuvre blood carbon dioxide (CO_2) tension is the norm for those who progress to Grade 3/4 HE. Although temporary hyperventilation can be undertaken to reduce ICP, prolonged periods of hyperventilation is not advised and can be deleterious. Mechanical ventilation exposes patients to a spectrum of ventilator-associated complications (VAC) -

such as ventilator associated pneumonias, acute lung injury (ALI), and acute respiratory distress syndrome (ARDS) - and the risks of baro, volu, and bio-trauma. Protective lung ventilation strategy is the gold standard for managing most acute lung injuries in the ICU but a careful balancing act is required when dealing with ALF patients. While high positive end expiratory pressure (PEEP) may be necessary to optimise recruitment of lung units and prevent atelectasis to improve oxygenation, high PEEP in conjunction with low tidal ventilation and low inflation pressure can adversely impact on cerebral oedema and ICH secondary to venous engorgement from high transmitted pressure, and cerebral vasodilation due to elevation in CO_2 (permissive hypercapnia).

Renal Support

Renal support is commonly required as >50% of patients develop acute kidney injury (AKI), with incidence of AKI rising to as high as 75% in patients with ALF due to acetaminophen overdose. The cause of renal failure in this setting is multifactorial, including dehydration, direct drug nephrotoxicity, hypotension, sepsis, and hepatorenal syndrome. Management includes avoidance of nephrotoxic agents, treatment of infections, and maintenance of euvolaemia and adequate renal perfusion. While the primary objectives of the renal support is to control azotaemia, treat electrolyte abnormalities and metabolic acidosis, and maintain fluid balance, it is also highly effective in removing ammonia, and achieving normo/hypothermia and control fever. Continuous modes of dialytic therapy are preferred to avoid large fluid shifts and cerebral complications associated with it.[32]

HE and ICH

HE and cerebral oedema with ICH is the most devastating complication of ALF, occurring more commonly and in a more severe form in hyperacute presentations. Despite significant improvement in the understanding of its pathophysiology, and the overall management of CNS complications of ALF, ICH still accounts for up to 25% of deaths in ALF. The basic tenets of cerebral management in ALF are similar to that in traumatic brain injury, with additional measures undertaken to control hyperammonaemia. Prevention and treatment of secondary insults, such as hypotension and hypoxia, are vital, along with maintenance of adequate cerebral perfusion and controlling ICP. Avoidance and treatment of hypo-osmolality, hyponatraemia being the main offender, through osmotherapy with mannitol or hypertonic saline to achieve higher serum sodium (>145 mmol/L) levels is associated with less severe cerebral oedema.[33]

Adequate sedation and treatment of subclinical seizures helps to reduce cerebral metabolic requirement for oxygen, thus preventing ischaemic injury. However, there is no role for prophylactic anticonvulsants.[34] ICH, in association with increased CBF (cerebral hyperaemia), is managed with short periods of hyperventilation to induce hypocapnoea and cerebral vasoconstriction, moderate hypothermia, and occasionally cyclo-oxygenase (COX) inhibition therapy (indomethacin).[35] Ammonia lowering strategies currently utilise extracorporeal removal techniques and induction of hypothermia. Hypothermia creates a state of 'hibernation' reducing metabolic rate and therefore ammonia production. It also leads to reduced ammonia uptake by the brain, and has been shown in animal studies and in small case series to effectively control refractory ICH.[36] Pharmaceutical interventions - such as lactulose, branched chain amino acid and non-absorbable antibiotic, and L-ornithine L-acetate (LOLA), which enhances ammonia detoxification in the muscle - whilst effective in cirrhotics, failed to show benefit in ALF.[37]

Prognosis and Selection for LT

LT is the mainstay of treatment in the poor prognosis group. Early identification of those with poor prognosis is crucial. Multiple scoring systems (Table 1) have evolved through the years to accurately prognosticate and act as selection criteria for LT. King's College Hospital (KCH) criteria[38] remains the most widely used and is based on factors indicative of poor prognosis, such as the extremes of age, rapidity of progress as defined by the jaundice-encephalopathy interval, and the aetiology of ALF with the spectrum ranging from spontaneous recovery most unlikely in WD to very good outcome for pregnancy-related ALF. The extended KCH criteria for acetaminophen poisoning has further improved its sensitivity and is based on refractory hyperlactataemia post fluid resuscitation.[39] The French Clichy criteria - which were developed in ALF patients with acute HBV infection,[40] and are based on the presence of confusion/coma, patient age, and Factor V levels - are used in difficult cases of non-acetaminophen aetiologies. Other indicators, such as serum phosphate levels, Gc-globulin, alpha-fetoprotein levels, and liver volume assessments (particularly in

subacute ALF), are occasionally used. Although the criteria developed at KCH are the most commonly applied in this setting, a recent meta-analysis of studies reporting their performance in non-paracetamol aetiologies concluded that different aspects of KCH criteria in this setting require further improvement.[41] Similarly, a systematic overview of the actual models for prediction of poor outcome in patients with ALF suggest that the models have shown inconsistent reproducibility, prognostic accuracy, and inability to predict mortality in ALF.[42] The need for a better prognostic model remains high.

FUTURE DIRECTIONS

Development of Novel Biomarkers to Monitor Progression of ALF

Current strategies under investigation include use of peripheral biomarkers to determine whether specific therapies, such as inhibitors of apoptosis (caspase inhibitors) or modulators of inflammation (Toll-like receptor 4 and 9 inhibitors), are likely to be useful for the prevention of progression of liver injury.[9,43] Ornithine phenylacetate (OP) promotes urinary excretion of glutamine produced in the

Table 1: Classification of acute liver failure (ALF) and criteria for liver transplant (LT).

Classification of ALF – clinical features and prognosis in the subgroups			
	Hyperacute	Acute	Subacute
Jaundice to Encephalopathy	0-1 week	1-4 weeks	4-12 weeks
Prothrombin time (PT) rise	Severe	Moderate	Mild
Cerebral oedema / Intracranial hypertension	Severe	Moderate	Mild
Prognosis (without LT)	Good	Moderate	Poor
Typical aetiology	Acetaminophen, Hepatitis E & A	Hepatitis B	Non-acetaminophen drugs, seronegative or indeterminate hepatitis
King's College Hospital (KCH) criteria for emergency or super-urgent LT			
Acetaminophen overdose 1. Irrespective of grade of encephalopathy: Arterial pH <7.25 following volume resuscitation >24 hours post overdose **Or** 2. All of the following: Grade 3 or 4 encephalopathy PT >100 sec Serum Creatinine >300 μmol/L **Or** 3. The extended KCH criteria Serum lactate >3.5 mmol/L after early resuscitation Serum lactate >3.0 mmol/L 24 hours post overdose, and adequate volume resuscitation **Non-acetaminophen aetiologies** 1. Irrespective of grade of encephalopathy: PT >100 sec **Or** 2. Presence of encephalopathy + any 3 of the following: Age <10 or >40 Aetiology NANB, drug reaction Jaundice to encephalopathy >7 days PT >50 sec Serum Bilirubin >300 μmol/L			
Clichy criteria for emergency LT (non-acetaminophen aetiology only)			
1. Confusion/coma + Factor V concentration <20% + Patient Age <30 yrs **Or** 2. Confusion/coma + Factor V concentration <30% + Patient Age >30 yrs			

muscles as phenylacetylglutamine (PAGN), and has been shown to significantly reduce serum ammonia levels and brain oedema in a chronic liver failure rodent model,[44] and to attenuate rise in ICP in a porcine model of ALF.[45] There are no studies yet in humans. Newer agents such as N-methyl-D-aspartate (NMDA) receptor antagonist memantine, an inhibitor of the glutamate NMDA receptor preventing binding of the extracellular brain glutamate and development of brain oedema,[46] etanercept, a TNF-α neutralising molecule,[47] and minocycline, a broad-spectrum tetracycline antibiotic, which is a potent inhibitor of microglial activation reducing neuroinflammation,[48] are some of the exciting therapeutic options which have shown promise in experimental models but are under further evaluation in the human setting.

New Targets for Pharmaceutical and Extracorporeal Interventions

Artificial and bioartificial liver support systems are aimed at supplementing standard intensive care for patients with liver failure, supporting either regeneration of the patient's native liver or bridging to transplantation. The premise and the concept of an ideal extracorporeal liver support device hinges on the ability to detoxify the blood, perform synthetic, metabolic, and immune functions, and remove and/or inhibit production of inflammatory signalling molecules (e.g. cytokines), thereby breaking the vicious circle of liver injury leading to production of inflammatory mediators and further propagation of liver injury; the ultimate aim being stimulation and promotion of liver regeneration. Unfortunately, as of now, none of the currently available artificial or bioartificial devices have shown much promise in the context of ALF, except a regime of enhanced or high volume plasmapheresis (>10 L of plasma removed and replaced per day). This demonstrated a clinical improvement in HE, hepatic and CBF, and even a survival benefit when used for up to 10 days.[49] Although this seems like a promising intervention, the definitive role of plasmapheresis needs further evaluation before it is adopted as a standard clinical care for ALF patients.

CONCLUSIONS

ALF is a reversible and, therefore, treatable condition. Huge in-roads have already been made in improving the understanding of the disease process and its treatment, as evidenced by a significant reduction in mortality and morbidity. Further research towards unravelling the pathophysiological processes underlying ALF, development of biomarkers for early diagnosis and prediction of disease progression and novel drugs to halt the disease process, and advances in the organ support system in intensive care, including the on-going search for an ideal extracorporeal liver support device, remain the unmet needs. The emergence of drugs that target apoptosis, inflammation, and the new stem cell strategies also provide exciting new opportunities to improve patient outcome.

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Sovaldi®: winning the battle against HCV

Interview with Mr Michael Elliott, Vice President of Medical Affairs, Gilead Sciences, Inc.

THE BACKGROUND

Michael Elliott, Vice President of Medical Affairs, Europe, Middle East, Australia, and Asia, for Gilead Sciences, Inc., spoke to the *European Medical Journal* exclusively about Gilead's new drug Sovaldi® (sofosbuvir), remarking: *"It is exciting to be able to offer a cure to a chronic virus."*

Sovaldi® is used for treating patients with chronic hepatitis C (CHC); it is a 400 mg tablet, nucleotide analogue polymerase inhibitor, and is used in combination with other antiviral medicines – ribavirin (RBV) and/or pegylated interferon (peg-IFN) - in adults with the hepatitis C virus (HCV).

THE PRODUCT

In the UK alone, it is estimated that HCV affects around 215,000 adults. When asked if it is exciting to see the product working in patients, Mr Elliott emphasised: *"If I talk to physicians, and I have heard a few patients speak as well...the opportunity to get more than a 90% cure is now becoming a reality and that, particularly for patients, is really exciting. They have been waiting a long time; and for physicians as well - they really care for their patients and their patients have been coming to the clinic, some of them for a number of years, some of them failed other therapies, and now at least they have the opportunity to consider a 90% cure; it is quite a milestone."*

How Does Sovaldi® Differ from Past Therapies?

Peg-IFN plus RBV offers a 40-60% cure; however the safety profile is quite marked and it can be hard to tolerate. The next generation of medicines, protease inhibitors boceprevir and telaprevir, presented a >60% cure, but patients reported skin rashes and anaemia, and the dose had to be modified depending on their response.

"So now," Mr Elliott described, *"you move forward to Sovaldi®; in those patients who have not been treated you can get more than a 90% cure; it covers all genotypes [1-6], whereas the protease inhibitors only covered just one. There is a very high barrier to resistance, and the treatment for a lot of patients is an oral treatment and can be as short as 12 weeks."*

Thousands of patients have been enrolled into trials and, as of yet, no new adverse events have been noted on top of those already witnessed in previous therapies.

How is Sovaldi® Tailored to Combat Each HCV Genotype?

Mr Elliott explained: *"It is really the line of the molecule itself. It is the first in its class; it is the first nuclear-type polymerase inhibitor so it has been targeted specifically to a very important enzyme in HCV...the expectation that by targeting this very specific enzyme you would affect all of the genotypes - it is an enzyme present in all genotypes - and that has been successful.*

"By designing a drug that is highly effective, and across genotypes, you can use a lower dose and also you can choose a safer molecule, and that, through the testing in clinical studies and physician experience, is what we end up with. The

molecule is being targeting to be covered in all cases, and it does do that successfully."

THE PHASE III STUDIES AND THEIR CONCLUSIONS

The main Phase III studies which have supported the licence for Sovaldi® are: NEUTRINO, FISSION, POSITRON, and FUSION; these studies supported the approval of the prescribing information. Another study – the PROTON study - assessed people who had both HIV and HCV.

"What the PROTON study showed was that [the effect of Sovaldi® treatment on] those co-infected, or double-infected patients, was the same as patients who just had HCV," mentioned Mr Elliott, "so that is good scientifically but it is also good for the doctor and the patients because it means that they do not have to think about different response rates or maybe treating differently,...a lot of patients who are co-infected will benefit substantially."

Sovaldi® is also beneficial for liver transplant patients, Mr Elliott said: *"We also found that we could significantly reduce the viral load...in those patients and improve the outcome."* The studies also determined that the number of infected livers decreased; *"that is very positive, so for actually some of the sickest patients we can provide some benefits,"* said Mr Elliott.

"The Sovaldi® molecule itself...was designed and developed specifically to hopefully achieve those aims. We found that also it is a credit to the investigators and the patients who joined those studies so quickly to help us get an answer quickly. Research is never predictable but if you do a large programme with a large number of patients and it is well designed then the data will tell you what it tells you – in this case it tells us that we have a very high degree of cure and a good safety profile," continued Mr Elliott.

Will resistance to Sovaldi® be a problem?

The prospect of developing resistance to a drug is a common concern, and although it is not guaranteed that the patients will not, Mr Elliott emphasised: *"In all of our clinical studies...we always look very carefully for resistance, particularly when a patient fails; if a patient fails you take a blood sample, you have the virus, and you look at the virus genetically for any signs of resistance.*

"In all of our studies we haven't had any patients developing resistance on treatment, or even when they relapse after treatment we haven't found resistance, or in patients treated fully with Sovaldi®, two patients have maybe missed their treatment and they have not surprisingly had a problem but they have successfully been treated with Sovaldi®. So at the moment it seems not to be a problem. We will of course continue to look very hard to look for resistance but again we think that the profile of this molecule we developed is very robust against resistance."

THE FUTURE OF SOVALDI®

Although Sovaldi® has been shown to be highly effective, more studies are being conducted, Mr Elliott mentioned: *"We are doing a study now looking at people with severe kidney problems/ renal dysfunction on dialysis; we are looking at lower doses and seeing which is the right dose to achieve good safety and the high cure rate. Again the studies will tell us what the right dose is and then we will be able to offer Sovaldi® to those patients."*

He added: *"It is really making sure that physicians and patients first of all understand HCV and understand that there is now a cure available and coming forward and being managed in clinics. The clinical practice is different all across Europe, and is different for every country working within their system to make sure that the right patients come through and get access to treatment."*

The US was the first country to receive approval for Sovaldi®, Mr Elliott said: *"All the feedback we have been receiving from physicians and patient groups has been very positive. In Europe, France and Germany have been the first two markets to receive it and again the feedback there is very similar."*

This year will illustrate how the medical community takes to this treatment. As Mr Elliott highlights: *"We are at the beginning of quite an exciting period and I am hoping that it will be a period which will turn out very well for patients as we bring Sovaldi® at least as an option to them."*

New liver transplant match model

> "We therefore propose an improvement that favours the principles of justice for the receiver and of utility of the transplant, matches the waiting times to the mortality risk on the active list and improves survival."
>
> *Ms María Pérez Ortiz, University of Córdoba, Córdoba, Spain*

MORTALITY could be reduced in patients on the transplant waiting list thanks to a new liver allocation model, based on a mathematical system. This has been developed by researchers to allow the most seriously ill organ receivers to have the maximum survival probability of each potential match assessed.

The model was created on the basis of automated learning techniques, and the application is based on 38 variables (age, gender, body mass index, existence of diabetes, etc.), gathered from approximately 1,500 donor/receiver pairs in 7 transplant units in Spain and 1 in London.

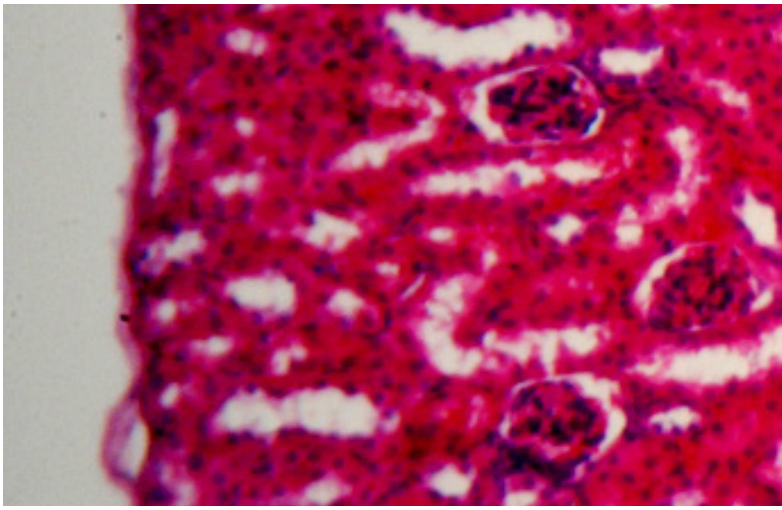
Survival time of the transplanted liver is also processed and learnt by the model and then used alongside the above-mentioned variables to produce suitable donor/receiver pairs, particularly based on the survival time suitability.

Liver allocation is currently calculated based on the donor characteristics and the patient's bilirubin, creatinine, and prothrombin time as predictors of mortality risk. However, researchers at University of Córdoba, Spain, believe that this method of allocation needs refining.

"We therefore propose an improvement that favours the principles of justice for the receiver and of utility of the transplant, matches the waiting times to the mortality risk on the active list and improves survival," explained Ms María Pérez Ortiz, one of the researchers and PhD student.

"This system respects the principle of urgency required by the MELD model and discriminates between receivers on a waiting list who theoretically would have a better prognosis but who, transplanted with a specific liver, would benefit from a better survival," explained the researchers, adding: "The interactions set up in the transplant procedure are more complex than those arising simply from matching a good donor with a very serious receiver."

1,093 liver transplants were carried out in Spain in 2013 alone, with an overall total of almost 22,000, cementing Spain as the worldwide transplant leader.



Transforming skin cells into liver cells

SKIN cells were transformed into mature, fully functioning liver cells that were identical to liver tissue cells. These cells were able to function independently and were transplanted into the animal models with liver failure.

This promising discovery can bring hope to millions of people suffering from liver failure, where a liver transplant is needed for survival.

Dr Sheng Ding, co-lead author and Professor of Pharmaceutical Chemistry, University of California, San Francisco (UCSF), USA, said: "Earlier studies tried to reprogramme skin cells back into pluripotent, stem cell-like state in order to then grow liver cells. However, generating these so-called induced pluripotent stem cells, or iPS cells, and then transforming them into liver cells wasn't always resulting in complete transformation."

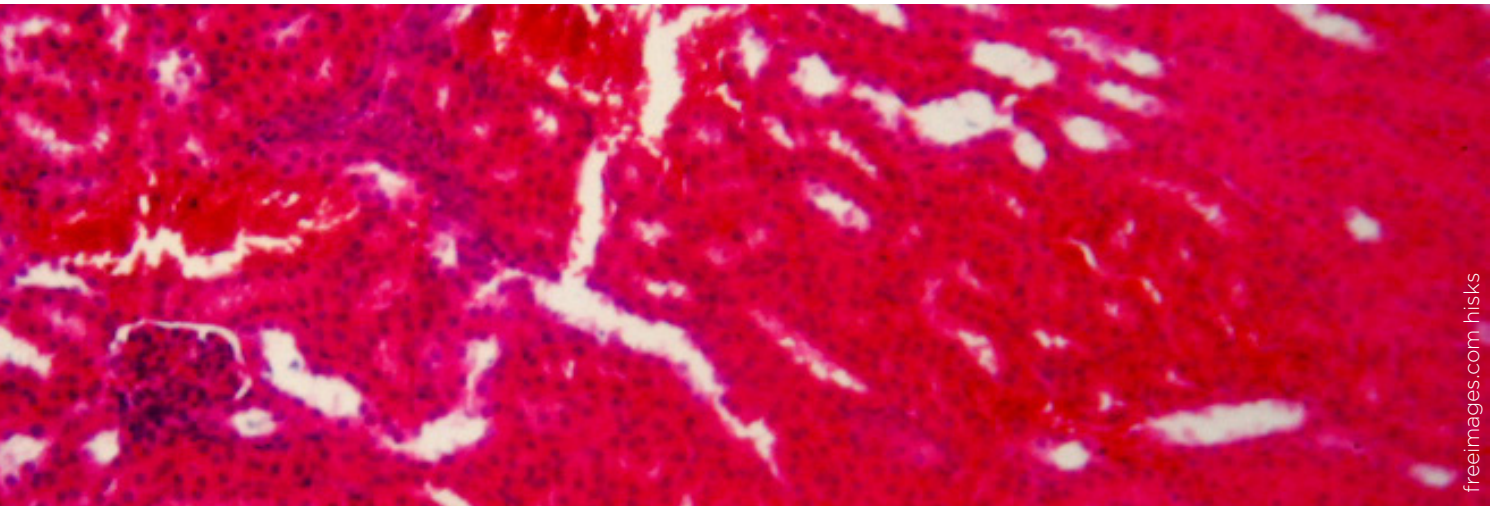
The researchers reprogrammed the cells back to the intermediate state instead of pluripotent state, which was achieved using a concoction of genes and molecules. The human skin cells were transformed back into endoderm cells which have the potential to mature into liver cells.

> "The cells began to take on the shape of liver cells, and even started to perform regular liver-cell functions."
>
> *Dr Milad Rezvani, Institute for Regeneration Medicine, UCSF, USA*

A large surplus of cells in the intermediate stage were easier to manipulate into liver cells, accomplished further by using another mixture of genes and molecules.

Dr Milad Rezvani, co-author and Postdoctoral Scholar, Institute for Regeneration Medicine, UCSF, explained: "The cells began to take on the shape of liver cells, and even started to perform regular liver-cell functions. They weren't fully mature cells yet – but they were on their way."

The cells were then transplanted into the live mice with liver failure. After 2 months, there was an increase in human liver proteins which signalled that the cells were maturing into functioning liver cells. After 9 months, the cell growth showed no signs of slowing down.



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Mysterious mutation causing liver cancer

> "Now that we know about this new chimera kinase, we can look for it in other cancers and work to develop new tools that will someday radically improve our ability to fight disease."
>
> *Dr Sanford Simon, The Rockefeller University, New York, USA*

AN UNUSUAL mutation has been linked to a rare liver cancer, called fibrolamellar hepatocellular carcinoma, which affects teenagers and young adults.

Approximately 200 new cases are diagnosed each year worldwide. There are no known causes, and the cancer is difficult to detect and treat since symptoms occur when the tumour has already developed.

"We reasoned that it would be easier to identify genetic mutations in the tumours of young patients than in older ones because in older people, the genome has been altered by years of aging and environmental factors," says Miss Elana Simon, a co-first author and a member of the research team, The Rockefeller University, New York, USA.

DNA and RNA, extracted from tumours that were surgically removed from 15 participants, were sequenced. The results revealed that there was one abnormality that was present in all of the participants' samples; there was a chimera, which is the result of a piece of DNA that has broken and re-joined, creating a mutated gene.

"These results were extremely encouraging," says Dr Sanford Simon, senior author and head of Rockefeller's Laboratory of Cellular Biophysics, The Rockefeller University. "It is uncommon for a genetic screen for a cancer to turn up such a strong candidate mutation, and for the mutation to be present in every single patient tested."

The effects of the chimera on human liver cells and mouse models will be the next focus of the investigation. There is hope of finding a genetic target to prevent cancer growth.

"Now that we know about this new chimera kinase, we can look for it in other cancers and work to develop new tools that will someday radically improve our ability to fight disease," remarked Dr Simon.

New budding treatment for hepatitis B

A NEW mechanism that targets persistent viruses such as the hepatitis B virus (HBV), which deposit their DNA into the cell nucleus to evade antiviral drugs, has been discovered.

"The degradation of viral DNA in the cell nucleus that we describe represents an important mechanism in the defence against the virus," said Prof Ulrike Protzer, Institute of Virology, Helmholtz Zentrum München, Neuherberg, Germany. "Moreover, for the first time, the results offer the possibility to develop a treatment that can heal hepatitis B."

The researchers were able to find an approach to attack and eliminate the viral genetic material within the nucleus without damaging the infected hepatocyte. In addition to interferons (the immune system's defence agents), the activation of lymphotoxin β receptor promotes proteins which can degrade viral DNA. This prevents viral reactivation even after treatment cessation.

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> "With the activation of lymphototoxin β receptor, also combined with substances that are already available, we have a very promising new therapy concept available."
>
> *Prof Mathias Heikenwälder, Helmholtz Zentrum München, Neuherberg, Germany*

According to the World Health Organization, more than 240 million people in the world are suffering from chronic hepatitis B infection, even though there is a vaccine available for the disease prevention. Chronic infection leads to the development of liver cirrhosis and liver cancer.

There are current antiviral drugs to clear the infection and stop viral replication, but as soon as the treatment is discontinued the HBV in the hepatocytes are reactivated. There is viral DNA stored within the nucleus of the infected hepatocyte, allowing the virus to escape the destructive effects of antiviral therapy. The DNA then serves as a template for new viral proteins and genomes.

"With the activation of lymphototoxin β receptor, also combined with substances that are already available, we have a very promising new therapy concept available," explained Prof Mathias Heikenwälder, Helmholtz Zentrum München.

Topiramate, curbing alcohol consumption

"Our study is the first we are aware of in which topiramate was evaluated as a treatment option for patients who want to limit their drinking to safe levels, rather than stop drinking altogether."

Dr Henry R. Kranzler, University of Pennsylvania, USA

TOPIRAMATE has been shown to reduce alcohol consumption in problematic drinkers who are trying to gradually abstain from their addiction. This anticonvulsant drug has been shown to be efficient among a specific population whose genetic makeup appears to respond particularly well to this therapy.

"This study represents an important next step in understanding and treating problem drinking," said Dr Henry R. Kranzler, lead author, Professor of Psychiatry and Director of Penn's Center for Studies of Addiction, University of Pennsylvania, USA. "Our study is the first we are aware of in which topiramate was evaluated as a treatment option for patients who want to limit their drinking to safe levels, rather than stop drinking altogether."

A total of 138 heavy drinkers took part in a randomised double-blind trial, where half received 12 weeks of treatment with topiramate at a maximum dose of 200 mg/day, and the other half received a placebo. Brief counselling was also provided to reduce drinking and increase abstinent days.

Breath alcohol concentration, weight, and vital signs of the participants were measured weekly during the first 6 weeks of treatment, followed by three bi-weekly visits.

Patients who received topiramate had fewer heavy drinking days than their placebo counterparts. The placebo group was five times more likely to experience a heavy drinking day than the topiramate group. There were more participants in the therapeutic group that experienced no heavy alcohol consumption in the last 4 weeks of treatment than the participants in the placebo group.

40% of European-Americans have a specific genotype which can benefit from the topiramate treatment. This is due to the presence of a certain form of the kainate (glutamate) receptor in certain individuals who reduced drinking with topiramate treatment. This receptor is a specific target for future drugs to reduce heavy drinking.

From stem cells to pure liver and pancreas cells

HUMAN pluripotent stem cells (hPSCs), derived from human embryos or human foetal tissue, have the potential to change the course of medicine due to their ability to self-replicate. This was demonstrated in ground-breaking research which aimed to obtain pure, highly sought-after tissue-specific cells.

The challenging outlook of the investigation was to produce a large number of pure, untainted, tissue-specific endoderms from hPSCs. This involved the use of highly-specific signals to ensure they would develop into the correct tissue type (such as liver and pancreas), and not a range of tissues.

The research team developed a highly systematic and novel screening method, which teased out proteins and signalling chemicals to coax the formation of a single desired cell type. The combination of signals used were then referred to as a 'signalling roadmap' for the pathways involved.

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> "This unprecedented access to highly pure population of endodermal cells attracts pharmaceutical companies, who are interested in further making human liver cells to test drug toxicities."
>
> *Dr Bing Lim, Genome Institute of Singapore (GIS), Singapore*

New insights into cell fates during stem cell differentiation were also discovered through the use of next-generation sequencing and bioinformatics. Dormant sections of DNA known as enhancers were found; they become active and switch on neighbouring genes when hPSCs differentiate.

"This unprecedented access to highly pure population of endodermal cells attracts pharmaceutical companies, who are interested in further making human liver cells to test drug toxicities," said Dr Bing Lim, Senior Group Leader and Associate Director of Cancer Stem Cell Biology, Genome Institute of Singapore (GIS), Agency for Science, Technology and Research (A*STAR), Singapore.

Prof Thomas Graf, Coordinator of the Differentiation and Cancer Programme, Centre for Genomic Regulation, Barcelona, Spain, commented: "Using this novel strategy, the work beautifully shows how hPSCs can be guided to differentiate into the endoderm cells at high efficiencies. The strategy described should be more widely applicable to other desired cell types."

Preventing the lethal consequences of paracetamol overuse

"Overdose of paracetamol is the most common cause of acute liver failure and the leading cause of liver damage requiring transplantation in developed countries. The precise mechanisms of liver toxicity due to paracetamol overdose, however, have remained unclear."

Dr Sanford Simon,
The Rockefeller University,
New York, USA

CALCIUM channel activation leading to liver failure, triggered by paracetamol overuse, has been discovered by researchers. This discovery can be utilised to develop specific therapies that target the channel to minimise the consequences of drug overuse.

Paracetamol (acetaminophen) is widely used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers. The maximum dosage for adults is 1 g (1000 mg) per dose and 4 g (4000 mg) per day.

In an overdose, the patient is asymptomatic for the first 24 hours or has nonspecific symptoms (such as nausea and vomiting). After this, specific symptoms such as jaundice, hypoglycaemia, and loss of co-ordination commonly occur.

"Overdose of paracetamol is the most common cause of acute liver failure and the leading cause of liver damage requiring transplantation in developed countries. The precise mechanisms of liver toxicity due to paracetamol overdose, however, have remained unclear," said Dr Grigori Rychkov, Senior Research Fellow, School of Medical Sciences, University of Adelaide, Australia.

The channel, called Transient Receptor Potential Melanostatine 2 (TRPM2), is responsible for calcium overload; once it is activated, there is an influx of calcium leading to hepatocellular death. If this is persistent and a large number of hepatocytes die, this leads to liver failure. The research had further shown that blockage of the channel leads to the protection of hepatocytes from damage.

"If we can block the TRPM2 channel we might be able to prevent the toxicity or extend this timeframe. If we can stop the calcium uptake and cell death, we'll be giving the liver a better chance for recovery and, hopefully, preventing complete liver failure," Dr Rychkov added.

Dr Rychkov also mentioned that this drug could be used for liver-damaging poisons.

Zebrafish model used to detect new cancer gene

ONCOGENE, UHRF1, has been discovered in an investigation involving zebrafish models and data on human tumours, in a comparison study. This gene is expressed in many cancers and is overexpressed in 40-50% of hepatocellular carcinomas, an occurrence associated with poor prognosis.

"It raises the hope that epigenetic drugs could be applied to liver cancer in the future."

Dr Kirsten C. Sadler, Icahn School of Medicine, Mount Sinai, New York, USA

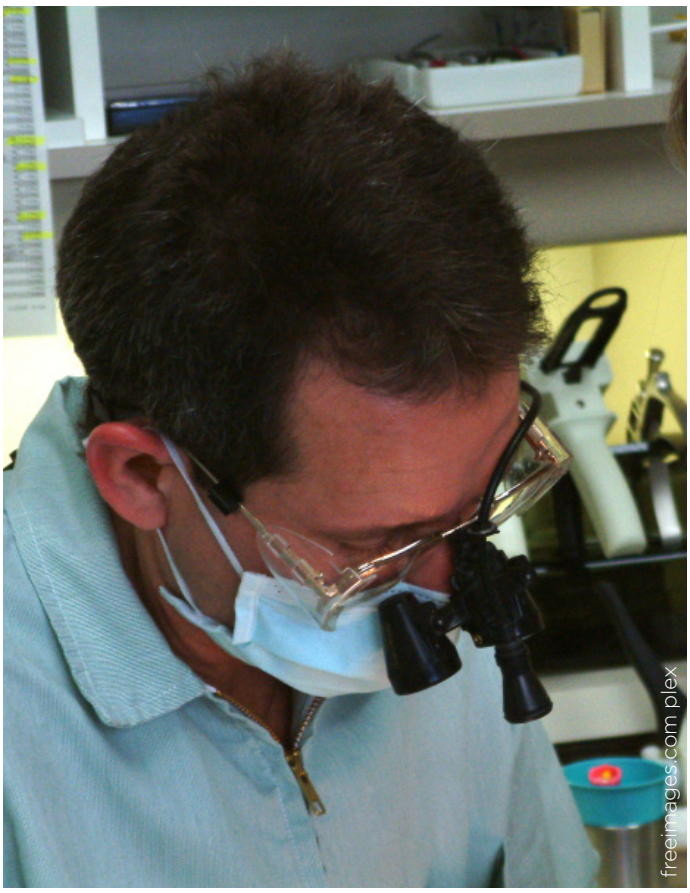
"This is the first time that UHRF1 has been shown to be sufficient on its own to cause any kind of cancer when it is highly expressed," said Dr Kirsten C. Sadler, lead investigator and Associate Professor Medicine, Division of Liver Diseases and of Development and Regenerative Biology, Director of the Zebrafish Research Facility, Icahn School of Medicine, Mount Sinai, New York, USA.

UHRF1 is a central regulator of epigenome, which determines gene expression and how DNA is transmitted during cell division. The difference in cancer cell epigenome with normal cells will be targeted, and there is further hope that certain changes, such as cancer aggressiveness, can be reversed.

High levels of UHRF1 were found in patient-derived liver tumours; a similar situation had also occurred in zebrafish, which suggests the ability to bypass tumour suppressive mechanisms. One potential therapy is to target UHRF1 into reactivating cellular senescence to halt cancer formation.

"We have little to offer people in the setting of advance disease – and this points to an entirely new direction," said Dr Sandler. "It raises the hope that epigenetic drugs could be applied to liver cancer in the future."

"This kind of comprehensive study not only uncovers a new approach to treating hepatocellular carcinoma, but also provides a vital roadmap to unlocking cancer's secrets more quickly and effectively," said Dr Scott Friedman, Dean for Therapeutic Discovery, and Fishberg Professor of Medicine, and Chief of the Division of Liver Diseases, Icahn School of Medicine.



Last-minute holiday deals may harm liver function

"People often leave too little time to get the advice and vaccinations they need to protect themselves from diseases while abroad."

Dr Peter Basile, Medical advisor for GlaxoSmithKline, London, UK

HOLIDAYMAKERS from the UK are not getting the full course of hepatitis A and hepatitis A/B vaccinations due to last-minute holiday plans.

A survey on travel health has revealed that only 14% of British people who received the first dose of combined hepatitis A and B vaccination, then went on to receive the subsequent three to four doses. Of those who did not complete the vaccination course, 17% mentioned that there was not enough time, while 12% said that they simply forgot. Amongst other international travellers, 32% received the full course of vaccination.

Individuals not receiving the full course of the vaccination, and travelling to countries where the disease is prevalent, are at risk of contracting the hepatitis virus. Hepatitis leads to inflammation of the liver; acute hepatitis will pass without causing permanent damage to the liver, while chronic hepatitis persists for many years, causing liver cirrhosis and, in the most serious cases, liver failure.

Dr Peter Basile, Medical Advisor at GlaxoSmithKline (GSK), London, United Kingdom, said: "Some people may be tempted by last-minute holiday deals this month. But our survey has revealed that they may be forgetting to think about their health when booking these holidays, and people often leave too little time to get the advice and vaccinations they need to protect themselves from diseases while abroad."

Survey results reveal that research into health risks in foreign countries and the necessary precautions associated with the holiday destination were not a priority; instead, more emphasis was placed on finding the best hotel deals.

Dr Basile continued: "It's important people are aware that they should visit their GP or travel health clinic ideally at least a month before going on holiday, if they're travelling to places where vaccinations are recommended."

Omega-3 fighting fatty liver disease

OMEGA-3 fatty acids, particularly docosahexaenoic acid (DHA), have been shown to have more impact in the prevention of fatty liver disease than was previously thought.

Researchers utilised metabolomics to investigate the biological effects of omega-3 fatty acids on the liver. Additionally, there were further investigations into the consequences of a Western diet, which has been significantly associated with liver inflammation, fibrosis, cirrhosis, and sometimes liver failure.

"Our metabolomics analysis indicates that the effects of omega-3 fatty acids extend beyond that, and include carbohydrate, amino acid, and vitamin metabolism," said Prof Donald Jump, College of Public Health and Human Sciences, Oregon State University, USA.

The results of recent research has produced much contradicting data, which are possibly due to the quantity of supplements used and the proportion of the two most common omega-3s, i.e. DHA and Eicosapentaenoic acid (EPA). Studies have validated that DHA has the capacity to prevent the formation of harmful metabolites better than EPA. In this incidence, there was a 65% reduction in the proteins involved in liver fibrosis with DHA supplementation.

Based on animal investigations, the equivalent amount of DHA supplementation for humans should be 2-4 g per day. The highest source of DHA can be found in oily fish such as salmon, mackerel, and sardines.

One focal point of the investigation was the effect of DHA, which is used in the control of inflammation, oxidative stress, and fibrosis in the liver – all characteristics of more progressively serious liver problems. The omega-3 fatty acids protect the cells from the effects of the source of inflammation and can improve liver health and other areas attributed to a Western lifestyle.

This investigation provides more information into the health benefits surrounding omega-3 supplementation.

> "Our metabolomics analysis indicates that the effects of omega-3 fatty acids extend beyond that, and include carbohydrate, amino acid, and vitamin metabolism."
>
> *Prof Donald Jump, College of Public Health and Human Sciences, Oregon State University, USA*

Stop blaming fructose for NAFLD

FRUCTOSE is no longer to blame, in itself, for an increase in non-alcoholic fatty liver disease (NAFLD), results revealed in human trials.

Fructose is a simple monosaccharide that forms sucrose when combined with glucose. Natural sources of fructose include fruits, vegetables, and honey. It is also found in common sweeteners, in commercially prepared foods such as sucrose, and high-fructose corn syrup.

Previously, consumption of fructose has been linked to the development of NAFLD, which is the most common chronic liver disease in developed countries.

> "The debate over the role of fructose in obesity, fatty liver, and other metabolic diseases has distracted us from the issue of overconsumption."
>
> *Dr John Sievenpiper, St. Michael's Hospital, University of Toronto, Canada*

The link was made since the disease is linked to obesity and type 2 diabetes, which suggested that diet is a major contributing factor to NAFLD. Due to fructose's unique biomolecular make-up and its metabolic activities, it was singled out as a potential cause for the obesity epidemic.

Dr John Sievenpiper, lead author, Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, University of Toronto, Canada, said: "We found it behaves no differently than glucose or refined starches. It is only when you consume excess calories in the form of fructose that you see a signal for harm, but no more harm than if you consume excess calories as glucose."

Another study published by Dr Sievenpiper mentioned that there was no benefit in replacing fructose with glucose in commercially prepared foods. The research supported the idea that when the portion sizes and calories are the same, both fructose and glucose have similar outcomes.

"The debate over the role of fructose in obesity, fatty liver, and other metabolic diseases has distracted us from the issue of overconsumption," Dr Sievenpipe said. "Our data should serve to remind people that the excess calories, whether they are from fructose or other sources, are the issue."

Mood-stabilising drug to treat ATD

"We found when we gave this drug for 3 weeks to mice with the disease, autophagy is activated, the abnormal protein load is diminished, and liver scarring is reversed."

Dr Gary Silverman, Pittsburgh School of Medicine, USA

FLUPHENAZINE, a typical antipsychotic drug used for the treatment of schizophrenia, has also shown to be effective in the treatment of α1-antitrypsin deficiency (ATD).

The classic form of ATD has an incidence of 1 in 3,000 and the gene mutation leads to the production of an abnormal protein called ATZ which is prone to clumping, explained Dr David H. Perlmutter, physician-in-chief and Scientific Director, Children's Hospital, Department of Pediatrics, Pittsburgh School of Medicine, USA.

A worm (*Caenorhabditis elegans*) model with ATD was used to screen more than 2,000 compounds to see which one causes a reduction of ATZ. Typically, worms that produce ATZ have a short lifespan of 20 days in comparison to healthy worms. Under the fluphenazine treatment, the worms had reduced ATZ accumulation and lived longer than the untreated worms. There was also an increase in autophagosomes, which suggested that autophagy (clearing of abnormal proteins out of the cell) was accomplished.

Further investigations involving mammalian-cell live models of ATD also showed a reduction in ATZ accumulation.

"We found when we gave this drug for 3 weeks to mice with the disease, autophagy is activated, the abnormal protein load is diminished, and liver scarring is reversed," said Dr Gary Silverman, Twenty-five Club Professor of Pediatrics, Cell Biology and Physiology, Pittsburgh School of Medicine.

"It's remarkable that you can take a completely unbiased, high-content screen using a primitive organism and end up identifying a drug that reduces the accumulation of an abnormal protein in mammalian cell lines and a living mouse. It's proof-of-principle of this research pipeline. Furthermore, this drug is very similar pharmacologically to carbamazepine, another mood stabiliser that we found to enhance autophagy and reverse liver fibrosis in the mouse model of α1-antitrypsin deficiency."

Breakthrough potential hepatitis C vaccine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HEPATITIS C surface protein has been discovered by researchers which could lead to the development of a vaccine.

According to Prof Joseph Marcotrigiano, Associate Professor of Chemistry and Chemical Biology, Rutgers University, New Jersey, USA, the outer region escapes immune detection, allowing chronic infection to persist, which can be attributed to the high mutation rate of the virus. He also mentioned that a vaccine which targets the immune system to vulnerable regions of the virus could prevent infection.

"Viruses are smart and it is a constant battle to keep them out," says Prof Marcotrigiano who collaborated on the research with colleagues from the Center for Advanced Biotechnology and Medicine at Rutgers and Emory University School of Medicine. "That's why the development of a vaccine is so important. It's always better to prevent infection through an effective vaccine than to treat after a chronic infection has been established."

> "It's always better to prevent infection through an effective vaccine than to treat after a chronic infection has been established."
>
> *Prof Joseph Marcotrigiano, Rutgers University, New Jersey, USA*

An estimated 160 million people worldwide are infected with the hepatitis C virus (HCV), which is approximately four times more than those with HIV, and more than 350,000 people die every year from hepatitis C-related liver diseases. In the UK, approximately 215,000 individuals are chronically infected with the virus.

This asymptomatic virus can cause scarring of the liver, which leads to cirrhosis and can further progress to liver failure; at this point a liver transplant is needed. HCV is spread mainly by blood-to-blood contact, which is associated with intravenous drug use, poorly sterilised medical equipment, and transfusions (prior to 1992).

The development of a vaccine will be the most cost-effective option to prevent more people from contracting the disease. This finding is a promising step towards potentially alleviating this condition on a global scale.

Saliva protein reducing mortality in ALF patients

SECRETORY leukocyte protease inhibitor (SLPI), a protein found in saliva, has been targeted to reduce the mortality rate in acute liver failure (ALF) patients. This protein is produced in response to injury.

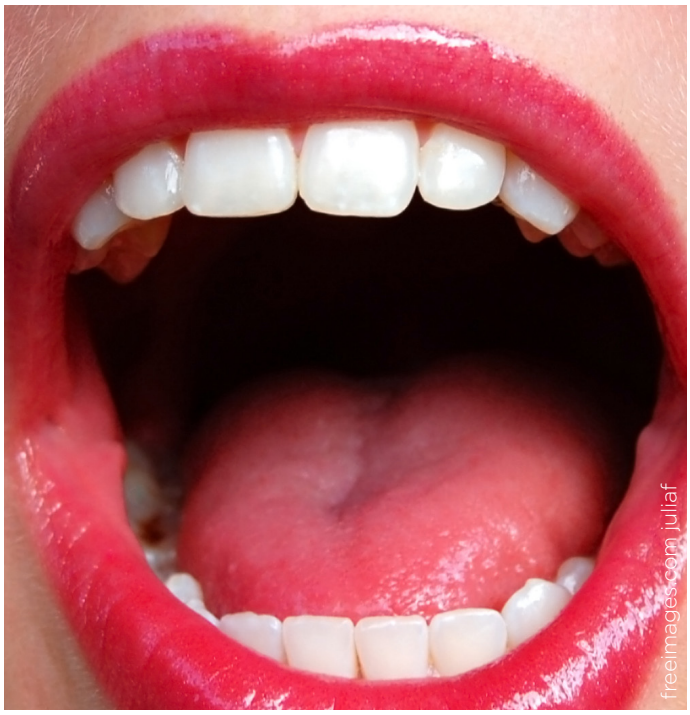
"Infection, namely sepsis, in patients with acute liver failure may be linked to an inadequate response of the body's immune system," said Dr C.G. Antoniades, Clinician Scientist, Imperial College London and King's College London, United Kingdom. "Our study is the first to investigate the role of this particular protein in liver failure patients."

ALF is the result of rapid death of hepatocytes and drug-induced liver injury, especially acetaminophen (Tylenol®); it is cited as the most common cause of hepatocellular death. Statistically, infection is the most common complication of liver disease, and premature death occurs in more than 50% of these patients.

"Our findings indicate that SLPI is a critical mediator of excessive anti-inflammatory responses in ALF, which explains the susceptibility to sepsis/infection in these patients."

Dr C.G. Antoniades, Imperial College and King's College, London, UK

Diagnosis is based on a wide range of information gathered from physical examinations, detailed patient history, laboratory findings, coagulopathy, etc.



The investigation included 98 ALF patients and 24 healthy participants. The results showed that SLPI levels were significantly increased in ALF patients in comparison to their healthy counterparts. It was observed that the circulation of the SLPI molecule resulted in the diminished defence ability of monocytes/macrophages. When the activity of the SLPI molecule was inhibited, the defence ability of monocytes/macrophages was restored. Correspondingly, when SLPI was introduced with healthy immune cells they were rendered irresponsive to foreign organisms.

"Our findings indicate that SLPI is a critical mediator of excessive anti-inflammatory responses in ALF, which explains the susceptibility to sepsis/infection in these patients," concluded Dr Antoniades. "Further study of therapeutic options to inhibit the activity of SLPI in the management of sepsis in liver failure are needed."

GPB shows promise for cirrhosis patients

"GPB reduced the risk of hepatic encephalopathy in patients with cirrhosis and further investigation of its therapeutic potential for patients with hepatic encephalopathy is warranted."

Dr Bruce F. Scharschmidt, Hyperion Therapeutics, San Francisco, USA

GLYCEROL Phenylbutyrate (GPB) has shown promise for reducing hepatic encephalopathy episodes in patients suffering from cirrhosis. The safety profile of this drug is also similar to that of a placebo, Phase II clinical trials have shown.

Hepatic encephalopathy causes various neuropsychiatric symptoms due to the decline in brain function that occurs when the liver is no longer able to remove toxic substances from the blood.

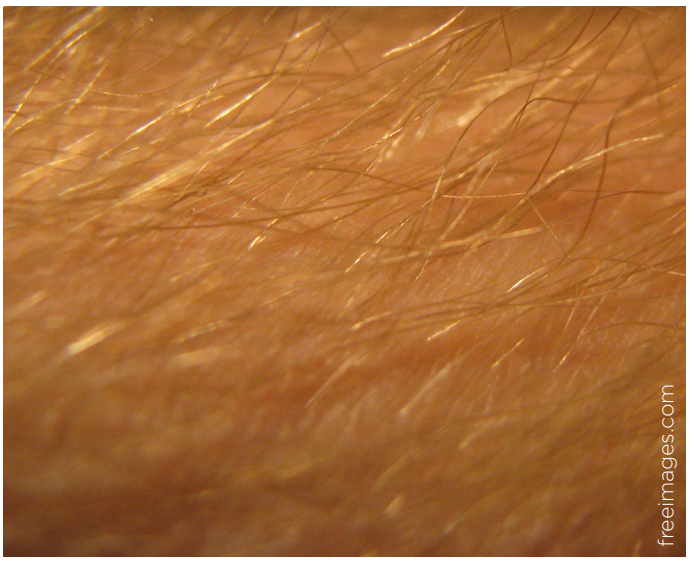
"GPB is approved to treat urea cycle defects that prevent the removal of ammonia from the body," said Dr Bruce F. Scharschmidt, Senior Vice President and Chief Medical Officer, Hyperion Therapeutics, San Francisco, California, USA. "Our trial was the first to investigate the efficacy of a direct ammonia lowering agent in patients with cirrhosis and hepatic encephalopathy."

There were 178 patients that took part in the Phase II clinical trial, which included 59 rifaximin patients. Also included in the study were patients that experienced two or more hepatic encephalopathy events within the 6-month period before the trial. Test patients taking 6 ml GBP twice daily were compared with their placebo counterparts, forming the main basis of the trial.

The results revealed that the GPB patients (36%) experienced fewer hepatic encephalopathy episodes than the patients in the placebo group (21%). There were also only 13 GPB patients that were hospitalised in comparison to 25 patients from the placebo group.

Ammonia levels in the blood of GPB patients were lower than those of their placebo equivalents.

"Our findings provide evidence that elevated blood ammonia plays an important role in the development of hepatic encephalopathy," concluded Dr Scharschmidt. "GPB reduced the risk of hepatic encephalopathy in patients with cirrhosis and further investigation of its therapeutic potential for patients with hepatic encephalopathy is warranted."



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Hepatology

Almirall is an international pharmaceutical company that aims to provide important medicines to patients worldwide. The Barcelona-based firm focuses on developing and making proprietary medicines and licensed products for areas which have yet to receive sufficient medical attention. Today, Almirall has products available across 70 countries in 5 continents, supplied through its 15 affiliates and a strategic partnership network. This includes agreements with other key companies in regions such as the United States, Japan, and China. Almirall strives to become one of the world's most innovative pharmaceutical companies, and hopes to place itself among the key players within its main therapeutic area.

Bristol-Myers Squibb (BMS) is an established giant of the biopharmaceutical industry, having led research and developed products for some of the most innovative medical products ever created. Across the world, the company's medicines have helped millions in their fight against raging diseases such as cancer, cardiovascular disease, hepatitis B, HIV/AIDS, and various psychiatric disorders. As part of its goal to provide patient access to healthcare worldwide, BMS has launched a ground-breaking $150m programme to help relieve HIV/AIDS in Africa, whilst in the USA, free medications are being provided to patients with financial hardship.

Gilead Sciences Inc. is a research-based biopharmaceutical company which discovers, develops, and markets innovative medicines in many areas. Gilead aims to transform the way treatments for life-threatening illnesses are distributed to patients worldwide. The company's own portfolio of products, as well as an extensive pipeline of investigational drugs, includes state-of-the-art treatments for HIV/AIDS, liver diseases, serious respiratory and cardiovascular conditions, cancer, and inflammation. Gilead's portfolio of marketed products includes a number of category firsts, including the first complete treatment regimens for HIV infection and the first oral antiretroviral pill.

Merck aspires to be the world's number one pharmaceutical company, improving the lives of people worldwide through a strategy involving the production, development, and marketing of innovative medicines, vaccines, biological therapies, consumer care, and animal health products. Merck currently employs a diverse workforce and has built its global empire on projects carried out with the utmost attention to ethics and integrity. Their numerous past achievements include the discovery of statins for treating high cholesterol, as well as unearthing the existence of vitamin B1 and a range of cold remedies and antacids.

VSL Pharmaceuticals, Inc. specialises in supplying a range of medical foods aimed at helping patients to manage their diets. The company's flagship product is VSL#3, a probiotic medical food which helps patients who are suffering from ulcerative colitis, irritable bowel syndrome, and ileal pouch. VSL#3 contains a number of beneficial live bacteria, which protect the gastrointestinal tracts and aid the dietary management of ulcerative colitis. VSL#3 is the world's most concentrated probiotic, with 450 billion beneficial bacteria in every sachet. The probiotic exceeds competitor products, with other probiotic dietary supplements possessing a much lower bacterial count.

Bristol-Myers Squibb:
at the Core of HCV Research

At Bristol-Myers Squibb, our commitment to the fight against HCV involves finding innovative new therapies that attack the virus at its core, targeting the non-structural proteins responsible for forming the viral replication complex[1,2]

Our GOAL … CURE [2,3]

References:
1. Penin F et al. Hepatology. 2004;39(1):5-19.
2. Lange CM et al. EMBO Mol Med. 2013;5:1-12.
3. European Association for the Study of the Liver. J Hepatol. 2014; 60(2):392-420.

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Hepatology

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International Liver Transplantation Society Joint International Congress 2014 (ILTS 2014)

4th-7th June 2014

London, UK

This Congress is designed for scientists, surgeons, anaesthesiologists, physicians, nurses, and organ procurement personnel in the field of liver transplantation and aims to allow specialists to compare and understand the differences in current procedures and therapy. Some topics of interest to be covered at the Congress include: fulminant liver failure, hepatitis C, immunosuppression, and liver transplantation.

47th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN 2014)

9th-12th June 2014

Jerusalem, Israel

The scientific programme of this meeting will include recent advances and state-of-the-art lectures covering genetic, immunological, microbiological, and clinical developments in the field of gastrointestinal disorders, liver diseases, and nutrition. There will be many opportunities to discuss clinical and research field interests, to present original works, to see old friends, and to meet new colleagues.

Latin American Association for the Study of the Liver 23rd Congress 2014 (ALEH 2014)

11th-13th September 2014

Cancún, Mexico

The focus of this Congress is to raise awareness of the new challenges and advances in the study of liver diseases. During the event there will be a revision of the guidelines for the diagnosis and treatment of the most common chronic liver diseases and presentation. The programme was developed with the advice of the scientific committee, composed of experienced hepatologists from around the world.

The 65th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD) - The Liver Meeting 2014

7th-8th November 2014

Boston, USA

This meeting will bring together all scientists and healthcare professionals involved in preventing and curing liver disease. It is a comprehensive and also highly customisable program, with a wide range of courses, workshops, plenary sessions, poster presentations, lectures, and symposia. Topics to be covered include: hepatitis, hepatotoxicity, paediatric hepatology, acute liver failure, and artificial liver support.

European Association for the Study of the Liver Clinical School of Hepatology Course 23: Liver Cirrhosis - A Systemic Disease

28th-29th November 2014

Belgrade, Serbia

The primary objective of this EASL course is to provide training and education to bridge the gap between the broad area of liver diseases and the peaks of medicine in its liver-related sub-specialities. Comprehensive care for patients with end-stage liver disease challenges hepatologists and internists, so discussions regarding state-of-the-art management of cirrhotic patients will be reviewed from a variety of perspectives.

EASL Basic School of Hepatology: Molecular Biology and Pathogenesis of Hepatitis Viruses

5th-7th February 2015

Lausanne, Switzerland

The EASL Schools of Hepatology covers diverse aspects in the field of hepatology. The course is divided into a balanced blend of lectures on theoretical, practical, and clinical case based discussions, presented during a residential course with limited attendance.. This is aimed at both young hepatology fellows and also more experienced clinicians who want to be exposed to the newest trends in hepatology.

50th Annual Meeting of EASL- The International Liver Congress (ICL 2015)

22nd-26th April 2015

Vienna, Austria

This Congress will attract specialists from around the world to discuss and analyse the latest developments in the clinical management and basic science aspects of liver diseases. Building on the success of the ICL 2014, the scientific programme will offer a wide range of symposia, workshops, postgraduate courses, forums, and webcasting services to pique the interest of all its participants.

The International Symposium on Viral Hepatitis and Liver Disease (ISVHLD)

25th-28th May 2015

Berlin, Germany

By active exchange of scientific information and lively discussion on important issues, solutions for old and new problems on viral hepatitis and liver diseases will be attained. This symposium will bring together the viral hepatitis community to promote better understanding of the causative viruses as well as pathogenesis, natural history, complications, treatment, and prevention of the diseases they cause.