EMJ EUROPEAN MEDICAL JOURNAL

HEPATOLOGY

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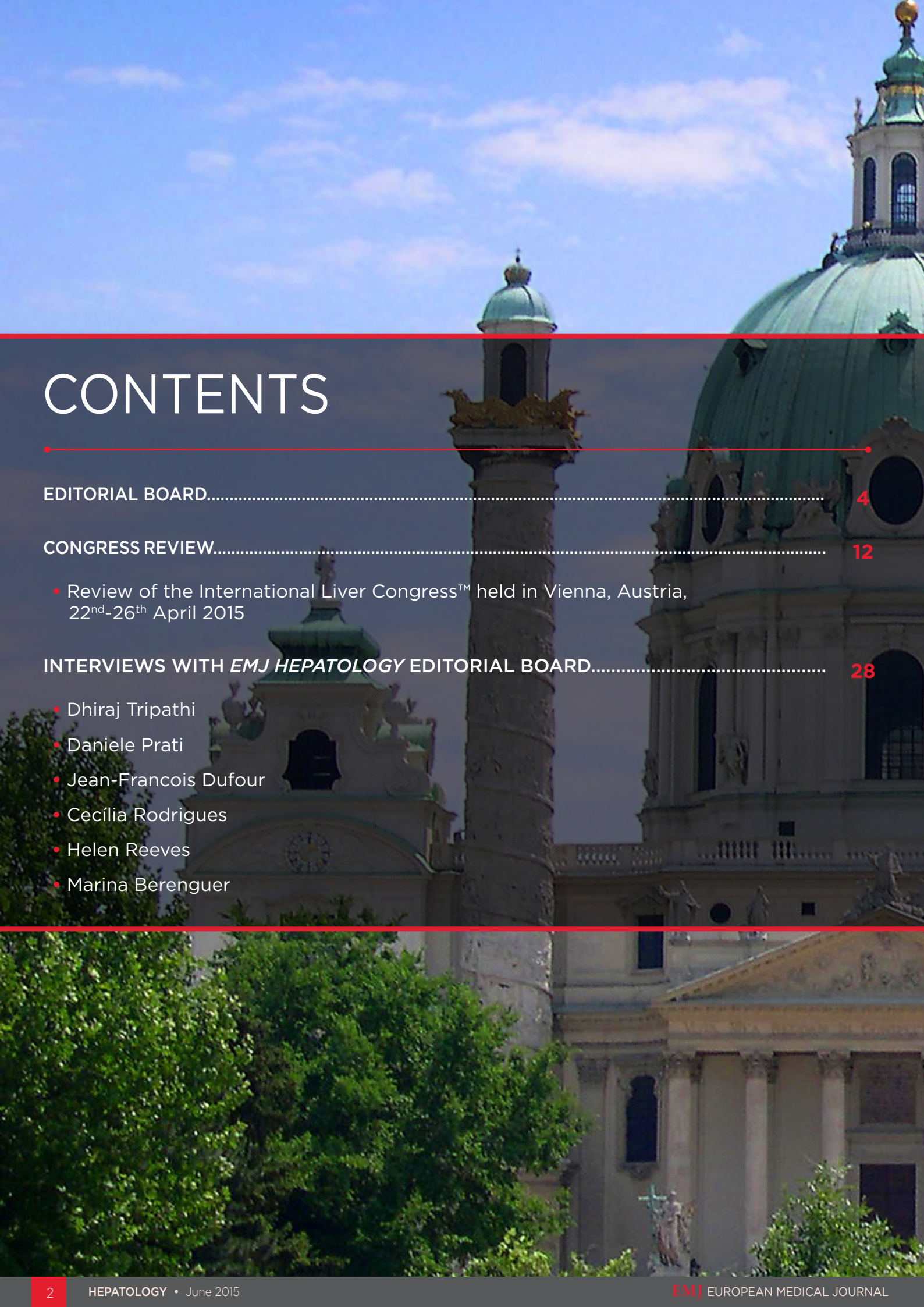
INSIDE

Review of

ILC 2015

Vienna, Austria



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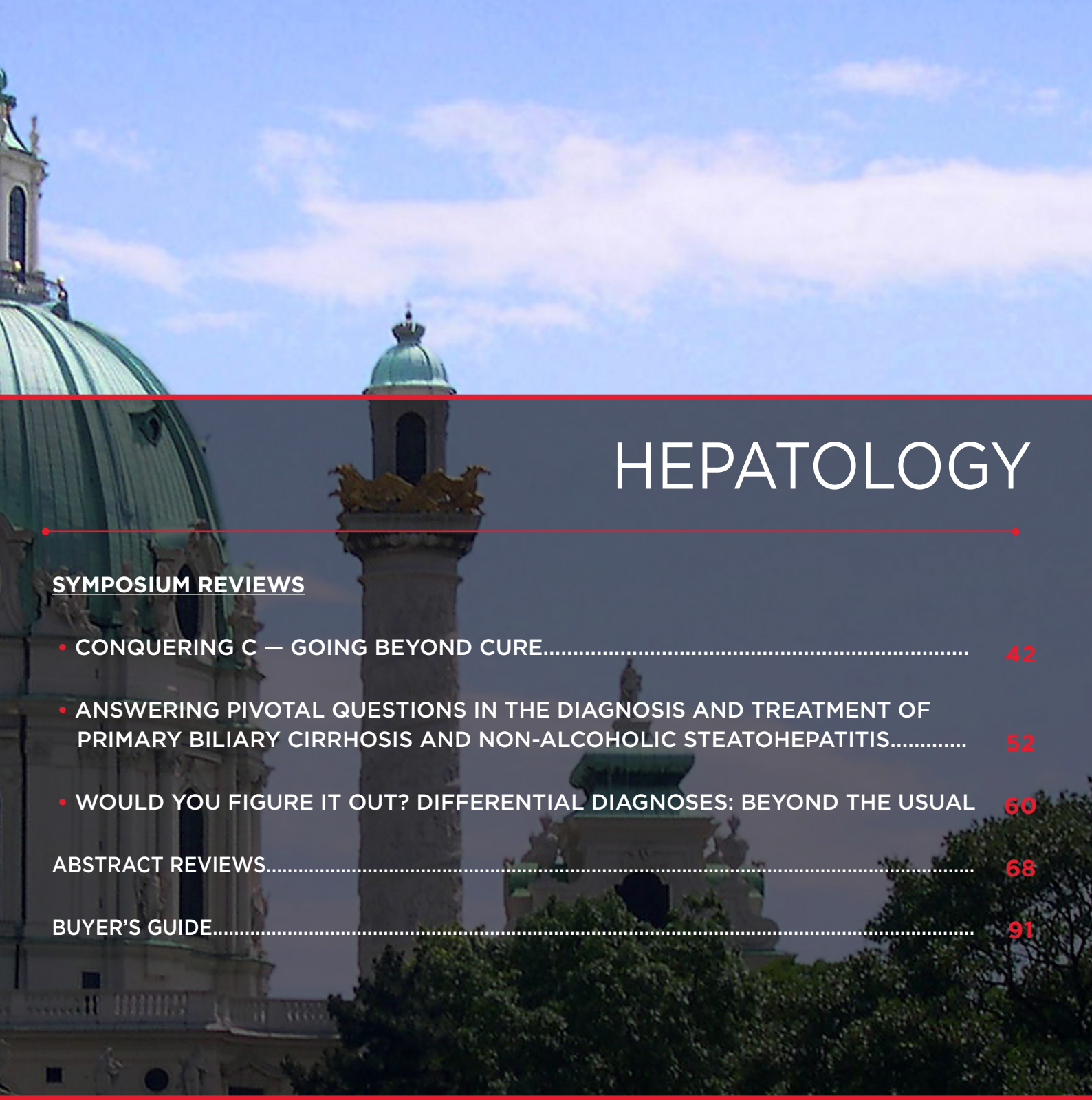

HEPATOLOGY

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EMJ GORELY NEW MEDIA

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Welcome

Hello and welcome to this year's second edition of *European Medical Journal Hepatology,* which we have devoted entirely to covering the major topics raised at the 50th anniversary of the International Liver Congress™ (ILC) organised by the European Association for the Study of the Liver and held in Vienna, Austria, in late April. As usual, this huge meeting presented a wealth of new data and in this issue of *EMJ Hepatology* we have not only included an overall review of the congress, but have also invited a host of delegates to recapitulate the key findings from their presentations in easy-to-digest summaries. As if that were not enough, we have also secured interviews with some of the most prominent hepatologists in attendance and provided coverage of a range of fascinating news stories that broke during the meeting.

Much of the scientific programme at the congress addressed the many clinical aspects of viral hepatitis and its sequelae: its epidemiology, prevention, screening and diagnostic testing, treatment, and monitoring. With this in mind, we are delighted to include in this edition of *EMJ Hepatology* three presentation summaries provided by members of the World Health Organization, who presented a symposium focussing on the themes of diagnostic testing and screening for viral hepatitis. Another key topic at the congress was the evaluation and management of liver fibrosis, which is a common endpoint for many diseases of the liver, and many groups presented data on novel non-invasive methods aimed at reducing the need for biopsies in liver disease patients. Other topics covered at the meeting, and which we have strived to cover in this edition, include genetic and autoimmune liver diseases, non-alcoholic fatty liver diseases, and primary biliary cirrhosis.

With so much new scientific content to take in, we hope that you enjoy reading our latest edition of *EMJ Hepatology* and that it provides you with a meaningful insight into all that transpired at the congress this year. Perhaps you are already beginning to look forward to our next edition, as well as the next ILC, which is to be held in the beautiful city of Barcelona in mid-April next year – you can rest assured that EMJ will be there to cover it, and we look forward to seeing you there!

Spencer Gore

Team Principal, European Medical Journal

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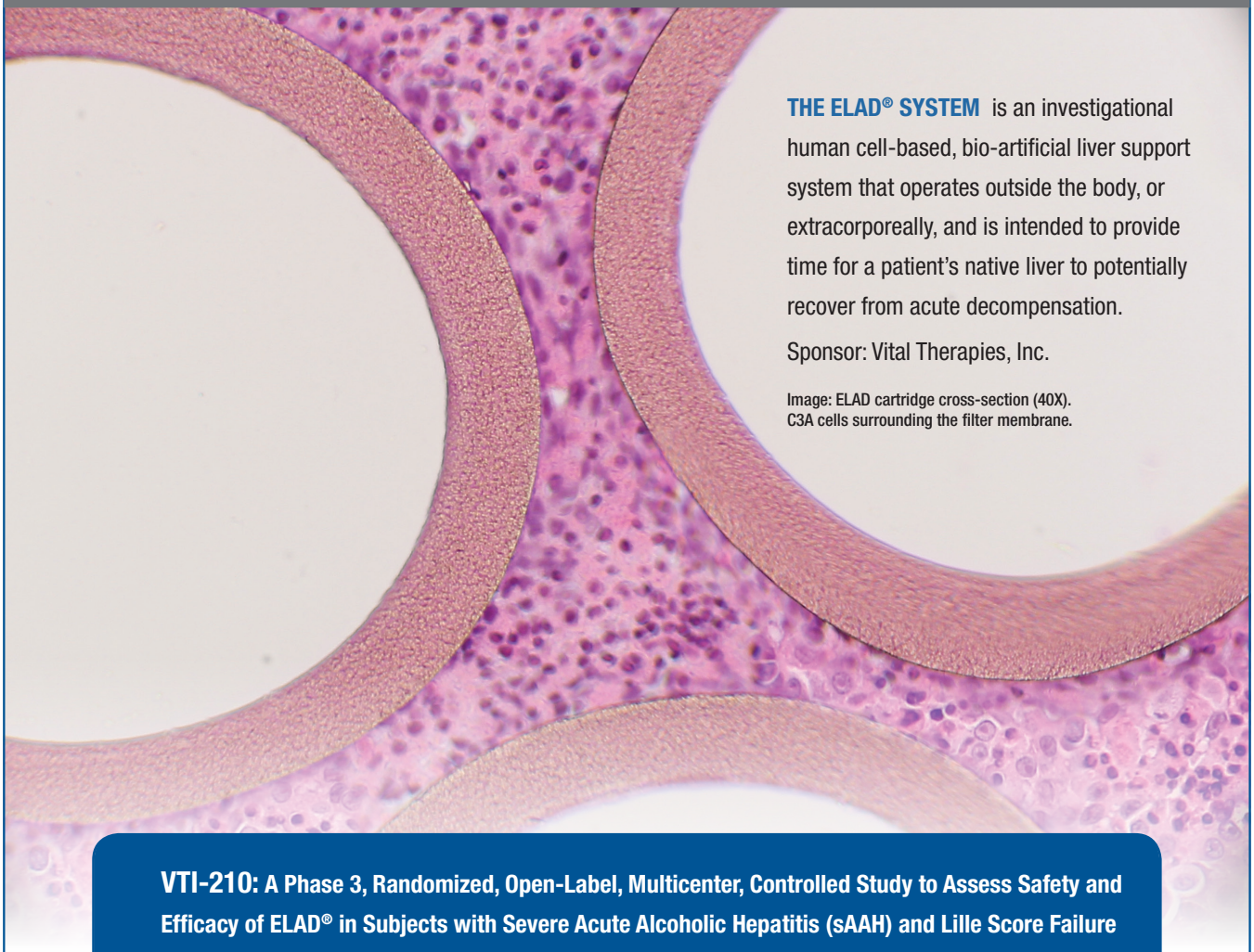

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Foreword

Prof 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* 2015. This year is again a very exciting year for EASL, the European Association for the Study of the Liver, as it is celebrating its 50th anniversary. At the same time, it also marks the beginning of a transitory era in hepatology, where eradication of hepatitis C has moved from the scientific into the public health arena.

> "Now we are witnessing the translation of these efforts into clinical drug development and in the end, relief or cure for our patients."

At the International Liver Congress (ILC), the official annual meeting of EASL in late April in Vienna, we witnessed a very interesting line-up of pivotal Phase III studies. These ranged from all-oral direct-acting antiviral treatments for chronic hepatitis C over large real-life cohort studies in the same field, all the way to novel drug trials for non-alcoholic fatty liver disease as well as rare but important paediatric diseases such as Alagille syndrome.

It is particularly satisfying to see that many of the new developments are a result of immense efforts in basic liver disease research undertaken by many members of the EASL scientific community worldwide. Now we are witnessing the translation of these efforts into clinical drug development and in the end, relief or cure for our patients. These changes have also been instrumental in reshaping the programme of ILC: now we see a strong basic science track reflecting the importance EASL is giving to the pivotal results in basic science that are driving the field.

Thus, I am happy to present to you the latest edition of *EMJ Hepatology,* and I also invite you to attend the next ILC in April 2016 in Barcelona, Spain, and become part of our ever-growing community of physicians and researchers dedicated to the advancement of knowledge in liver disease and how to tackle it.

Yours sincerely,

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 the American Association for the Study of Liver Disease (AASLD), the European Association for the Study of the Liver (EASL), the Austrian Transplant Association, the Austrian Society for Infectious Diseases and Tropical Medicine (OEGIT), the Austrian Association for Gastroenterology and Hepatology; Currently serving as the Secretary-General of EASL.

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Includes a selection of the
latest articles for hepatology.

Featured articles:

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- Benedetta Campana et al.

Alcohol Dependence and Alcoholic Liver Disease

- Karl Mann and Sebastian Mueller

The Liver Meeting 2014: Summary of Presentations on Genotype 3 HCV Infection from the 65th Annual Liver Meeting of the American Association for the Study of Liver Diseases (AASLD), held in Boston, MA, USA, on 7th-11th November, 2014

- Markus Peck and Stanislas Pol

Definitions of Acute-On-Chronic Liver Failure: The Past, the Present, and the Future

- Roland Amathieu and Ali Al-Khafaji

Hepatic-Based Inborn Errors of Metabolism

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Diagnosis and Management of Occult Hepatitis B Virus Infection: A Short Review

- Javier Martínez González et al.

Occult HBV Infection Reactivation in Non-Hodgkin's Lymphoma: An Update on Prevalence and Management

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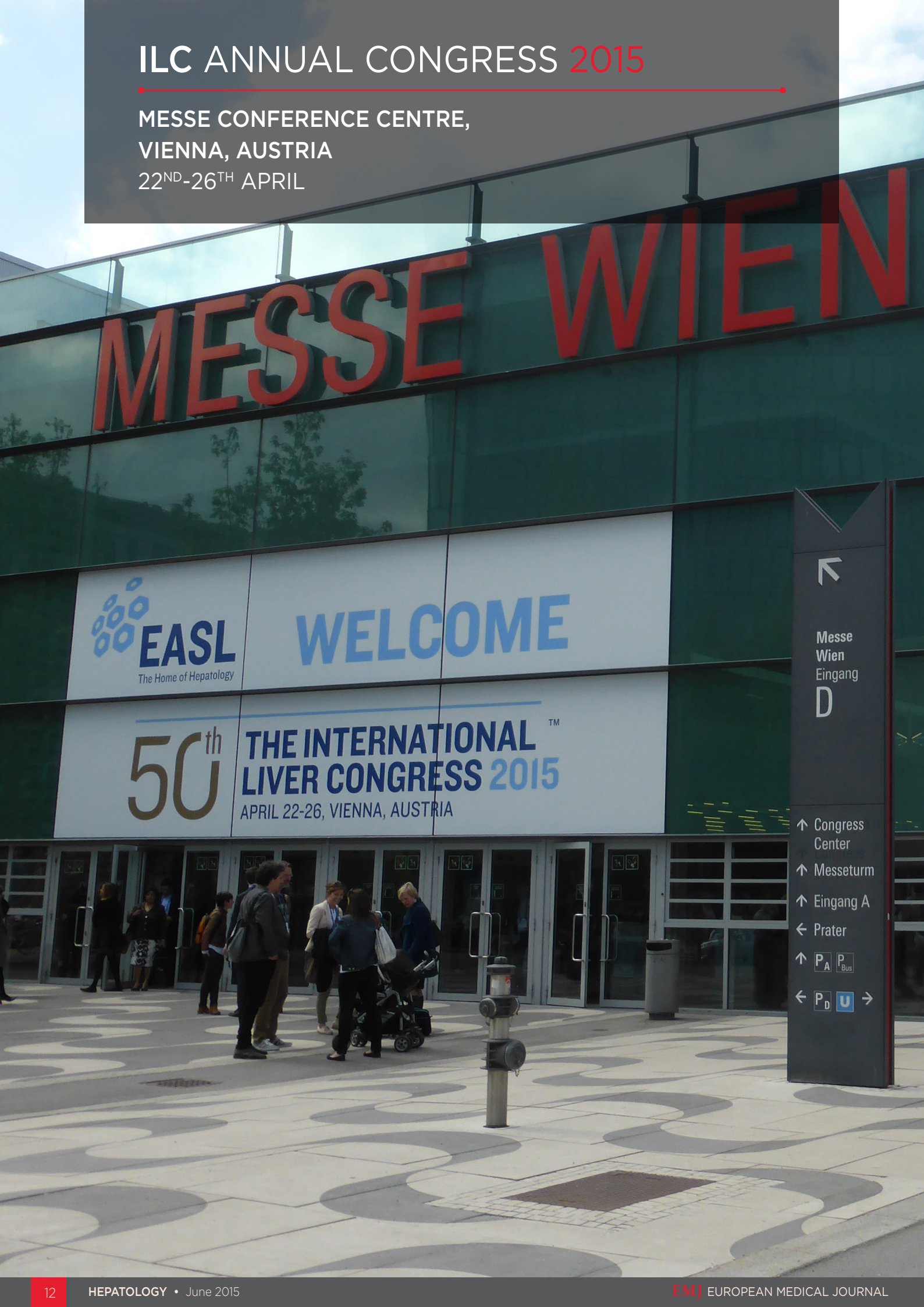


Welcome to the *European Medical Journal* review of the 50th International Liver Congress™ 2015

The 50th International Liver Congress™ (ILC) 2015 lived up to its substantial reputation by playing host to a fascinating display of ground-breaking exhibitions, all aimed at broadening our knowledge and understanding of an ever-changing medical terrain. Taking place on 22nd-26th April 2015 in Vienna, Austria, the congress was steeped in the grandeur of one of the world's most decorated cities. Vienna has overseen the birth of Prof Hans Popper, arguably the founding father of hepatology and its most esteemed practitioner, while Mozart, Haydn, and Beethoven make the city a veritable hotbed of culture.

The promotion of research is the outstanding goal of the European Association of the Liver (EASL), relentlessly pursued through a combination of top-quality presentations at the ILC and extensive educational activities. These have contributed to attracting an enormous level of interest in the annual congress, underlined by the presence of a record-breaking 10,800 attendees in London last year, and this expanding enthusiasm was evident throughout ILC 2015. All of this is proof of EASL's unrelenting drive to inspire and educate to eliminate the global burden of liver-related conditions. "These are very exciting times for hepatology and for our society in particular.

"EASL was founded 50 years ago as a society to promote and exchange science in the field of liver diseases. Still today, this remains the most important task for our association," said Prof Markus Peck-Radosavljevic, EASL Secretary-General, in his pre-congress welcome.

The winners of the EASL Recognition Awards were announced during the congress. Distributed to individuals who have made an outstanding contribution to hepatology in Europe, the awards represent a major achievement in the careers of the recipients, whose work and bearing in the community are sure to inspire future generations. The awards were presented to Prof Roberto de Franchis (Italy), Prof Dominique-Charles Valla (France), and Dr Shiv Kumar Sarin (India).

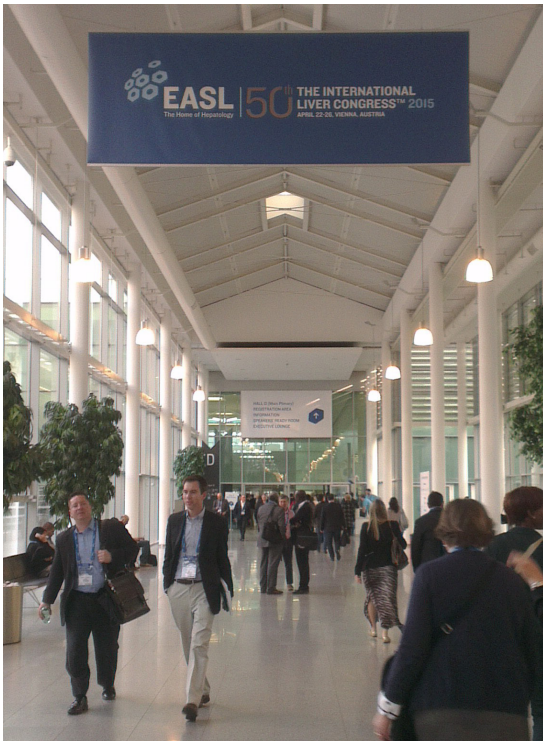
A large portion of ILC 2015 focussed on the longstanding worldwide battle against the hepatitis C virus (HCV). Sessions focussed on every step of the clinical pathway, including screening, diagnosis, and treatment, with a variety of combination treatments designed to tackle HCV in the difficult-to-treat subset. Those proven successful so far include ledipasvir/sofosbuvir in combination with ribavirin and sofosbuvir/daclatasvir combination, administered to difficult-to-treat patients with chronic HCV, and grazoprevir/elbasvir, administered to chronic HCV patients. This wave of new therapies could help usher in an era of fast and effective treatment for an extremely prevalent and serious condition, potentially bringing relief to the millions of people who suffer from HCV worldwide.

Due to the oncogenic make-up of HCV, the chances of developing cancers such as non-Hodgkin's lymphoma and liver cancer are greatly elevated in HCV sufferers. The results of a trial showed that the rate of developing all cancers in HCV sufferers is 2.5-times higher than in non-HCV patients, helping oncologists to peel away more layers of uncertainty and further unveil the complex and destructive nature of this terrible illness.

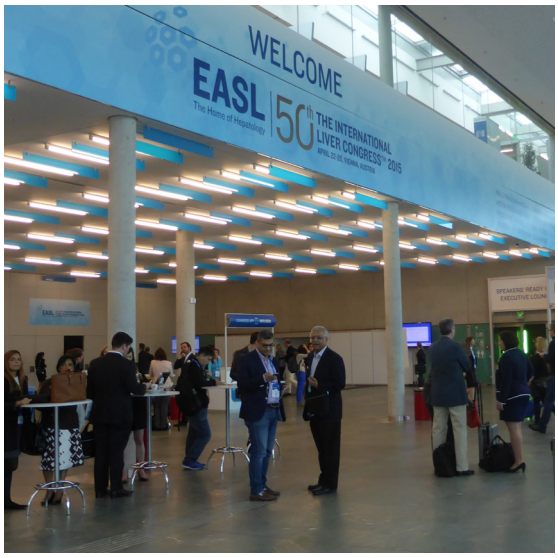
"EASL was founded 50 years ago as a society to promote and exchange science in the field of liver diseases. Still today, this remains the most important task for our association."

The use of genomic analyses, including exome sequencing to decipher the patterns of carcinogenic mutations in patients with hepatocellular carcinoma, could unearth new targets for therapy, potentially lighting the way towards effective personalised treatment. The importance of screening for HCV was also underlined in the NHANES study, which showed that advanced fibrosis is no less prevalent in undiagnosed HCV sufferers than in diagnosed patients who are under clinical management.

The findings at ILC 2015 will reinforce research into the best therapies and strategies required to combat liver-related conditions. While many of the goals set out at last year's congress have been accomplished, there is still much ground to cover before the scientific community as a whole can claim to have defeated globally debilitating illnesses such as HCV and hepatocellular carcinoma. With that in mind, hopes and stakes at ILC 2016 will likely be higher than ever.



HIGHLIGHTS



Advanced Liver Fibrosis in Undiagnosed HCV Cases Underlines the Importance of Screening

GUIDELINES advocating screening for hepatitis C virus (HCV) infection, especially in high-risk demographic groups have been reinforced by new research indicating that the prevalence of advanced liver fibrosis is similar irrespective of whether an individual has received a formal diagnosis or not.

Analysing data obtained from 30,140 respondents to the National Health and Nutrition Examination Survey (NHANES) in the USA between 2001 and 2012, researchers from Stanford University, Stanford, California, USA presented their findings at ILC 2015.

The researchers' hypothesis was that individuals unaware of their HCV infection (undiagnosed) would have less severe liver fibrosis than those who had already been diagnosed. However, they found no significant difference in the mean clinical grade of fibrosis displayed by each group, as assessed by two common scoring systems (FIB-4 and APRI).

A total of 130 survey respondents had completed the follow-up HCV questionnaires and had the laboratory data necessary for calculation of the FIB-4 and APRI scores: 62 had been diagnosed and 68 were unaware of their infection before participating in the survey. The proportion of survey respondents with a high, intermediate, or low probability of advanced liver fibrosis was 14.5%, 40.3%, and 45.2%, respectively, in the diagnosed group compared with 19.1%, 30.9%, and 50.0%, respectively, in the undiagnosed group. The two groups were also similar with regard to age, sex, aminotransferase activity, and platelet count, although patients who were already diagnosed displayed a higher body mass index, the clinical significance of which is unclear.

The consequences of untreated, chronic HCV infection are grave and include progression to end-stage liver disease and hepatocellular carcinoma. Current clinical guidelines recommending HCV screening of individuals, particularly from high-risk demographic groups, would seem justified given that the extent of liver fibrosis present in unaware individuals is similar to that in individuals who have been diagnosed and are under clinical management.

HCV Patients at Higher Risk of Developing Cancer

CANCER rates are significantly higher in patients with the hepatitis C virus (HCV) than those who are not infected with the virus, suggesting that an extrahepatic manifestation of HCV could cause an increased risk of cancer, according to results presented at ILC 2015.

A retrospective study at Kaiser Permanente, Oakland, California, USA was conducted; it aimed to report the rates of HCV-associated cancers, including non-Hodgkin's lymphoma, renal and prostate cancers, and liver cancer (LC), in an HCV patient cohort compared with a non-HCV group. The study recorded all cancer diagnoses in patients over 18 years of age with or without HCV throughout 2008-2012. Within this timeframe, 145,210 patient years were included in the HCV cohort, and 13,948,826 patient years were included in the non-HCV cohort.

The results were stark, with the rate of cancer diagnosis 2.5-times higher in the HCV cohort compared with the non-HCV cohort. Even when LC was excluded the rate was still almost two-times greater. In the HCV cohort there were 2,213 cancer diagnoses (1,524/100,000) during the 5-year period and 1,654 cancer diagnoses when LC was excluded (1,139/100,000). In the non-HCV cohort there were 84,419 cancer diagnoses (605/100,000) throughout this same 5-year period and 83,795 (601/100,000) when LC was excluded.

"The results suggest that cancer rates are increased in the cohort of HCV patients versus the non-HCV patients, both including and excluding LCs. These findings certainly point to the suggestion that HCV may be associated with an increased risk of cancer. However, the findings must be interpreted with caution, as the study also showed that confounding factors such as alcohol abuse, tobacco, obesity, and diabetes modified the results," explained Dr Lisa Nyberg, senior author of the study, Kaiser Permanente.

"These data add to the evidence bank linking HCV with an increased risk of cancer, and highlight that there is still a long way to go in order to fully understand this complex and devastating disease," said Dr Laurent Castera, EASL Vice-Secretary.

Alcohol-Use Disorders a Stronger Predictor of Mortality than Chronic HCV Infection

PATIENTS with hepatitis C virus (HCV) infection have a significantly higher risk of death if they have an alcohol-use disorder (AUD) or another serious comorbidity such as HIV infection, cancer, or chronic kidney disease, according to results of a study presented at ILC 2015.

"These results show that AUDs are a much more accurate indicator of mortality in chronic HCV infection, and highlight the need to encourage alcohol withdrawal and abstinence in all patients."

The study, which is the largest study of the impact of alcohol on liver-related clinical disease and death ever reported, established that chronic HCV has a limited impact on mortality unless the patient also suffers from other severe comorbidities such as HIV infection, cancer, or chronic kidney disease. However, individuals with AUDs are at significant risk of death, with a greater mortality risk observed across all of the study subgroups.

During 2008-2012, 28,953,755 adult patients living in Metropolitan France were hospitalised, with 1,506,453 patients dying in hospital. Chronic HCV infection was present in 112,146 (0.39%) hospitalised patients, AUD in 705,259 (2.44%), and both chronic HCV infection and AUD in 23,351 (0.08%, i.e. 20.8% of HCV and 3.3% of AUD). The investigation discovered that people with HCV were six-times more likely to have an AUD than other hospitalised patients, and 2.4-times more likely to have at least one serious comorbidity.

Of all liver-related events in individuals with HCV, 46% occurred in those with AUD, approximately one-third in those with at least one serious comorbidity, and just 14% in people with HCV who had neither a comorbidity or an AUD. Within the general population, those with an AUD were three-times more likely to die in hospital than those who did not drink alcohol. Therefore, the rates of progression and resultant medical costs may be far lower than current models indicate, thus disputing the cost-effectiveness of direct-acting antiviral treatment of HCV in individuals without AUD.

"These results show that AUDs are a much more accurate indicator of mortality in chronic HCV infection, and highlight the need to encourage alcohol withdrawal and abstinence in all patients," said Prof Tom Hemming Karlsen, Scientific Committee Member, European Association for the Study of the Liver.

Combination Therapy Provides Breakthrough for Difficult-To-Treat Chronic HCV Patients

PATIENTS with chronic hepatitis C virus (HCV) who have decompensated liver disease or have undergone liver transplantation have received a major boost after a study showed that the use of the fixed-dose combination ledipasvir/sofosbuvir (LDV/SOF) in conjunction with ribavirin (RBV) is well tolerated and displays high sustained virologic response rates 12 weeks post treatment (SVR12) in these patients.

SOLAR 2 data were presented at ILC 2015 for 328 HCV genotype (GT)1 or 4 treatment-naïve or treatment-experienced patients with decompensated liver disease or recurrent HCV following a liver transplant. The participants were randomised to receive either 12 or 24 weeks of LDV/SOF plus RBV.

The results were encouraging: 96% (n=72/75) and 98% (n=57/58) of GT1 patients with compensated or no cirrhosis achieved SVR12 at 12 and 24 weeks, respectively, while 91% (n=20/22) and 95% (n=19/20) of patients with decompensated cirrhosis achieved SVR12 at 12 and 24 weeks, respectively. Of the 32 GT4 patients, 27 (84%) achieved SVR12.

"Current treatment options are limited for HCV patients with decompensated liver disease or in those where the virus persists even after having a liver transplant," explained Prof Michael Manns, Professor and Chairman, Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany. "We are therefore pleased that the combination of LDV/SOF+RBV has proved to be so effective, and consider this a significant step forward in the management of these difficult-to-treat patients."

"As we constantly seek to improve the lives of people with chronic HCV, results from trials such as SOLAR 2 give hope to those with an advanced form of the disease such as cirrhosis and disease persisting even after a liver transplant," commented Prof Markus Peck-Radosavljevic, EASL Secretary-General.

Much-Needed Boost Achieved Through the Application of Sofosbuvir/Daclatasvir Combination for Difficult-To-Treat HCV Patients

EFFORTS to apply sofosbuvir (SOF)/daclatasvir (DCV) combination treatment to hepatitis C virus (HCV) genotype (GT)1 mono-infected patients in real life situations have been bolstered by successful study results.

While real-life data of the SOF/simeprevir combination are well publicised, results concerning the SOF/DCV combination are scarce. However, the French observational cohort ANRS CO-22 HEPATHER, which involved giving the new oral antivirals to 3,003 patients, has proved the combination's effectiveness in HCV patients, providing another tool for combatting this prevalent disease.

409 HCV GT1 mono-infected patients were administered a combination of SOF (400 mg/d) and DCV (60 mg/d) without ribavirin (RBV) (n=318) or with RBV (1-1.2 g/d, n=91). A total of 318 patients had cirrhosis and 306 were previously treated with peginterferon-ribavarin (PR) (n=134) or PR + a first-generation protease inhibitor.

The sustained virologic response rate at 4 weeks (SVR4) for SOF/DCV was 81.6% and 93.9% after 12 and 24 weeks of treatment, respectively. The SVR4 rate for SOF/DCV with

RBV was 100% and 96.6% after 12 and 24 weeks, respectively. The 12-week combination of SOF/DCV/RBV attained a 100% SVR4 rate in cirrhotic patients without the additive effect of extension of the therapy to 24 weeks with or without RBV (95.7% and 92.5%, respectively); this was also true for experienced patients. All non-cirrhotic patients attained 100% SVR4 at 12 weeks, confirming the 12-week combination of SOF/DCV as a fruitful therapeutic choice. Significant adverse events were reported in 9% of patients, and treatment discontinuation related to adverse events in 3.1%.

"The cohort study has found that the SOF/DCV combination is associated with a high rate of SVR4 in difficult-to-treat patients infected with GT1 hepatitis C. We also found that the combination with RBV increases the SVR rate in cirrhotic or experienced patients without the additive effect of the extension of the treatment from 12-24 weeks. We hope that this helps support further treatment options for difficult-to-treat patients," said Prof Stanislas Pol, Head of the Liver Department, Hôpital Cochin, Paris, France, principal investigator of the cohort, presenting the findings at ILC 2015.

Once-Daily Grazoprevir/Elbasvir Proves Successful in Patients Infected with Chronic HCV

EFFICACIOUS and well-tolerated therapy of treatment-naïve (TN) patients infected with chronic hepatitis C virus (HCV) genotypes (GT)1, 4, or 6, including those with compensated cirrhosis, is possible through a 12-week oral regimen of once-daily single tablet grazoprevir/elbasvir (GZR/EBR).

"These initial results show that once-daily GZR/EBR offers significant advantages over older treatments, demonstrating the ideal combination of high efficacy with good tolerability and convenience in TN patients infected with chronic HCV."

Testing the safety and efficacy of a once-daily regimen of GZR (NS3/4A protease inhibitor) and EBR (NS5A inhibitor), the Phase III C-EDGE TN study is an international, randomised, blinded, placebo-controlled, parallel-group trial of an oral fixed-dose combination of GZR 100 mg/EBR 50 mg once-daily in TN patients infected with HCV GT1, 4, or 6, including cirrhotic and non-cirrhotic patients.

The study allocated patients in a 3:1 ratio to receive either immediate or deferred treatment after stratification by GT and fibrosis stage evaluated by biopsy or non-invasive methods. HCV RNA levels were recorded by the COBAS TaqMan v2.0 assay. The primary efficacy endpoint was prespecified as the number of treated patients in the immediate GZR/EBR arm with unquantifiable RNA levels (<15 IU/ml) 12 weeks after the conclusion of study treatment (SVR12).

Overall, 421 (90%) of the 469 screened patients were registered, randomised, and administered with ≥1 dose of the study drug.

According to preliminary data from 316 GZR/EBR recipients in the immediate treatment arm, 299 patients (95%) attained SVR12. In the active (immediate treatment) and placebo (deferred treatment) arms, significant adverse events occurred in 10 (3.2%) and 3 (2.9%) patients, respectively.

"These initial results show that once-daily GZR/EBR offers significant advantages over older treatments, demonstrating the ideal combination of high efficacy with good tolerability and convenience in TN patients infected with chronic HCV," said Dr Rajender Reddy, Professor of Medicine, Professor of Medicine in Surgery, Director of Hepatology, Medical Director of Liver Transplantation, University of Pennsylvania, Philadelphia, Pennsylvania, USA, upon presenting the findings at ILC 2015.

Novel Combination Therapy Potentially Effective for HCV Patients

OPTIMUM outcomes for hepatitis C virus (HCV)-infected genotype 2 (GT2) and genotype 3 (GT3) patients have become an achievable goal in the wake of the ground-breaking BOSON trial.

Sofosbuvir (SOF) in combination with ribavirin (RBV) and peginterferon (PEG) achieved the greatest sustained virologic response rates at 12 weeks (SVR12) in HCV-infected GT3 patients with and without cirrhosis, according to results from the BOSON Phase III trial presented at ILC. This study compared for the first time the efficacy of SOF+PEG/RBV and SOF+PEG in HCV-infected GT2 and GT3 patients, with all patients receiving SOF 400 mg daily and RBV 1,000-1,200 mg in a divided daily dose. PEG was delivered as a 180 μg weekly injection.

Among GT3 patients, SVR12 rates were highest in those receiving SOF+PEG/RBV combination for 12 weeks (93%) as compared with SOF+RBV for 24 weeks (84%, p=0.008) or 16 weeks (71%, p<0.001).

"SOF in combination with RBV and with and without PEG have never been directly compared before to determine SVR12. This study highlights that SOF with RBV and PEG should be considered for interferon-eligible GT3 patients, particularly for those with cirrhosis and/or prior treatment failure," said Prof Graham Foster, Professor of Hepatology, Queen Mary University of London, London, UK.

Of 592 patients randomised and treated, 92% had GT3 HCV, 67% were male, 84% were white, 53% were treatment experienced, 62% had non-CC IL28B genotypes, and 37% had cirrhosis.

The study also tested the safety and efficacy of SOF+PEG/RBV for 12 weeks versus SOF+RBV for 16 or 24 weeks in treatment-experienced GT2 HCV-infected patients with cirrhosis. High SVR12 rates were

"This study highlights that SOF with RBV and PEG should be considered for interferon-eligible GT3 patients, particularly for those with cirrhosis and/or prior treatment failure."

recorded in treatment-experienced GT2 patients with cirrhosis in all treatment arms, including those receiving SOF+RBV for 16 weeks (87%), those receiving SOF+RBV for 24 weeks (100%), and those receiving SOF+PEG/RBV for 12 weeks (94%). Fatigue, headache, insomnia, and nausea were the most common adverse events in all arms.

Non-Alcoholic Steatohepatitis Carries a Drastically Increased Mortality Risk

MORTALITY risk conveyed by non-alcoholic steatohepatitis (NASH) is 50% higher than that conveyed by non-alcoholic fatty liver disease (NAFLD), according to the results of a large population-based cohort study presented at ILC 2015.

The large trial analysed the overall burden of cardiovascular disease (CVD) and all-cause mortality across the spectrum of NAFLD (the four stages of NAFLD are steatosis [or simple fatty liver], NASH, fibrosis, and cirrhosis). Data from 929,465 patients in England were obtained from a local computerised hospital activity analysis register during 2000-2013. Patients with NAFLD, NASH, and NAFLD cirrhosis were identified, and cardiovascular comorbidities were coded and their prevalence examined over 14 years.

During the study period, 2,701 patients were diagnosed with NAFLD-spectrum conditions: 1,294 with NAFLD, 122 with NASH, and 1,285 with cirrhosis. All-cause mortality was higher in patients with NASH than NAFLD (22.1% versus 14.5%) and in those with cirrhosis than NAFLD (53.1% versus 14.5%). Congestive cardiac failure was less prevalent in NAFLD than in NASH and cirrhosis.

"NAFLD is recognised as a risk factor for CVD. Our results suggest that NASH conveys an even greater risk. This study provides important new insights into mortality and burden of CVD in patients across the NAFLD spectrum," said Dr Jake Mann, Department of Paediatrics, University of Cambridge, Cambridge, UK.

NAFLD involves fat build-up in the liver, which can trigger inflammation and, eventually, permanent scarring. This study shows a clear association between the severity of NAFLD and an increased risk of CVD and death. It is therefore imperative that practitioners seek to diagnose the condition in its earliest possible stages, so that they can provide diet and lifestyle interventions to prevent their illness from becoming potentially fatal.

Liraglutide Successful in Treatment of NASH

TREATMENT options for non-alcoholic steatohepatitis (NASH) could become more diverse, according to the results of a recent randomised controlled trial studying the adaptation of a drug already approved to treat Type 2 diabetes. Data presented at ILC 2015 underlined the success of liraglutide in meeting the primary endpoint of histological clearance of NASH and in curtailing fibrosis progression.

In the Liraglutide Efficacy and Action in NASH (LEAN) trial, overweight patients with biopsy-confirmed NASH were randomised to receive a 48-week treatment with once-daily subcutaneous injections of either 1.8 mg liraglutide or liraglutide-placebo (control). The key result was improvement in liver histology, defined as 'resolution of definite NASH' and no worsening of fibrosis from baseline to end-of-treatment.

Of the 52 randomised patients, 45 underwent end-of-treatment liver biopsies. A total of 9 (39%) of 23 patients taking liraglutide had resolution of definite NASH with no worsening of fibrosis compared with 2 (9%) of 22 patients taking placebo. Just 2 (9%) patients taking liraglutide experienced worsening of fibrosis compared with 8 on placebo. Furthermore, liraglutide appeared to reduce weight, body mass index, and fasting glucose compared with placebo. No drug-related serious adverse events were reported in patients on liraglutide.

Dr Matthew Armstrong, co-investigator, NIHR Birmingham Liver Biomedical Research Unit, University of Birmingham, Birmingham, UK, explained: "Although NASH is the most common cause of chronic liver disease, there are still no licensed drugs to treat it. Results from the LEAN trial are a major breakthrough and point towards a potential treatment option for this disease. A Phase III trial is now needed to confirm the potential of this class of medication, known as human glucagon-like peptide-1 analogues, as a valid therapeutic option for patients with NASH."

Prof Philip Newsome, LEAN Chief Investigator, NIHR Birmingham Liver Biomedical Research Unit, said: "Clearance of NASH in this study was very encouraging and means we are a step closer to new treatments for patients suffering with non-alcoholic fatty liver disease."

Hepatitis Sufferers Reveal Extent of Discrimination

DISCRIMINATION is encountered by as many as half of viral hepatitis (VH) patients, and one-quarter admit that family members avoided physical contact with them after finding out that they were infected, according to the results of a new survey presented at ILC 2015.

The survey, carried out in collaboration with the Brazilian Ministry of Health, was completed by 1,217 people infected with hepatitis B or C from Europe and America using an online survey tool.

"Clearance of NASH in this study was very encouraging and means we are a step closer to new treatments for patients suffering with non-alcoholic fatty liver disease."

"Not only are people dealing with the illness, it is very evident that VH infection still has a major stigma attached to it across all areas of people's lives, including their family life and even the workplace."

This research aimed to discover, from those infected, when and with what intensity stigma and discrimination impede their quality of life (QoL).

Research revealed that approximately half (49.6%) of all patients have suffered some form of discrimination. Of the 94.1% who informed their family about their infection, one quarter (24.6%) said that their relatives began to avoid physical contact. Moreover, of the 57.4% who informed their partners about their condition, 33.3% said it had an impact on their relationship and almost half (42.7%) said it affected their sex life. Stigma is also present in the workplace, with 10.1% of patients revealing that they lost their jobs after telling their colleagues.

"This shocking survey highlights the true toll VH can have on people's lives. Not only are people dealing with the illness, it is very evident that VH infection still has a major stigma attached to it across all areas of people's lives, including their family life and even the workplace," explained Dr Marcelo CM Naveira, Secretariat for Disease Surveillance, Ministry of Health, Brazil.

Discrimination also stems from healthcare professionals, with 24.6% of those who administer care admitting to remaining distant from the patient and 6.9% refusing to provide care altogether. Friendships are also impacted; of the 73.7% of patients who informed friends of their condition, 46.9% experienced discrimination. The survey has therefore highlighted the implications of discrimination towards hepatitis patients, and solutions can now be created to counter these negative perceptions and improve sufferers' overall QoL.

New Therapeutic Candidate Targets Crucial Driver of HCC

A POWERFUL treatment option for up to 30% of hepatocellular carcinoma (HCC) patients has emerged in the wake of preclinical studies. BLU-554, a potent and highly selective small molecule inhibitor of fibroblast growth factor receptor 4 (FGFR4), is a novel therapeutic candidate for a genomically defined subset of HCC patients with an aberrant FGFR4 pathway, according to findings presented at ILC 2015.

Overexpression of fibroblast growth factor 19 (FGF19), the ligand for FGFR4, can stimulate liver tumour formation. However, by knocking out the *FGFR4* gene, this process can be hindered, suggesting that FGFR4 inhibition may represent an effective treatment strategy in HCC patients whose tumours have an active FGF19/FGFR4 signalling axis.

The research team, from Blueprint Medicines, Cambridge, Massachusetts, USA, discovered that BLU-554 was well tolerated at the highest dose level and showed significant anti-tumour activity in liver cancer models dependent on the FGFR4 signalling pathway.

Dr Klaus Hoeflich, Director of Biology at Blueprint Medicines, explained: "HCC is a disease with a high unmet need and no approved genomically targeted therapies. These findings support the investigation of BLU-554 in clinical studies of patients with HCC driven by aberrant FGFR4 signalling. By identifying patients most likely to respond to therapy based on the molecular profile of their cancer, we hope to make a meaningful difference for HCC patients."

Treatment options are currently limited for HCC patients, with the multi-kinase inhibitor sorafenib still the only approved drug. These findings therefore provide a timely boost, and Phase I clinical trials with the drug are due to begin in mid-2015. "Most people are diagnosed

with HCC once the cancer is at an advanced stage and the outlook is poor. Median survival from the time of diagnosis is about 6 months. Finding new disease drivers and treatment options for patients with HCC is critical to make strides against this devastating disease," said Dr Laurent Castera, Vice-Secretary, EASL.

Exome Sequencing Offers Possibility of Personalised Healthcare for Liver Cancer Patients

GENETIC analysis of liver tumour biopsies may provide physicians with an insight into the events leading to the development of the tumour, as well as appropriate targets for personalised therapy. Researchers from INSERM/Paris Descartes University, Paris, France, used exome sequencing to identify specific mutational signatures associated with exposure to known environmental risk factors, such as alcohol and tobacco consumption, and reported their findings at ILC 2015.

The researchers identified eight mutational signatures, with hierarchical clustering analysis revealing six groups and four singletons significantly associated with specific environmental risk factors, mainly combined alcohol and tobacco consumption and exposure to aflatoxin B1. A total of 11 recurring molecular pathways associated with 161 putative driver genes were also identified, as were three groups of genes centred on the *CTNNB1*, *TP53*, and *AXIN1* loci. Furthermore, associations between mutational signatures and the clinical stage of tumour progression suggest that mutations in the *TERT* promoter represent an early event, whereas amplification of *FGF/CCND1* and alterations in *TP53* and *CDKN2A* occur in aggressive tumours at a more advanced clinical stage. Overall, 28% of the patient samples harboured at least one harmful mutation potentially targetable by an FDA-approved drug, and 86% by an investigational drug studied in Phase I-III trials.

"Genomic analyses, such as exome sequencing, allow us to better understand the mutational processes involved in the development of cancers."

The potential of the new findings was highlighted by Prof Markus Peck-Radosavljevic, EASL Secretary-General, who commented: "Hepatocarcinogenesis is a multi-step process in which pre-cancerous lesions can ultimately transform into liver cancer. Genomic analyses, such as exome sequencing, allow us to better understand the mutational processes involved in the development of cancers. This detailed knowledge then helps us to unravel the mutagenic processes and to optimise personalised patient care."

Drinking Just One or Two Alcoholic Beverages a Day Connected to Liver Disease

DAILY alcohol consumption has proved to be a more potent predictor of alcoholic cirrhosis than total annual amount drunk per person. According to the World Health Organization (WHO),

"The weight of alcohol burden in a country's cirrhosis burden is therefore most significantly and independently affected by the presence of heavy daily drinkers in a population, a finding that reinforces the argument for reducing heavy drinking through effective public health monitoring and policies."

excessive alcohol consumption is the predominant cause of cirrhosis worldwide. Data from a worldwide study presented at ILC 2015 have proved the powerful effects of daily drinking on the cirrhosis burden.

The main focus of most studies evaluating the prevalence of alcohol abuse as a risk factor for alcoholic cirrhosis centre on total annual amount drunk per person. However, it has been noted that clinical trials indicate that strong daily drinking is the strongest predictor of alcoholic cirrhosis. The WHO's Global Status Report on Alcohol and Health reports that approximately 6% of global deaths are caused by alcohol consumption, mostly from alcoholic cirrhosis. Furthermore, of diseases not clearly defined by alcohol consumption, cirrhosis carries the highest alcohol-attributable fraction (AAF), comprising 50% of all cases worldwide.

The researchers thoroughly analysed the WHO's Global Status Report on Alcohol and Health, which included parameters of alcohol consumption and drinking patterns from 193 countries. The team then classified countries by heavy or moderate drinking utilising the average daily consumption among drinkers, according to the US Dietary Guidelines threshold for daily drinking (up to one drink per day for women and two drinks per day for men).

The greatest influence on the weight of alcohol in the cirrhosis burden was the classification of countries by moderate or heavy daily drinking. Moreover, the cirrhosis burden caused by alcohol increased by 11.13% when moving from the moderate to heavy classification. In contrast, total yearly per capita consumption bore a correlation coefficient of just 2.22 with the AAF of cirrhosis.

The weight of alcohol burden in a country's cirrhosis burden is therefore most significantly and independently affected by the presence of heavy daily drinkers in a population, a finding that reinforces the argument for reducing heavy drinking through effective public health monitoring and policies.

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Dhiraj Tripathi

Consultant Hepatologist, Queen Elizabeth Hospital Birmingham, Birmingham, UK.

Q: Was there anything in particular that made you decide to specialise in hepatology?

A: I was very interested in hepatology following an attachment in a gastroenterology unit as a medical student. I was particularly fascinated by interventions such as transjugular intrahepatic portosystemic stent shunting (TIPSS) and endoscopy.

Q: Your research interests include non-cirrhotic portal hypertension and the clinical aspects of portal hypertension. How far has our knowledge progressed in these areas since you first began your research?

A: I started my research 15 years ago and a lot has changed in that time. There have been significant advances in drug therapy, endoscopic treatments, and interventional radiology. Through direct involvement in laboratory-based and Phase III trials, I have increased my knowledge of non-selective beta-blockers (NSBBs) in portal hypertension and was involved in seminal work on carvedilol. This drug has the potential to be more effective than propranolol and we showed it was as effective as band ligation in primary prevention of variceal bleeding. I have also greatly increased my knowledge of TIPSS in the management of variceal bleeding and in Budd–Chiari syndrome (BCS), and have also reported very good outcomes following TIPSS for BCS through publication of the largest single-centre study in the literature to date. I have also become very involved with the management of gastric varices and have considerable experience in the use of human thrombin for their treatment.

Q: In a recent article that you co-authored it was found that, contrary to other data, the use of NSBBs in patients with ascites and refractory ascites and listed for liver transplantation are not detrimental, and instead are associated with reduced waitlist death. Since publication, have those findings had a significant impact on research or clinical practice in this area?

A: The role of NSBBs in patients with advanced cirrhosis and ascites is controversial. There is a lack of consensus due to conflicting data, all of which originate from observational studies with recognised inherent limitations. We have shown that outcomes are better for patients on NSBBs, and are similar to those in a previous large retrospective study. We carefully selected patients and I think that is the key. The practice is not to stop NSBBs in all patients with refractory ascites, but only to consider withdrawal after spontaneous bacterial peritonitis, in hypotension, and after acute kidney injury.

Q: In another article that you co-authored recently, you found that carvedilol was not superior to variceal band ligation in the prevention of rebleeding following a first variceal bleed, although you stated that further exploration was required. Do you and your fellow authors have any plans to undertake any follow-up research in this area?

A: One of the limitations of this trial was the lack of a treatment arm receiving a combination of banding and drug therapy; this is the present standard of care in secondary prevention. We would like to compare banding alone with banding + carvedilol.

Q: Are there other areas of research within hepatology that you feel you would like to move into at some point in the future?

A: I would be keen to be involved in liver transplant research, although I have been involved briefly in the past. I would also be keen to explore portal hypertension research at a more basic level, with haemodynamic and laboratory-based work.

Q: Do you believe that enough funding/support is provided for research in hepatology, could more help be provided by governments in this respect?

A: Absolutely! The funding streams often promote basic-science research and cancer. It is very difficult to obtain funding for clinical trials in hepatology outside of pharmaceutical industry sponsorship.

The greatest success has been the STOPAH NIHR-funded UK trial, but this took years. There is an urgent need for independent funding for the study of therapies for complications of cirrhosis and alcoholic liver disease; these conditions can have an extremely high mortality.

Q: In your view, what is the greatest challenge that hepatologists face today?

A: As mentioned above, funding for clinical trials outside of pharmaceutical industry sponsorship. There needs to be increased resources for hepatology training so that there can be a hepatologist or a gastroenterologist with an interest in liver disease in every acute hospital. This is recommended by the Lancet Commission blueprint. There are major challenges here, but urgent action is necessary to curb the rise of mortality from liver diseases. These measures should also improve access to liver transplantation for patients.

Q: What changes/improvements in the treatment of patients have been particularly noticeable during your years as a consultant hepatologist?

A: There has been a large increase in the burden of liver disease, which is reflected in record numbers of liver transplants. As an example, the number of liver transplants in our institution has increased by more than 40% over the past 7 years. This is due to the increased use of marginal organs and organ preservation techniques, and perhaps increased expertise, leading to more borderline patients being accepted for listing than in the past.

There has been a complete paradigm shift in the management of patients with hepatitis C virus (HCV) infection due to the explosion of extremely effective direct-acting antiviral drugs. Chronic HCV infection looks like it may be eradicated. However, significant challenges remain with regards to case detection, offering timely therapy, and funding for these very expensive drugs.

"Chronic HCV infection looks like it may be eradicated."

Q: You have previously been an active member of the Nepalese Doctors Association (NDA) (UK). How far has this organisation grown in size and influence since you first became a member?

A: NDA (UK) has around 150 members and has grown in recent years. I have been active with regards to promoting the journal, website, and charity work. NDA (UK) is presently focussing on helping the people of Nepal after the devastating earthquakes. We have sent a number of doctors to remote parts of Nepal to do what they can; they have already provided surgery for some of the people, but much more needs to be done.

Q: What advice would you give to any young medical students thinking about specialising in hepatology?

A: Hepatology is a fascinating specialty and one that continues to grow. There are tremendous opportunities to make significant contributions to research and, most importantly, to stemming the rise in mortality from liver disease in the UK.

Q: Do you believe that, in general, people are now more aware of how to reduce the risk of liver damage and disease, and has this been reflected in the number of patients who suffer from such problems?

A: I think much more needs to be done to educate people. The role of alcohol and obesity remains poorly understood. There is more to be done politically, with a lot of the recommendations from the hepatology community, e.g. minimum per unit pricing of alcohol, requiring further reinforcement.

Q: What has been your greatest professional achievement during your career?

A: Career-wise, it would have to be my appointment as a Consultant Hepatologist in Birmingham, which is one of the most prestigious liver units in the world. Birmingham has the largest solid-organ transplant programme in the European Union.

Academically, it would be as lead author of "UK guidelines on the management of variceal haemorrhage in cirrhotic patients", which has just

been published in Gut under the auspices of the Liver Section of the British Society of Gastroenterology. This was exhausting and required many meetings and discussions and a very thorough literature review. I led a 13-strong guidelines development group. The guidelines will be implemented through a number of quality markers and 'Liver Quest'.

Daniele Prati

Director of the Department of Transfusion Medicine and Hematology, Ospedale Alessandro Manzoni, Lecco, Italy; Scientific Committee member of the European Association for the Study of the Liver and Member of the Board of Directors, Italian Foundation for Research in Hepatology (2010-2013), Secretary-General of the Scientific Board of the Italian Association for the Study of the Liver (2007-2010), and Member of the Steering Committee of the Italian Society of Transfusion Medicine and Immunohematology (2011-2014).

Q: Why did you choose to specialise in hepatology during your medical training? Are there any differences in the types of cases you see now compared with when you first started working in the clinic? What changes/improvements in the treatment of patients have been particularly noticeable during your career as a hepatologist?

A: I started to be interested in hepatology when I was a medical student. In the late 1980s, I used to work in a haematology clinic for patients with thalassaemia and I was interested to see that most of the patients who started to be periodically transfused at the time developed acute hepatitis after a few treatments. At that time it was called 'non A, non B hepatitis'. The identification of the hepatitis C virus (HCV) a few years later led to the introduction of screening for blood donations, and post-transfusion hepatitis almost disappeared. That is probably the greatest clinical breakthrough that I have had the opportunity to observe in my career.

Q: Has there been a large increase in the prevalence of HCV infection in Europe in recent years, and if so, are any specific demographic groups at increased risk?

A: I think that it is very difficult to answer a question focusing on HCV epidemiology in Europe because what we lack is a reliable epidemiological study. I know that some health authorities have published some data indicating that the number of cases diagnosed each year is increasing, but this could simply reflect a change in screening policy rather than a changing epidemiology.

Q: What kind of measures can be taken in order to prevent the spread of HCV in Europe?

A: Actually, the epidemiological features of HCV spread in Europe are likely to be quite heterogeneous. In resource-rich countries, the transmission of HCV through infected blood components or other unsafe medical procedures has virtually disappeared. In these countries, the vast majority of new infections occur in intravenous drug users. Therefore, prevention programmes dedicated to these individuals appear to be of primary importance. These include periodic screening followed by the treatment of those who are carriers. In fact, intravenous drug users are potentially very efficient spreaders of the virus (so-called 'super spreaders'), and their treatment is expected to contain the transmission of HCV to other individuals. In some regions of the continent, however, unsafe medical injections and surgical or endoscopic procedures, as well as suboptimal screening of blood donations, still leads to virus transmission.

"In my opinion, whether you are a clinical hepatologist or a scientist studying liver disease, you simply cannot miss The International Liver Congress™."

Q: With so much migration to and within Europe in recent years, has there been a change in the HCV genotypes that you tend to see in the clinic?

A: In Europe we are used to seeing patients infected by almost all viral genotypes. Infections imported from abroad can, of course, cross-circulate among communities of high-risk individuals, for example intravenous drug users.

Q: There have been some large developments in the treatment of HCV infection recently — will the introduction of new direct-acting antivirals mean that chronic HCV and its sequelae will be very rare in the clinic of the future?

A: Of course the availability of new drug regimens represents the second major clinical breakthrough after the one that coincided with the identification of the virus approximately 25 years ago. At that time, the possibility of screening blood components meant that the incidence of transmission dropped dramatically, almost arresting virus circulation among blood recipients. With the new drugs, we will be able to prevent the development of long-term consequences of HCV infection (liver decompensation, liver cancer) among chronic carriers. The main uncertainty, however, is when this will occur. At present, the costs of these new treatments are very high and this hampers their widespread use in all HCV patients, especially those with mild disease.

Q: Liver transplantation is slightly unusual in not being as dependent on the human leukocyte antigen matching of tissues as other types of allograft — does this mean that there are enough livers available for transplant in Europe?

A: In several European countries, especially those with the highest anti-HCV prevalence, waiting lists for liver transplantation and the mortality of patients needing a transplant are still a cause for concern. In order to improve the survival of these patients, we need safe and effective treatments to contain the rate of progression towards end-stage liver disease (such as the new interferon-free regimens) combined with more effective policies of organ donor recruitment for HCV treatment. Both aspects are needed in order to observe improvements.

Q: What is your opinion of the introduction of an 'opt-out' system of organ donation compared with the more common 'opt-in' systems?

A: It is one way of increasing organ availability, although not all European countries are ready to introduce this policy in their healthcare systems.

Q: There is a well-recognised and unmet need for donor organs for transplantation — are the blood stocks available to Italian and other European hospitals also insufficient? How stringent are current blood screening systems both in Italy and across Europe, and how do these compare with other regions of the world?

A: Here again, Europe is divided into two. In resource-rich countries, people have access to a safe and sufficient blood supply. However, in some low-income countries, especially in Eastern and Central Europe, the national blood systems are less developed, and both the availability and the safety of the blood supply remain an issue. Too many donations are collected from replacement or family donors, and in areas of high risk anti-HCV screening has been implemented relatively late.

Q: In your opinion, what is the greatest challenge that hepatologists face today?

A: As a physician, I would say that it is important to ensure comparable levels of access to prevention, diagnosis, and care across the world. But we are still far away from such a goal. It is a paradox that in regions of the world where people have the highest rate of HCV infection, only a few can be identified and treated.

Q: How important is the annual European Association for the Study of the Liver (EASL) congress for helping hepatologists to combat liver disease?

A: The mission of EASL is that all who are involved with liver disease can realise their full potential to cure and prevent it. In my opinion, whether you are a clinical hepatologist or a scientist studying liver disease, you simply cannot miss The International Liver Congress™. If for some reason you were not able to attend this year then you can still keep track of the news by using the webcast sessions.

Q: Are there any other areas of hepatology that you would like to research in the future?

A: The plans of my group are to continue to explore the spectrum of subclinical liver diseases. Since the impact of hepatitis virus infection is declining, there will be more interest in diseases due to the excessive intake of calories and alcohol. In addition, we need to refine our diagnostic tools by improving clinical reasoning and making decisions based on evidence. I think that research on clinical methodology is of great importance, and absolutely no less relevant than research performed at the laboratory bench.

Q: Could you speculate on what is likely to be the 'next big thing' in the field of hepatology? What advice would you give to young medical students thinking about specialising in hepatology?

A: Predicting the future is a job for fortune tellers and not for physicians! The margins of error are too high and too many predictions have failed, or been proven wrong years after they have been formulated. Anyway, my suggestion for young fellows is always to take alcoholic disease into great consideration. It is widespread in Europe, and we need to prevent and treat it better than we do now.

Jean-Francois Dufour

Clinic Director and Professor of Hepatology, University Clinic Visceral Surgery and Medicine, Inselspital, Bern, Switzerland; Member of the American Association for the Study of Liver Disease, the American Gastroenterology Association, the American College of Gastroenterology, the European Association for the Study of the Liver (EASL), the Swiss Association for the Study of the Liver, the Schweizerische Gesellschaft für Gastroenterologie und Hepatologie; Founding Member of the International Liver Cancer Association; Member of the Governing Board of EASL, Educational Councillor for EASL; Member of the Educational Committee of United European Gastroenterology.

Q: What was it that made you decide that you wanted to work in medicine, having previously studied mathematics at the University of Geneva?

A: I actually completed my mathematical studies in parallel with my medical studies. I find there is an incredible abstract beauty and intellectual challenge in mathematics, which I was ready to take at that time. Studying in two different faculties was only possible with the great support of good friends who shared their notes with me.

Q: What in particular made you decide to specialise in hepatology during your medical studies?

A: I opted for hepatology because you deal with all aspects of internal medicine when you treat patients with liver diseases. I also found the combination of basic research and clinical research attractive. Moreover, I felt that the field would advance in the next decades and I am happy to see that I was right on this one: we can now treat hepatitis B infection and we can cure hepatitis C infection. These are major progresses in medicine.

Q: Many of the clinical studies you have been involved in have focussed on non-alcoholic steatohepatitis and hepatocellular carcinoma (HCC). How far has our understanding in these areas improved since you first began research into them?

A: Non-alcoholic fatty liver disease (NAFLD) is a relatively new disease. I have seen how important it has become in the last years and its strong link with HCC makes it a priority in hepatology. If recognised, NAFLD is still a black box and a heterogeneous entity. We urgently need to have a positive diagnosis for this disease and better understand what in our lifestyle is putting us at so much risk.

Q: In a recent publication that you co-authored, you concluded that selection of liver transplant candidates from those with HCC could be expanded to the total tumour volume (≤115 cm^3)/AFP (≤400 ng/ml) criteria in centres with at least 8-month waiting times. Do you think that this is likely to happen in the near future?

A: Certainly! The Milan criteria have been a milestone and widely accepted because they propose a simple set of rules for listing or not listing patients for transplantation, which is an extremely important decision. So these rules were and are very helpful to define limits. Nevertheless, we know that these rules can be improved. Dr Christian Toso took this approach several years ago and is now publishing a prospective validation of the rules he proposed. I believe that it is only with such scientific testing and validation that we will move the field forward.

Q: In what ways do you feel more people could be persuaded to donate their liver for transplantation? Would an 'opt-out' system be something that you would advocate, for example?

A: This is helpful and makes a difference. It is also very important to have a working culture in intensive care units so as to not miss any possible donor. Such an approach greatly increased the rate of donation in Spain. Moreover, the effort needed to look for potential donors should be recognised and better valued.

Q: In your view, what is the biggest challenge that hepatologists face today?

A: Medicine is changing. The amount of relevant new information is increasing at a dizzying speed, although this is not specific to hepatology. I think the biggest challenge for our specialty is to take advantage of new technologies in order to better diagnose and treat our patients.

Q: What are the major changes that you have seen in hepatology research since you first began your career? Are there more resources available to researchers now, for example?

A: I entered the field 25 years ago when the hepatitis C virus was discovered. Now this has become an easily curable disease and that is clearly the most amazing change in the field. This change was driven by fundamental research and also industry-sponsored research. Such a combination is essential in order to achieve decisive changes. Researchers now have access to more resources than before but the competition is also much stronger.

Q: How important is the annual EASL congress for hepatologists in the battle against liver disease?

A: This is the largest liver meeting worldwide. This is where the most important advances are presented, where you can meet any hepatologist, and where big studies are planned, discussed, and put forward.

Q: What lessons could other countries learn from the way healthcare is provided in Switzerland?

A: Each country has to develop a healthcare model that suits its population's needs best; no system is perfect. This is a very difficult topic with many different competing interests. Switzerland has a long history and culture of compromise, which helps the different stakeholders to find common solutions.

Q: How does the Swiss system compare with the USA healthcare system, having lived and worked in both?

A: I had the chance to live and to train in the USA many years ago. I am sure that the system has changed since then. Both systems face the challenge of preventing healthcare costs from increasing too massively.

Q: What advice do you have for young medical students thinking of specialising in this area of medicine?

A: My advice is to join in! The diversity of diseases and the progress made are fascinating. Hepatologists are well-educated people and great colleagues. Patients with liver diseases come from all sides of society and we get to know them and their families quite well over the years. Hepatology is a great specialty.

Q: Having lived and worked in different areas of the world, as well as being an active member in many different hepatological associations, how co-ordinated do you believe that the strategies for fighting liver disease worldwide are? In your opinion, is there more that could be done to co-ordinate responses?

A: This is an ongoing process. The societies are better co-ordinating their efforts and are teaming up with other partners, such as foundations and

political bodies. Policies and approaches are getting more and more global.

Q: What would you say has been the proudest moment of your career to date?

A: Having the chance to work with a fantastic team of great individuals alongside me in Bern.

"This is where the most important advances are presented, where you can meet any hepatologist..."

Cecília Rodrigues

Head of the Department of Biochemistry and Human Biology; Coordinator of Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; Governing Board, European Association for the Study of the Liver.

Q: Why did you decide to specialise in the field of hepatology during your education?

A: As a basic scientist, I became interested in liver cell biology because the liver is gifted with an amazing capacity to regenerate in response to loss of functional liver mass. This regenerative ability reflects essential metabolic and detoxifying functions to protect the liver from injury induced by xenobiotics, toxins, or infection, where rapid regeneration represents an efficient mechanism to avoid loss of key hepatic function. In the context of chronic disease processes such as chronic viral hepatitis, non-alcoholic fatty liver disease, and alcoholic liver disease, the hepatic response to cell death restores hepatic architecture and function, but may also become maladaptive and promote the development of liver fibrosis, cirrhosis, and hepatocellular carcinoma. Building on the fundamental role of cell death in hepatic diseases, a precise knowledge of cellular and molecular mechanisms regulating cell death and cell death responses is essential to understanding the pathophysiology of liver disease and further developing new therapeutic approaches, and this has been a key driver for my specialisation in the field of hepatology.

"Liver disease is a very important cause of chronic disease but also death."

Q: What changes and developments in hepatology have been the most impactful during your career? How close do you think we are to fully understanding the majority of mechanisms responsible for liver disease? How much progress has been made in understanding the gene expression and signalling networks involved in cell death, differentiation, and proliferation during your time in research?

A: Much progress has been made in understanding the pathophysiology of liver disease, as well as in unveiling the role that gene expression and signalling networks regulating cell death, differentiation, and proliferation play in this context. The discovery of novel modes of cell death and specific pathways that regulate cell death has significantly improved our understanding of the pathophysiology of acute and chronic liver disease. Even though revolutionary progress has been made in hepatitis C virus (HCV) therapy, we have not yet been successful in halting progression in other relevant liver diseases. Despite new insights into key pathways through which cell death drives liver disease, clinical translation of these findings is still eagerly awaited. In addition, future studies will certainly focus on understanding the regulation of cell death mechanisms in the liver, as these pathways might hold important clues to pave the way for personalised treatment of liver disease. Finally, novel biomarkers of cell death may allow more accurate predictions for patient selection, response to therapy, and treatment decisions.

Q: You recently co-authored an article showing how targeting mutant *KRAS* at the genetic level using G4-binding small molecules is a promising anti-cancer strategy. How long do you think it may be until such a strategy becomes translated from bench to bedside?

A: *KRAS* is one of the most frequently mutated oncogenes in human cancer, yet it remains undruggable. In this study, we unravel the potential of G-quadruplex structures in the *KRAS* promoter region and mRNA as novel therapeutic targets, and identify a new anti-cancer drug scaffold. It is now possible to further develop this potential therapeutic approach, which is still in its infancy, but may ultimately lead to novel therapeutic targeting, thus contributing to the improvement of human health and benefiting quality of life.

Q: Are there any other areas of hepatology that you would like to research in the future?

A: Liver diseases need to be proactively tackled, at the level of both diagnosis and treatment. Indeed, better biomarkers and novel approaches for early diagnosis and treatment of both liver failure and liver cancer are expected to emerge in the near future. In this regard, biomarker discovery will be key to identifying patients at risk and those with advanced disease, monitoring disease progression, and monitoring response to therapy, while genomics may lead to customised therapy based on individual treatment response. Other areas of interest include further investigation of future promising therapeutic agents and their potential molecular mechanisms and modes of action. Deeper insights into approaches to address the complexity of liver metabolism, cell signalling, gene transcription, and epigenetic control are also eagerly needed.

Q: In your opinion, what is the most challenging area in hepatology at this time?

A: The availability of drugs that can cure HCV infection will revolutionise the field of hepatology within the next decade, although the benefit of the new drugs in reducing death rates is still expected to take years. Despite the progress in various areas of liver research, about 29 million people in the EU have chronic liver disease. Alcohol consumption, viral hepatitis B and C, and metabolic syndromes linked to overweight and obesity are reported to be leading causes of liver cirrhosis and primary liver tumours. Liver cirrhosis is responsible for around 170,000 deaths in Europe annually. The substantial burden of liver disease-associated mortality in Europe means that governments and health-care providers must tackle liver disease in a much more proactive fashion. This should include placing liver disease higher in public health priority, implementing prevention programmes, using non-invasive tests to screen for early stage disease, and finally supporting the research needed to develop novel treatments.

Q: You supervise many postgraduate research students – how do you feel about this aspect of your work?

A: Supervising postgraduate research students is a key part of my work where I clearly see my contribution to promoting and exchanging liver research among young investigators. This has been a truly rewarding experience.

Q: Is there any advice that you would give to aspiring students thinking about specialising in hepatology?

A: I would openly advise aspiring students to specialise in the challenging area of hepatology, to be passionate about the field, creative, and honest - in summary, be good at what you do and you will succeed!

Q: What is the scientific achievement that you are most proud of?

A: My laboratory has contributed to the evidence for a role of cell death including apoptosis in promotion of liver disease in experimental animal models and in patients, which has provided the basis to therapeutically target cell death in liver disease. We have developed bile acids as a group of promising endogenous modulators of cell death and survival, including in organs other than the liver, protected by global coverage patents and licensed by spin-off pharmaceuticals.

“Liver diseases need to be proactively tackled...”

Q: How important is the annual European Association for the Study of the Liver (EASL) congress for helping hepatologists to combat liver disease?

A: The International Liver Congress by EASL is the main educational event in liver disease to promote and exchange the best quality science in liver disease prevention, management, and treatment. It is also a unique occasion for basic scientists to network and present their latest findings in the pathophysiology of acute and chronic liver disease and the development of new therapeutic approaches and technology breakthroughs.

Q: What is the most important advice that can be given to people in order to help them maintain a healthy liver?

A: Liver disease is a very important cause of chronic disease but also death. Fighting liver disease will require education about the importance of liver health for general wellbeing, including the promotion of active and healthy living.

Helen Reeves

Senior Lecturer and Honorary Consultant Gastroenterologist, Northern Institute for Cancer Research, The Medical School, Newcastle University and the Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne; Fellow of the Royal College of Physicians, London, UK; Scientific committee member of the European Association for the Study of the Liver.

Q: How did your time at university influence your choice to specialise in hepatology?

A: Clinical exposure was introduced very early on at Nottingham Medical School, which I loved. Probably it was the people rather than the subject that were more important initially. These included Prof Chris Hawkey during my pre-clinical years. In my senior clinical attachment, Dr Richard Long was very influential. Both were gastroenterologists. Managing gastrointestinal haemorrhage and liver failure were what fascinated me the most at that stage. I chose my house jobs so I that could do more gastroenterology, and my training rotation so that I could do hepatology.

Q: Which areas of hepatology did you begin your career in?

A: This was general hepatology on the liver unit at the Freeman Hospital, before the transplant unit there existed. Prof Oliver James was my key mentor. My first research project was helping to run a clinical trial giving patients with cirrhosis s-adenosylmethionine. The compound didn't work, unfortunately, but I learnt a lot about clinical trials during that time and also began working on immunohistochemistry of fatty liver disease with Prof Alastair Burt. His interest was liver fibrosis and I spent hours and hours counting activated hepatic stellate cells and quantifying fibrosis with an early computer-based electronic algorithm. I published a paper on that and also learned how to isolate hepatic stellate cells from rats in Loranne Agius' laboratory in Newcastle. I won a Wellcome Trust clinical training fellowship around about that time, and began my PhD. This was to study cell signalling cascades involved in hepatic stellate activation. That was supervised by Prof Alastair Burt and Prof Loranne Agius, as well as Prof Chris Day.

Q: How did you meet Prof Oliver James and how big an influence did he have in your career decision making?

A: I met Oliver when I started my first Senior House Officer (SHO) job on the liver unit. On a post-take ward round I introduced him to my house mate, Russell Roberts. He was also an SHO on the rotation, but was working on the renal ward. He had been on call with me the night before. Because he had nothing to do, I had persuaded him to clerk some of the medical admissions for me as I was so busy. Oliver thought that it was marvellous that I should get someone else to do my work for me. A bit random, admittedly, but he remembered me and always supported me after that. He introduced me to Prof Alastair Burt and Prof Chris Day, setting me off on my PhD studies. Years later, when I failed to get a travelling fellowship to go to the

United States as a postdoctoral scientist, and was at the point of marrying my partner at that time and accepting a consultant job in one of our big peripheral hospitals, he offered me a second chance. He gave me one year's funding to go to work with Dr Scott Friedman at Mount Sinai, on the understanding that I would have to get the rest of the money myself if I wanted to stay on longer. So off I went. I will always thank him for believing in me at a time when I doubted myself. No-one before or after has ever had the same influence on my decision making.

Q: What aspects of American life did you enjoy when you worked at Mount Sinai Medical School in New York?

A: I loved the people I worked with in the lab. I met some of the most intelligent and stimulating people I have ever known. We lived and breathed science. I was in the lab by 8 am and there until 9 or 10 pm most nights. We published a paper in the journal *Science*, as well as other journals. I won an American Association for Study of Liver Diseases fellowship so I could stay on. The city was always alive, so there was always somewhere to go when we left the lab. It would not have been the same if I had been there with a partner or a family. While I would not want to live like that now, it was three of the best years of my life. I was also at work at 8 am on the 11th floor of Mount Sinai on 11th September 2001, when the planes struck the World Trade Center. We watched the smoke fill the skyline from the top of our building, about 80 blocks north, unable to contact people we knew because of electronic blackout and total communication failure. We waited for people to arrive at work who had escaped the underground and walked the rest of the way. Nothing was the same after 11th September, as it had a deep emotional impact on all the inhabitants of the city. But certainly the times before and afterwards were equally memorable.

Q: Do you see a big contrast between the ways in which liver cancer (LC) is handled in the USA compared with Europe?

A: Not when I was in America, no. I did no clinical work at all when I was a postdoctoral scientist. And I went to America to work on liver fibrosis. My focus was changed by the science when I was there, rather than the clinical experience. I had to learn the clinical stuff when I got home! Prof Oliver James and Prof Chris Day dispatched me to the experienced hands of Dr Jordi Bruix for 2 weeks, as an observer at the Barcelona Clinic for Liver Cancer, for a crash course in 2004. For that I will always be extremely grateful to Dr Bruix. Ten years down the line, having done little else but look after patients with LC, I think I know pretty well what I am doing. My impression now is that there are centres of excellence in the USA, but that overall, treatment in Europe is evidence based, guideline driven, and more often available to all of those affected.

Q: What is your opinion of the way in which healthcare is provided in the USA, for example, the lack of an equivalent to the NHS?

A: Suffice it to say I am a great supporter of the NHS.

Q: Why did you decide to specialise in hepatocellular cancer?

A: As above really – I always loved hepatology as my clinical specialty. The molecular pathogenesis of liver disease and cancer fascinated me and I was excited by the prospect of spending my working life exploring its biology and translating science to the clinic.

Q: What impact do you hope your research will have on liver-related conditions?

A: I want to deliver novel targeted therapies to patients with LC. I want these to be safe drugs that we can use in patients whose cancer has affected their liver function.

Q: In what way has your work with organisations such as the Northern Institute for Cancer Research inspired you in your work and research?

A: I work with the Cancer Research UK Drug Discovery team, including chemists and biologists, in Newcastle. Their approach to cancer has a different perspective, helping to focus biology on identification of good therapeutic candidates, as well as their validation at multiple levels.

Q: Are there any new areas of research that you feel you would like to move into in the future?

"I want to deliver novel targeted therapies to patients with LC."

A: I believe we will use circulating tumour cells and cells of the immune system as targets and biomarkers for treatment stratification and monitoring.

Q: Do you enjoy working in Newcastle?

A: I love Newcastle. I am married now, with a husband I adore and a little girl who is seven-and-a-half years old and as 'Geordie' as they come - 'Posh Geordie' maybe. I live in Jesmond, between the hospital and the university. I can be wherever I need to be in 10-15 minutes. My clinical colleagues are great and my academic life is a passion that keeps me occupied and extremely fulfilled.

Q: When did you realise that you wanted to teach?

A: I did not. It came with the research job, but actually inspiring students is extremely rewarding. It is a very important part of my job.

Q: Do you see yourself working abroad in the near future?

A: Probably not. Newcastle, the people of the North East, as well as the quality of my home and work life, would be very hard to beat.

Q: A recent forecast predicts that one in two people in the UK will develop cancer at some point in their lives. What has been the trend in LC incidence in recent years, and does this follow the overall cancer trend?

A: Mortality from most other cancers is falling. For LC, mortality has doubled in the last 10 years and continues to rise.

Q: What are the underlying mechanisms responsible for the slowly emerging nature of distinct LC symptoms?

A: The liver is very large with a unique capacity to regenerate and heal in the face of chronic injury. It probably takes 20 years to develop cirrhosis and another 10-20 years to develop a cancer. To become an advanced cancer, there are an estimated 40-60 mutations. The cancer arises from a regenerative nodule that becomes a dysplastic nodule, then a more dysplastic nodule, and then an early cancer, etc. Each stage of progression is associated with more genetic damage. For a LC to be symptomatic, it has to be big enough to stretch the liver capsule and cause pain, or impact liver function.

Q: Would you recommend chemotherapy to treat primary LC?

A: No. There is no evidence that it does any good. Any treatment that makes liver function worse will shorten a person's life rather than prolong it, even if it shrinks the cancer a bit. We have a medical therapy called sorafenib which is well tolerated and can prolong life in those whose disease is too advanced to be treated by other means.

Q: Is a significantly greater financial contribution from governments worldwide required for scientists to achieve a higher level of understanding of the pathology of LC, and how effective is the current lobbying for this funding?

A: Governments do invest a significant amount of money in research, even at this economically difficult time. It is up to our liver community to unite more effectively and persuade/win a greater amount of the funding available for all research into LC. Lobbying is not something most doctors are particularly good at. However, recognising its importance and enlisting the help of those who are good at it is a start. That is what the European Association for the Study of the Liver (EASL) has tried to do, having recently appointed Ms Fiona Godfrey as Director of European Public Affairs at EASL.

Q: With referrals to the Hepatopancreatobiliary Centre in Newcastle NHS Trust increasing 10-fold in the last decade, would you say that the service has adapted well to this increase and what trend do you expect to see in the next few years? Has the service adapted?

A: Not yet! Resource is limited and we manage the increasing numbers of patients by improving

efficiency and trying to communicate effectively. But since we published our data on the increasing numbers of referrals, they have increased further – to nearly 200 patients per year. It is too much work now to manage with the team we have. Newcastle plans to invest in the LC service however, so I am sure both our scientific advances and clinical impact will continue to improve.

Marina Berenguer

Professor-Consultant Hepatology, Faculty of Medicine, Department of Digestive Diseases, Hepatology and Liver Transplantation Unit, La Fe University Hospital; Coordinator, CIBER-ehd, Valencia, Spain.

Q: Has there been a dramatic increase in the prevalence of hepatitis C virus (HCV) infection in recent years? Once diagnosed, what are the major sequelae that can arise from this disease?

A: No, there has not been a dramatic increase in the prevalence of HCV infection in recent years. What we have seen is an increase in the major long-term consequences of chronic (HCV) infection, especially cases of decompensated cirrhosis and hepatocellular carcinoma.

Q: People who inject drugs have, in the past, been labelled as a group in which the prevalence of HCV infection is highest. Has this changed or is this still true today?

A: The prevalence of HCV infection is higher in certain 'at-risk populations', such as intravenous drug users. However, this number is highly dependent on the country, and when changes regarding safer drug injection practices were put in place.

Q: Many individuals remain unaware that they are infected with HCV because the symptoms are so common, what are the long-term health implications of this?

A: Most patients infected with HCV are unaware of their infection because chronic infection is seldom associated with clinical symptoms until severe sequelae develop, such as advanced cirrhosis or hepatocellular carcinoma. Before these final stages of disease, people are usually diagnosed following a regular blood test requested for other reasons.

"Most patients infected with HCV are unaware..."

Q: Do you consider the new all-oral direct-acting antivirals, upon which the subject of your presentation for ILC 2015 is based, to be your preferred method of treating recurrent HCV infection after liver transplantation? If not, which method do you prefer to use in your practice?

A: An all-oral direct-acting antiviral combination is the most effective and safest way to treat HCV infection after liver transplantation, regardless of the timing of treatment or severity of graft disease.

Q: What proportion of liver transplants that you see in your daily practice are treated successfully without the recurrence of HCV infection? In addition, with Europe facing a shortage of organ donors, what, in your opinion, can be done to encourage individuals to donate their livers?

A: Patients in the transplant setting can be treated either before or after liver transplantation with new oral antiviral agents that are highly effective and safe. Most physicians treat the patients before liver transplantation in order to prevent recurrence of HCV infection after liver transplantation. In some cases, treatment is not feasible before transplantation because the patient is too sick, and therefore post-transplantation therapy is attempted with very good results obtained in clinical trials and in our daily clinical practice.

There are several ways to encourage organ donation, but one of the few strategies that has been shown to be particularly useful is the Spanish model of organ donation whereby 'Transplant Coordinators', generally critical-care or emergency-room doctors, are trained and localised in all healthcare centres that have an intensive care unit.

Q: Would you like to see the organ donor system become an 'opt-out' system in all countries?

A: The opt-out system, whereby presumed consent is present within a country, is not a fundamental pillar to achieving higher numbers of donations. Indeed, countries that have tried to apply a 'hard' opt-out system have failed to show that it is associated with an increase in donations. A 'soft' opt-out system, such as that put in place in Spain (where families have always had the last word), has been shown to be much more effective because people confide in the system, and, at the same time, the presumed consent is useful in certain situations, such as in donation after circulatory death, Type II or III donation, or in cases with an absence of family or last will from the individual.

Q: In order to prevent the spread of HCV, what kind of measures need to be taken?

A: Prevention of HCV spread starts with the detection of undiagnosed cases and treatment of those diagnosed. Treatment can now be achieved in most patients with the use of new oral antivirals. Detection of undiagnosed cases is still something that each country needs to work on, particularly in deciding whether to screen all of the population, only those with a history of risk factors, or those belonging to high-prevalence groups.

Q: Are there any differences between the prevalence, treatment, and survival rates of those who suffer from HCV infection in developed and developing countries?

A: Currently, with the advent of highly effective but very expensive anti-HCV drugs, the treatment of HCV infection differs substantially between developed and developing countries, but, even in developed countries, patients with a lack of insurance cannot access these drugs.

Q: What was it that inspired you to pursue a career in the field of hepatology? Which parts of your training did you enjoy the most?

A: My father was a hepatologist and an inspiration to me during my initial training. Further down the line I was lucky to meet other great hepatologists, such as Prof Teresa Wright and Dr Norah Terrault in San Francisco, who still continue to inspire me. Within hepatology, I was absolutely amazed from my initial years in the hospital about how liver transplantation could completely change the outcome of severely ill liver patients. This is the part that I most enjoyed in the first years and the reason that I took the path to becoming a hepatologist focusing on liver transplantation.

Q: For young and aspiring hepatologists, what advice would you give to prepare them for a career in the field and in medicine in general?

A: The two most important messages would be, firstly, to truly believe in what they do and believe that 'yes – I can make a difference', and secondly, to look out for 'good' mentors, not only from a scientific point of view, but also from a human point of view.

Q: What will be the next stage of your research on HCV?

A: My current interest is to establish the best HCV-screening strategy that should be applied in Spain. Furthermore, establishing the 'point-of-no-return' at which, despite sustained clearance of HCV infection, disease severity is not changed and patient outcome is not improved, which is also an important aspect of research.

Q: Was there any particular message or theme that ILC emphasised this year? What piqued your interest at ILC 2015?

A: This year was, like last year, mostly devoted to anti-HCV therapy, particularly the efficacy, tolerability, and impact of these drugs in certain classical 'difficult-to-treat' and 'difficult-to-cure' populations, such as patients with advanced decompensated cirrhosis or those with renal insufficiency.

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TS-102

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Main Inclusion Criteria	Progression with first-line chemotherapy (oxaliplatin- or irinotecan-based regimen)
Arms	Second-line chemotherapy +/– TheraSphere™
Primary Endpoint	Progression-free survival

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STOP-HCC

TS-103

Evaluating yttrium-90 trans-arterial radioembolization (TheraSphere™) in the treatment of patients with unresectable hepatocellular carcinoma (HCC)

Main Inclusion Criteria	Unilobar or bilobar disease, Child-Pugh score ≤7, eligible to receive sorafenib
Arms	Sorafenib +/– TheraSphere™
Primary Endpoint	Overall survival

www.TheraSphereTrials.com/STOP

www.clinicaltrials.gov (NCT01556490)

YES-P

TS-104

Evaluating yttrium-90 trans-arterial radioembolization (TheraSphere™) versus the standard of care (sorafenib) for the treatment of advanced HCC with portal vein thrombosis (PVT)

Main Inclusion Criteria	Branch PVT without extrahepatic metastases, unilobar disease, Child-Pugh A
Arms	TheraSphere™ vs sorafenib
Primary Endpoint	Overall survival

www.TheraSphereTrials.com/YES

www.clinicaltrials.gov (NCT01887717)

BTG

CONQUERING C – GOING BEYOND CURE

Summary of presentations from the Gilead Satellite Symposium, held at the International Liver Congress™ 2015, the 50th Annual Meeting of the European Association for the Study of the Liver, Vienna, Austria, 24th April 2015

Chairperson

Stefan Zeuzem[1]

Speakers

Nezam H. Afdhal,[2] Marc Bourlière,[3] Graham Foster,[4] Charles Gore,[5] Jean-Michel Pawlotsky[6]

1. J.W. Goethe University Hospital, Frankfurt, Germany
2. Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA
3. Hôpital Saint Joseph, Marseille, France
4. Queen Mary University of London, London, UK
5. World Hepatitis Alliance, Geneva, Switzerland
6. National Reference Center for Viral Hepatitis B, C and Delta, Hôpital Henri Mondor, Université Paris-Est, Créteil, France

Disclosure: All the authors have been speakers for Gilead Sciences Europe Ltd. Stefan Zeuzem has provided consultancy and participated in speakers' bureaus for Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen, and Merck. Nezam H. Afdhal has received research support for hepatitis C virus studies from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Merck, and Vertex Pharmaceuticals; has been a consultant and/or has participated as an advisory board member for AbbVie, Achillion Pharmaceuticals, Echosens, Gilead Sciences, GlaxoSmithKline, Janssen, Kadmon Corporation, Ligand, Merck, Springbank Pharmaceuticals, and Sandhill Scientific; holds stock or stock options with Springbank; and is currently the editor of the *Journal of Viral Hepatitis*. Marc Bourlière has consulted and advised for Bristol-Myers Squibb, Merck, Roche, AbbVie, Gilead, Janssen, and MSD, and also advised for Vertex. Graham Foster has received funding from AbbVie, Achillion Pharmaceuticals, Alnylam Pharmaceuticals, Bristol-Myers Squibb, Boehringer Ingelheim, Gilead Sciences, Merck, Novartis, Roche, Spring Bank Pharmaceuticals, and Tekmira Pharmaceuticals. Charles Gore is currently the President of the World Hepatitis Alliance in Switzerland. Jean-Michel Pawlotsky has received research grants from Gilead Sciences and served as a scientific advisor for Abbvie, Achillion, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Idenix, Janssen, Merck, Novartis, and Roche.
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MEETING SUMMARY

Prof Zeuzem opened the symposium by acknowledging that there is a new era in hepatitis C virus (HCV) treatment, due to the availability of efficacious treatments that could eradicate the disease. Prof Pawlotsky outlined recent advances in the field of HCV and discussed the European Association for the Study of the Liver (EASL) Recommendations on Treatment of Hepatitis C 2015, which were released at the congress. These recommendations prioritise the available HCV treatments in Europe, from treatment-naïve to treatment-experienced patients and in the context of patients with various stages of HCV disease, and highlight the need to remain vigilant for possible drug–drug interactions (DDIs) between HCV direct-acting antiviral agent (DAA) treatments and regular pharmaceutical medications. Dr Bourlière then described the remaining challenges in HCV relating to treatment of certain patient populations, such as those with advanced disease and specific contraindications. Prof Foster presented the real-life challenges of treating

a patient population that can have heterogeneous characteristics and presented the recent outcomes of nationally implemented programmes for HCV. Mr Charles Gore, a patient advocate, described the World Health Organization (WHO) policies in HCV and highlighted that government lobbying by physicians and patients was required to improve awareness and prioritise HCV treatment. Prof Afdhal then summarised the current impact of HCV on productiveness and patient outcomes, and spoke about the benefits of patient access programmes in expanding the pool of patients who can be treated along with the cost implications of the global eradication of HCV. Finally, Prof Zeuzem emphasised how HCV is currently perceived as a lower global priority compared with other viral diseases and that lobbying will be required to demonstrate how investments into the treatment of HCV patients would dramatically reduce the prevalence and long-term costs of the disease.

Conquering C – Looking Beyond Cure

Professor Stefan Zeuzem

There is a need to treat HCV-infected adults due to the increased risk of premature death[1] and curability of the chronic viral disease.[2-6] The efficaciousness of DAAs on mortality, morbidity, and sustained virological response (SVR) rates >90% have been demonstrated in recent clinical studies.[2,5-7]

Real-world data have also demonstrated the effectiveness of such treatments and their successful transition from a trial to a clinical setting.[8] However, the translation of SVR to long-term outcomes and eradication of the disease may present some challenges. Patients with advanced stages of HCV infection can be treated successfully; however, long-term surveillance is still required for hepatocellular carcinoma (HCC).

Although new treatments for patients with HCV have ushered in a new era where the disease can be eradicated, this is dependent on certain aspects such as treatment access, policy changes, and patient factors that include existing disease status.

Conquering C – Solutions For All Patient Types

Professor Jean-Michel Pawlotsky

The goal of therapy is to cure HCV infection, preventing complications including compensated or decompensated cirrhosis, HCC, severe extra-hepatic manifestations, and death. The EASL Recommendations on Treatment of Hepatitis C 2015 defined the HCV therapy endpoint as SVR with undetectable HCV RNA (≤15 IU/ml), 12 or 24 weeks after the end of treatment.[9] The simple life cycle of HCV has resulted in effective treatments that are well tolerated and can be grouped into four classes: protease inhibitors that inhibit polyprotein processing (i.e. the maturation of viral proteins), nucleotide analogues and non-nucleoside inhibitors of the HCV RNA-dependent RNA polymerase that affect HCV replication, and non-structural protein 5A (NS5A) inhibitors that both indirectly inhibit viral replication and block the assembly and release of the virus.[10]

Efficacious treatments are available and the EASL Recommendations on Treatment of Hepatitis C 2015 now highlight the need to prioritise specific groups of patients, due to the current cost of certain medications and the large number of individuals with an indication for HCV therapy (Table 1).[9] The EASL recommendations describe treatments for patients with a high severity of HCV disease, clinically significant extra-hepatic manifestations, or debilitating fatigue. Patients with specific risk factors should also be prioritised treatment for HCV, including HIV or hepatitis B virus (HBV) co-infection and those at higher risk of transmitting HCV (people who inject drugs, men who have sex with men with high-risk sexual practices, and prisoners).

Recently approved DAAs in the EU include sofosbuvir (SOF), a nucleotide analogue that is active against all genotypes (GT), simeprevir (SIM), a protease inhibitor against GT1 and 4,[11-13] and daclatasvir (DCV), a pan-genotypic inhibitor approved for GT1, 3, 4, 5, and 6.[11] A fixed-dose combination of ledipasvir (LDV)/SOF active against HCV GT1 and 4 has been approved by the European Medicines Agency, while the 2015 EASL recommendations also advise the use of LDV/SOF for GT5 and 6.[9,14] A combination of ombitasvir (OMB)/paritaprevir (PTV)/ritonavir (RIT) should be prescribed for GT1 and 4, which can be combined with dasabuvir (DSV) for GT1 patients.[14-16] A variety of recommended treatment options according to HCV GT are shown in Figure 1.[9]

Table 1: Treatment prioritisation of patients with hepatitis C virus according to recommendations from the European Association for the Study of the Liver.

Treatment priority	Patient group
Treatment should be prioritised	- Patients with significant fibrosis (F3) or cirrhosis (F4), including decompensated cirrhosis - Patients with HIV co-infection - Patients with HBV co-infection - Patients with an indication for liver transplantation - Patients with HCV recurrence after liver transplantation - Patients with clinically significant extra-hepatic manifestations - Patients with debilitating fatigue - Individuals at risk of transmitting HCV
Treatment is justified	- Patients with moderate fibrosis (F2)
Treatment can be deferred	- Patients with no or mild disease (F0-F1) and none of the above-mentioned extra-hepatic manifestations
Treatment is not recommended	- Patients with limited life expectancy due to non-liver-related comorbidities

From Jean-Michel Pawlotsky, presentation at the Gilead Satellite Symposium, held at the International Liver Congress (ILC), Vienna, Austria, 24th April 2015.
HBV: hepatitis B virus; HCV: hepatitis C virus.

IFN-free regimens	HCV genotype
Sofosbuvir + RBV	2, 3
Ledipasvir/sofosbuvir (±RBV)	1, 4, 5, 6
Ombitasvir/paritaprevir/ritonavir + dasabuvir (±RBV)	1
Sofosbuvir + dimeprevir (±RBV)	1, 4
Sofosbuvir + daclatasvir (±RBV)	All
Ombitasvir/paritaprevir/ritonavir (±RBV)	4
IFN-containing regimes	
PEG-IFNα + RBV + sofobuvir	All
PEG-IFNα + RBV + simeprevir	1, 4

Figure 1: Treatment options for patients with hepatitis C virus (HCV), according to recommendations from the European Association for the Study of the Liver.[9]
From Jean-Michel Pawlotsky, presentation at the Gilead Satellite Symposium, held at the International Liver Congress (ILC), Vienna, Austria, 24th April 2015.
IFN: interferon; PEG-IFNα: pegylated interferon alpha; RBV: ribavirin.

Treatment recommendations are provided as numbered options to address the needs of all patient types, with various criteria to inform the selection of each specific DAA regimen, such as HCV GT (including GT subtype for some options), severity of liver disease, patient comorbidities, the DAA pharmacokinetics profile, DDIs, and the patient's prior treatment experience. For the interferon (IFN)-free fixed-dose combination of LDV/SOF with or without ribavirin (RBV), treatment recommendations for patients with GT1 apply across a broad range of patient characteristics,[9] including non-cirrhotic and certain cirrhotic patients,[17,18] patients with compensated cirrhosis who are treatment-experienced or treatment-naïve,[19,20] and those who are HIV–HCV coinfected. OMB/PTV/RIT+DSV with or without RBV can also be used for patients with GT1 (subtypes 1b and

1a) both with and without cirrhosis, with studies showing SVR rates of >90% in patients who had GT1, cirrhosis, and were either treatment-experienced or treatment-naïve.[20] IFN-free regimens can also be used in HIV–HCV coinfected patients as per HCV monoinfected patients, as described by Osinusi et al.[21]

Patients with compensated cirrhosis and who had failed prior treatments were treated with LDV/SOF+RBV and demonstrated high SVR rates >95%,[19] as well as those with GT1 and decompensated cirrhosis (SVR rates >85%).[22,23] Post-transplantation patients with a fibrosis score between F0–3 or with Child-Turcotte-Pugh (CTP) Stage A, and HCV recurrence, were given LDV/SOF+RBV and showed an SVR rate of 96% after 12 weeks of treatment.[22,24] SVR rates were reduced in patients with CTP Stages B and C who were prescribed the treatment regimen of LDV/SOF+RBV. Treatment-naïve patients with GT4 displayed an SVR rate of 95% in a recent Phase II trial when prescribed LDV/SOF,[25] while patient populations with GT4 who were treatment-naïve or treatment-experienced and had not shown any cirrhosis presented with 100% SVR after 24 weeks of treatment with OMB/PTV/RIT.[26,27]

Although the efficacy of some treatment regimens has been established in certain patient populations, remaining treatment challenges include options for patients with severe chronic kidney disease or end-stage renal disease.[9] DDIs can also present difficulties when treating certain patients taking prescribed and/or over-the-counter medications, therefore guidance from EASL and drug interaction charts from the website of the Department of Pharmacology, University of Liverpool[28] may assist in the determination of an optimal treatment regimen. However, physicians should remain vigilant for any adverse events that may result from certain treatment combinations. Furthermore, recommendations for patients who have failed an IFN-free regimen are based upon indirect evidence, and real-life studies will again be useful for the confirmation of efficacious treatment strategies. Current re-treatment regimens should contain SOF and RBV along with one or two other DAAs for a duration of 12 or 24 weeks.[9]

In summary, IFN-free therapies have provided physicians with a curative and tolerable toolbox with which to treat patients with HCV. Remaining challenges include how to implement treatment strategies in the most optimal, effective, and cost-effective way as well as how to treat certain patient populations, such as those who have failed IFN-free regimens.

Conquering C – Solutions For Advanced Disease

Doctor Marc Bourlière

The translation of SVR improvements into a curative treatment for patients requires consideration of several factors, which include the stage of fibrosis. HCV accounts for one-in-four cases of cirrhosis or HCC in the global population, rising to 90% in certain high-incidence populations such as in Egypt or Japan.[29] A US-based study found that compared to matched patients without HCV, mean Fibrosis-4 scores doubled during the first 4 years after HCV infection.[30] Subsequently, 18% of this population developed cirrhosis within 10 years of having HCV, highlighting the need for early treatment. It has recently been established that achieving an SVR is associated with significantly decreased risk of mortality, and reduced risks of HCC and requirement of liver transplant (Figure 2),[31] and studies have shown that the risk of disease progression is also linked to fibrosis stage.[32] Achieving SVR is therefore not sufficient to prevent HCC in patients who are already cirrhotic; ongoing monitoring is then required.

As described above, recently available DAA combinations have enabled the treatment of patients who are compensated cirrhotic and also in some decompensated patients. The treatment regimen of OMB/PTV/RIT+DSV and RBV showed an SVR of 89–100% in patients with GT1 who were compensated cirrhotic,[15] and the regimen is also recommended for cirrhotic patients who have GT1a or 1b.[15,16,20,33,34]

A post-hoc analysis of data from seven clinical trials has shown that laboratory parameters improve along with SVR for a treatment regimen of LDV/SOF that was prescribed with or without RBV to patients who were treatment-naïve or treatment-experienced and with compensated cirrhosis.[19] A similar safety profile was reported in non-cirrhotic patients. Albumin, bilirubin, alanine aminotransferase levels, the international normalised ratio, and platelet counts significantly improved along with SVR in these patients, indicating early benefits for the compensated cirrhotic patients. A correlation of improved laboratory parameters

with SVR was also shown in cirrhotic GT1 patients who had previously failed protease inhibitor triple therapy and were treated with LDV/SOF, with or without RBV.[35] The SOLAR-1 study also demonstrated improved rates of SVR (>85%) with decompensated CTP B and C patients who were receiving LDV/SOF+RBV, with an improved Model for End-Stage Liver Disease (MELD) score after 12 and 24 weeks.[23] Improvements in SVR, laboratory parameters, and MELD score were observed in post-transplantation patients using the same treatment regimen after 12 and 24 weeks.[24] Results were similar in the SOLAR-2 study of pre and post-transplantation patients where a high rate of SVR was achieved,[36] demonstrating the immediate benefits of treating patients with severe HCV disease.

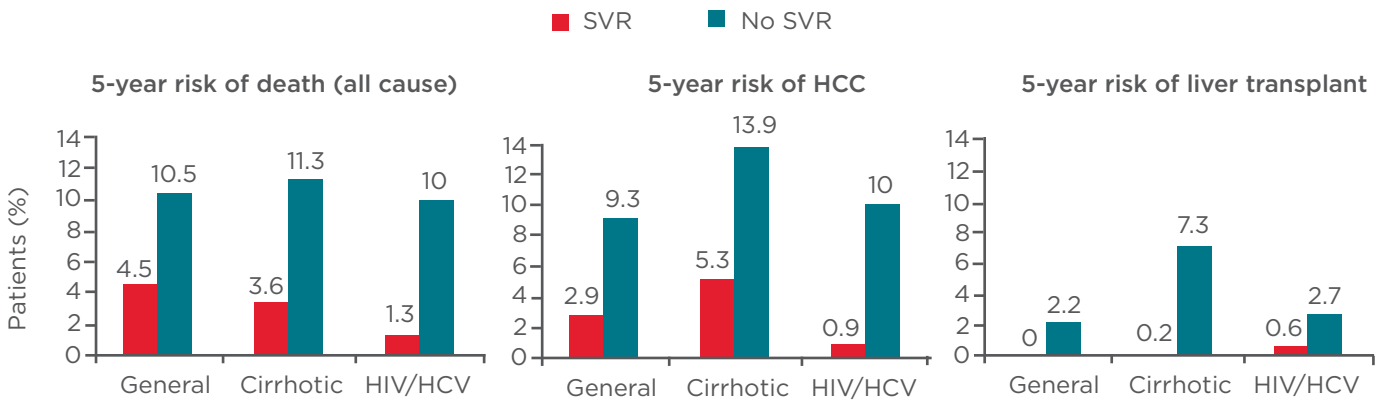
Moreover, in the SOLAR-2 trial, 48% of the patients who had been initially classed as CTP C at baseline were reduced to Class B and 5% to Class A at Week 4 of follow-up.[36] Furthermore, of the patients in CTP Class B at baseline, 35% were CTP A by the end of treatment. This beneficial effect of treatment on the CTP class has also been reported for the regimen of DCV with either SOF or SIM, both with and without RBV. Results in GT1 post-transplantation patients showed significant improvements in MELD and CTP scores as well as the stabilisation of laboratory and clinical status.[37]

In conclusion, treating patients as early as possible to ensure that cirrhosis does not occur and selecting treatments with an optimal efficacy for a particular patient profile should result in the ideal outcome. Although there have been positive outcomes and improved liver function in patients with a higher severity of HCV disease, the ideal situation would be to treat patients as soon as possible.[38]

Conquering C – Solutions For Real Life

Professor Graham Foster

Although the recent EASL recommendations serve as an invaluable user guide for HCV and advocate the use of IFN-free therapies, there can be some complications with implementing the guidance in routine clinical practice.[9] While it is agreed that patients should be treated as early as possible, treatment access may not allow for therapies to be administered until the disease has reached a later stage. However, patient factors that include comorbidities, diabetes, obesity, and alcohol problems can also contribute to the exacerbation of HCV disease, and clinical advice can therefore contribute towards lessening these issues.



- Achieving SVR was associated with:
  - 62-84% reduction in all-cause mortality
  - 68-79% reduction in risk of HCC
  - 90% reduction in risk of liver transplant

Figure 2: Sustained virological response is associated with reduced mortality, hepatocellular carcinoma, and liver transplant, as reported by a meta-analysis of 129 studies of IFN-based therapy in 34,563 patients with hepatitis C virus.[31]

From Marc Bourlière, presentation at the Gilead Satellite Symposium, held at International Liver Congress (ILC), Vienna, Austria, 24th April 2015.

IFN: interferon; SVR: sustained virological response; HCC: hepatocellular carcinoma; HCV: hepatitis C virus.

Table 2: A summary of the possible drug–drug interactions that can result from certain treatment combinations.[11–16,28,75–78]

	Victim of DDI	Perpetrator of DDI	DDI potential
Telaprevir	Substrate for CYP 3A4, P-gp	Inhibits CYP 3A4, P-gp & OATP 1B1/2	High
Boceprevir	Substrate for aldoketoreductase, CYP 3A4, P-gp, BCRP	Inhibits CYP 3A4 & P-gp	High
Ombitasvir, paritaprevir, dasabuvir, ritonavir	Inhibits CYP 3A4; substrate for CYP 3A4, CYP 2C8, OATP 1B1/3, P-gp & BCRP enzyme inducer	Inhibits CYP 3A4, OATP 1B1/3, OATP 2B1, OCT 1, BCRP, P-gp, UGT 1A1, CYP 2C19	High
Simeprevir	Substrate for CYP 3A4, P-gp & OATP 1B1	Inhibits OATP 1B1 & P-gp; mild inhibitor of CYP 1A2 & gut CYP 3A4	Moderate
Daclatasvir	Substrate for CYP 3A4 & P-gp	Inhibits OATP 1B1, OCT 1, P-gp & BCRP	Moderate
Ledipasvir/sofosbuvir	Substrate for P-gp & BCRP	Inhibits P-gp, BCRP, gut CYP 3A4 & UGT 1A1; induces CYP 3A4 & UGT 1A1	Low
Sofosbuvir	Substrate for P-gp & BCRP (affects prodrug but not active metabolite)		Low

From Graham Foster, presentation at the Gilead Satellite Symposium, held at the International Liver Congress (ILC), Vienna, Austria, 24th April 2015.
BCRP: breast cancer resistance protein; CYP: cytochrome P; OATP: organic anion transporter polypeptide; OCT: organic cation transporter; P-gp: P-glycoprotein; UGT: uridine 5'-diphospho-glucuronosyltransferase; DDI: drug–drug interaction.

To facilitate patient outcomes further, treatment regimens can be adapted around the lifestyle of the patient to improve adherence, with consideration given to possible DDIs (Table 2).[28] Patient drug histories are very pertinent and over-the-counter medications such as St John's Wort should be assessed, alongside any existing prescription medications. The reduction in complexity and duration of newly available oral therapies compared with previous therapies may also assist with patient adherence,[39] as well as extending the range of patients who can be treated to include those with a milder form of HCV if authorised by the healthcare provider.[38]

Although there is a larger selection of efficacious treatments available that enable a greater proportion of patients to be treated, the order of prioritisation of these patients still requires agreement. The uptake of DAA regimens during 2014–2015 has varied between countries, with physicians in the USA switching to SOF+SIM or SOF+RBV as soon as possible.[40] SVR rates in an observational study by the Hepatitis C Therapeutic Registry and Research Network (HCV-TARGET) were >80% after the 12-week treatment regimens in patients with GT1 and who included cirrhotics and non-cirrhotics, with some patients having previously experienced decompensation or treatment failures. SVR rates in GT2 patients were 90% after 16-week regimens.[41] Another benefit of the DAA treatments in a real-world setting is the low discontinuation rates observed (<4%).

Overall, there is a trend of fewer IFN-based therapies and an uptake of DAA in both cirrhotic and non-cirrhotic patients.[40] Germany and France have also shown increased treatment rates compared with the UK, with 16 and 12 patients treated per 100 prevalent cases compared with 3 patients, respectively.[42,43] Treatment strategy across the UK has centred on treating the most urgent patients, including those on a transplant waiting list and/or with CPT B and C.[44] Interestingly, high percentages of SVR (>80%) have been shown in a cohort of real-life patients with GT1 who were

treated with LDV/SOF with or without RBV, as well as those treated with SOF+DCV with RBV. Although the SVR was lower in patients with GT3 at around 60–70% after treatment, other patients who were predominantly GT2 and 4 demonstrated SVR rates ≥85%.[44]

Real-life patient cohorts also demonstrated improvements in MELD after 60 weeks of therapy, as well as the removal of some patients from the transplant waiting list and patients with ascites having no symptoms after no detection of the HCV. The treatment of patients with SOF plus RBV ± peginterferon alpha is recommended for certain patients across all GTs with chronic HCV in the UK, while recommendations for SIM plus RBV ± peginterferon are provided for patients with GT1 and 4.[45] Although there have been promising results from national patient cohorts, the prioritisation of patients with HCV for treatment after those with a high severity of disease requires agreement.[46] It has been suggested that patients who show a high risk of disease transmission should be targeted after those of a higher severity, in order to contain the epidemic of HCV. The final patient cohort treated with the new oral therapies would be those with milder disease states.

In summary, current recommendations have been very useful in guiding treatment decisions with oral therapies and matching patients to the optimal therapies according to their lifestyle, GT, simplicity, and treatment access. However, the next challenge in the area of HCV will be to decide which patient groups would receive the greatest benefit from oral therapies, for which real-world clinical data will be important.

Conquering C – Solutions From the Patient's Perspective

Mister Charles Gore

One of the challenges of making efficacious treatments available for people living with HCV is the limited budget allocation for viral hepatitis by governments, compared with other infectious diseases such as HIV. This is despite the greater mortality from viral hepatitis, as reported in 2013.[47,48] Furthermore, few countries have national hepatitis strategies and there is an uncertain political will that seems to be linked to the associated stigma, resulting in a major impediment to a strong advocacy movement.

However, there is a global drive to improve hepatitis treatment. In 2014, the World Health Assembly adopted the resolution WHA67.[49] This resolution called for governments to put comprehensive national plans in place for the prevention, diagnosis, and treatment of hepatitis, and asked the WHO to assess the feasibility of eliminating HBV and HCV with a view to set targets and devise a monitoring system. As a result, the WHO has developed a Global Hepatitis Strategy and proposed targets of 90% of those with HCV to be diagnosed, 90% of those who are eligible to be treated, and 90% of these patients to be cured by 2030.[50]

Although patient advocacy is essential in lobbying governments to allocate more spending to HCV, physicians not only need to lend their support, but must also become actively engaged advocates for improvement in access to highly effective HCV treatments. Results from strong lobbying would include a higher prioritisation of HCV, increased prestige to the area, and more funding, as well as increased support, equipment, and research opportunities. Alongside epidemiology and economic reports to support the clinical and cost-effectiveness of national HCV treatment strategies, highlighting the emotional aspects of HCV infection and media involvement are required to effectively justify the multiple benefits of HCV treatment to governments.

Conquering C – Solutions To Address Access

Professor Nezam H. Afdhal

Current healthcare costs for HCV are increasing due to long-term effects such as HCC, liver decompensation, and the requirement for liver transplantations,[51,52] which have a median (range) annual cost of €109,075 (€38,594-€326,233).[53] Although SVR has been associated with a reduction in liver-related mortality and HCC,[5,54] as well as lower associated costs and improved quality of life,[55,56] the implementation of treatment access for all patients can be difficult.

Previously, only around 11% of patients with HCV in the USA were treated and 6% would show an SVR.[46,57] There are still global barriers to HCV treatment that include affordability and the healthcare systems available. The stigma of HCV disease combined with unwilling providers, a lack of screening, the location of clinics, and

a heterogenous population of patients can cause challenges when implementing treatment programmes.[58-60] Although targeted screening programmes have been effective at improving the detection and referral of patients with HCV, 40-85% of infected persons may not be identified depending on the location and current screening practices.[61,62]

A previous challenge of healthcare systems in treating patients with HCV was the complexity and expense of IFN treatments.[61] However, the improved efficacy of current treatments has shown a higher rate of SVR non-detection, with subsequently lower numbers of patients who require retreatment and a lower overall cost per SVR.[51,52,63] Modelling has shown that global implementation of the DAA treatments could cause HCV to be classed as a rare disease within 22 years.[64] Increasing DAA treatments to 165,000 patients per year by 2018 in the USA would eliminate the disease and cost under US$10 billion (as per calculations performed in 2014).[51,52] As 82% of patients with GT1 and moderate Stage 2 or cirrhotic diseases in the USA were treated with LDV+SOF between October 2014 and March 2015, treatments are being implemented in some areas and for patients with moderate-to-severe disease activity.

As well as the roll-out of DAA treatments across the USA, treatment access programmes have been developed in other countries. A Gilead HCV access programme has been set up with the aim to invest in long-term collaboration with governments, to implement public health plans, and to support treatment strategies.[65] Egypt has the highest global HCV prevalence of 9.8% of the population, of whom 90% are GT4 and 10% are GT1.[66-70] A 5-year action plan has been agreed upon to target around 300,000-350,000 patients per year with a 90% SVR, which could lead to eradication of the disease within 15 years and significantly reduced cirrhosis, HCC, and mortality.[66] Georgia has also implemented an eradication programme over 3-5 years along with the Centres for Disease Control and Prevention and support from Gilead, which could be used as a case study for other countries.[71]

In conclusion, the burden of HCV is still present but could be reduced substantially through DAA-based therapy, which has been shown to be effective and cost-effective. Access programmes to further improve the proportion of patients with HCV who are treated could transform the current prevalence and global consequences of the disease.

Conquering C – Going Beyond Cure

Professor Stefan Zeuzem

HCV meets all the established criteria for a disease that can be eliminated, including the absence of a non-human reservoir, an environment in which the virus cannot amplify, practical interventions that can be implemented to interrupt the transmission of HCV, and a cure.[72] As the current budget allocations for HCV are lower than HIV despite the greater mortality in HCV,[73] lobbying by patients and clinicians is required to demonstrate that current interventions are cost-effective, and that diagnosis rates of patients with HCV need to be improved.

If the treatment rate of countries was increased by 10% through a 3-5-fold increase in the diagnosis and treatment of patients, the strategy could result in a 90% decrease in total infections by 2030. Firstly, specific patient populations should be targeted with the treatment strategy, focussing initially on those with a high severity of disease. Patients with a high risk of transmission, including those who are HIV/HCV co-infected, injecting drug users, and prisoners would then be targeted with HCV treatment eradication strategies.[51]

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ANSWERING PIVOTAL QUESTIONS IN THE DIAGNOSIS AND TREATMENT OF PRIMARY BILIARY CIRRHOSIS AND NON-ALCOHOLIC STEATOHEPATITIS

Symposium Report from the Intercept Pharmaceuticals, Inc. Supported Satellite Symposium, held at the International Liver Congress™ 2015, the 50th Annual Meeting of the European Association for the Study of the Liver, Vienna, Austria, 22nd–26th April 2015

Chairperson

Michael Trauner[1]

Speakers

David E.J. Jones,[2] Vlad Ratziu[3]

1. Medical University of Vienna, Vienna, Austria

2. Newcastle University, Newcastle upon Tyne, UK

3. Hôspital Pitié-Salpêtrière Université Pierre et Marie Curie, Paris, France

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MEETING SUMMARY

Professor Trauner introduced the subject of liver disease and its burden within the European Union (EU) and across the globe. Professor Jones summarised the progress made in understanding the pathophysiology of primary biliary cirrhosis (PBC), current unmet needs in the ursodeoxycholic acid (UDCA) era, and novel therapeutic options for PBC treatment. Professor Ratziu discussed the emerging understanding of the complex multisystem pathophysiology of non-alcoholic steatohepatitis (NASH), summarised the available therapeutic targets, and detailed the trials of novel agents currently underway.

Opening Remarks From the Chair

Professor Michael Trauner

Professor Trauner welcomed the audience and thanked the sponsors, Intercept Pharmaceuticals, Inc., for allowing the opportunity to discuss PBC and NASH and answer some key questions in diagnosis and treatment. The audience were invited to engage in the discussion.

PBC and NASH: Serious Liver Diseases with Unmet Needs

Professor Michael Trauner

There is little need to remind an audience of specialists of the importance of liver disease; nevertheless, statistics on its impact on society make for stark reading. Liver disease is a major cause of morbidity and mortality in the EU,

affecting 6% of the population.[1] Chronic disease leads to cirrhosis, hepatocellular carcinoma, and liver transplantation. In the EU, liver cancer mortality stands at 47,000 deaths annually, and more than 5,500 liver transplants are carried out each year.[2] Overall, liver disease is the fifth-most common cause of mortality in the EU and is implicated in one in six deaths.[1]

PBC and NASH stand out amongst the various aetiologies of liver disease, due in part to the recent major advances that have been made in understanding their pathobiology. Increased knowledge of the role played by bile acids in both conditions has helped to develop novel therapeutic targets and has led to improvements in the evaluation and assessment of patients. However, difficulties persist in patient management due to the lack of reliable biomarkers to assist in risk stratification and assessment of patient prognosis in these broad-spectrum diseases. Perhaps the most pressing challenge in the successful treatment of PBC and NASH is the failure of early diagnosis and concomitant lag in treatment, common in both conditions.

PBC Challenges: What is Treatment Success and What Will Emerging Therapies Offer?

Professor David E.J. Jones

There remains significant unmet need in the PBC patient population despite the existence of proven primary therapy in the form of UDCA, as illustrated by the deficit in transplant-free survival in UDCA non-responders compared with age and sex-matched community controls.[3] There are a number of possible reasons for the impaired survival of patients treated for PBC: treatments may be used sub-optimally; the effectiveness of current treatments may be overestimated or may be restricted to a subpopulation of patients, and the distribution of treatments to those in need may be sub-optimal.

In 2008 in the UK, 20% of PBC patients did not receive treatment with UDCA,[4] and unpublished data indicate that many patients received doses now regarded as insufficient. UDCA also has issues with patient adherence, with barriers including weight gain, nausea, and hair loss. Addressing the above treatment-related issues, using a simple and consistent message underscoring UDCA's effectiveness and the need for all patients to at least receive it at the correct dose, is a logical first step in addressing unmet need.

To identify those UDCA-treated patients with unmet need, patients responding successfully must firstly be characterised. The two principal systems developed to identify treatment response are the Paris and Barcelona criteria (Table 1).[5,6] These and related criteria (Toronto criteria) have been independently validated using the large UK-PBC cohort, confirming their ability to predict transplant-free survival and consequently the need for their incorporation into routine clinical use.[7]

Recent data from the Global-PBC Group indicate that baseline biomarkers are predictive of treatment outcome. Researchers showed that both elevated alkaline phosphatase (ALP) and bilirubin predict poor clinical outcome.[8] Thus, both baseline characteristics and treatment response are important to predict event-free survival. This was confirmed by a univariate and multivariate analysis of the UK-PBC cohort, with baseline cirrhosis (as measured by albumin and platelets) and response to UDCA at 12 months (bilirubin, alanine aminotransferase [ALT], and ALP) predictive of transplant-free survival at 15 years. These data are further backed by a 50% treatment failure rate in younger patients in the UK-PBC cohort, despite apparently high overall response rates.[9]

Table 1: UDCA treatment-response criteria.

Paris Criteria	Barcelona Criteria
Bilirubin ≤1 mg/dl + AST ≤2 × ULN + ALP ≤3 × ULN after 1 year of UDCA at 13—15 mg/kg/day	ALP decreased by 40% or normalised after 1 year of UDCA at 13—15 mg/kg/day

UDCA: ursodeoxycholic acid; AST: aspartate aminotransferase; ULN: upper limit of normal; ALP: alkaline phosphatase.

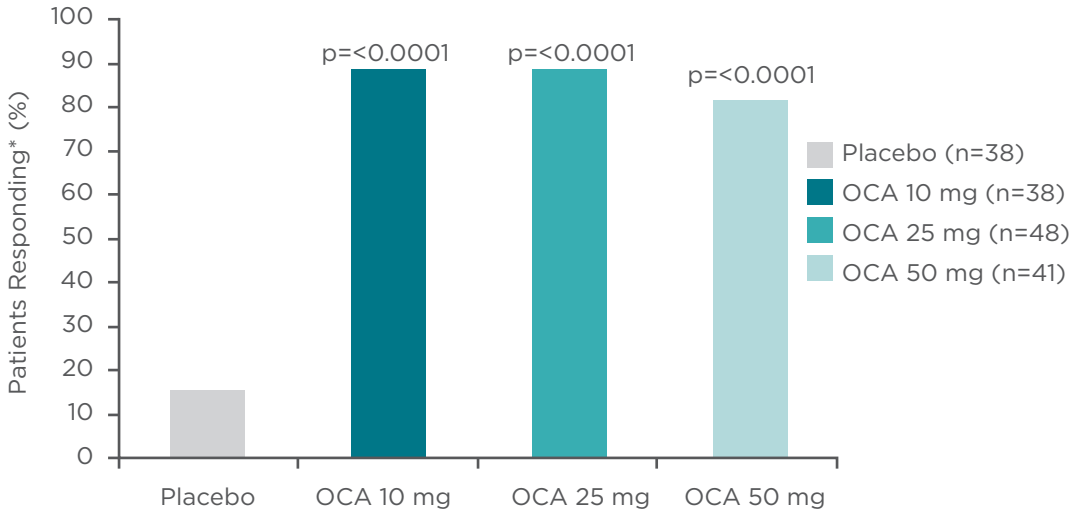

Figure 1: Efficacy of OCA in PBC patients on stable UDCA treatment.
*Primary efficacy endpoint was percentage change in plasma alkaline phosphatase (ALP) from baseline; patients with a placebo-subtracted ALP reduction of ≥10% were defined as responders.
OCA: obeticholic acid; PBC: primary biliary cirrhosis; UDCA: ursodeoxycholic acid.
Adapted from Hirschfield GM et al.[14]

These younger patients represent a high-risk group in which novel therapies may be most useful.

The above described unmet clinical need necessitates new therapies. There are four elements of the PBC disease process that may offer novel therapeutic targets. The autoimmune response may be addressed through targeted immunosuppression. The secondary cholestatic phase may be amenable to 'second-line' bile acid therapies or manipulation of the microbiota. Biliary epithelial protectant agents may offer a novel route to preserve bile ducts. Finally, those patients who already have fibrosis/cirrhosis may be targeted using antifibrotics. Research into novel therapeutics is complicated by the incomplete picture in terms of biomarkers, difficulties in identifying early stage patients who may respond to immunosuppressant therapies, a lack of clarity regarding therapy-specific response criteria, and the inherent difficulties of trial design caused by a lack of hard endpoints and validated histological measures.

Despite these challenges, it is currently an exciting time for novel PBC therapeutics. Drugs targeting peroxisome proliferator-activated receptor (PPAR)-α (fibrates) and Farnesoid X receptor (FXR) (obeticholic acid [OCA]) systems are joined by norUDCA, which may protect via the creation of a bicarbonate 'umbrella' and has anti-inflammatory and anti-fibrotic effects, and rituximab (RTX), which targets B cell depletion. A number of other new therapies are also in the early stages of development, including the ileal bile acid absorption blockers A4250 and LUM001, and the immunological agents NI-0801 and ustekinumab.

The fibrates act via PPAR-α agonism, which has been linked to the regulation of bile acid synthesis and detoxification and the modulation of phospholipid secretion, which helps to protect the bile duct epithelium through the formation of micelles.[10] Currently, there is an inadequate number of well-designed trials examining fibrates in PBC. The trials that exist, and associated meta-analyses, have failed to demonstrate clinical efficacy despite biochemical improvements, and have also shown possible safety concerns.[11–13] As a result, despite a logical mechanistic basis, fibrates lack a solid evidence base for efficacy and are associated with possible adverse outcomes in the long term.

The FXR agonist OCA is the most extensively evaluated of the second-line therapies. OCA represents the logical extension of bile acid therapy beyond UDCA, sharing a number of properties (choleretic, anti-apoptotic, and antioxidant effects) as well as a number of additional direct and indirect, FGF19-mediated effects on bile acids. In a recently published Phase II trial (n=165) involving UDCA non-responders, OCA achieved an approximate 90% response rate at all doses tested (Figure 1).

Discontinuation due to pruritus (itch) was an issue in this study,[14] but has been addressed by a dose reduction at Phase III.

Quality of life (QoL) is often the key outcome from the perspective of patients and does not appear to be modified by current treatments. Currently, 35% of PBC patients perceive their QoL as impaired, and almost half feel that their health is worse than it was a year earlier.[4] B cell depleting agents such as RTX may have a role in reducing fatigue. In summary, the actions needed to improve QoL for PBC patients begin with improving community, patient, and first-physician awareness of the disease and its presentations. Improved physician awareness of the need for therapy with UDCA (≥95%) and identification of non-responders must be matched with a systematic approach to management. Built-in triage for high-risk/non-responding patients should migrate these individuals into clinical trials and onto second-line therapies as they become available. In parallel, continued evaluation of second-line therapies and their integration into stratified management pathways is required. Finally, improvement of awareness, assessment, and treatment of symptoms in PBC using systematic approaches and a focus on patients' QoL in addition to the above measures has the potential to dramatically improve the lives of PBC patients.

NASH: Diagnostic Challenges, Therapeutic Targets, and New Paths to Treatment Success

Professor Vlad Ratziu

Recent strides in the understanding of non-alcoholic fatty liver disease (NAFLD) and NASH should soon begin to translate into improved therapeutic options. However, diagnostic challenges still exist, both in terms of disease recognition and risk stratification. Many patients are still underdiagnosed and undermanaged, as in the 61% of patients in retrospectively confirmed cases from a recent database analysis who received no NAFLD care.[15] Beyond recognition of the disease itself, the nature of NAFLD as part of a multi-organ metabolic syndrome must also be recognised. NAFLD is a multi-system disease, and extra-hepatic comorbidities such as Type 2 diabetes (T2D), sleep apnoea, and arterial dyslipidaemia must be addressed. Direct effects of these comorbidities have been demonstrated, for example, sleep apnoea-related hypoxia modifies the progression of liver fibrosis in NASH. In terms of the liver condition itself, assessment of cofactors of fibrosis in conjunction with disease severity (steatosis/NAFLD or steatohepatitis/NASH), disease stage, and an estimate of prognosis are essential first steps for adequate management.

Identification of patients at risk of progression is a further diagnostic challenge. Recent evidence from serial biopsies suggests that the presence of inflammation and steatosis alone, and not necessarily the full necroinflammatory histology characteristic of NASH, are enough to put patients at risk of progression.[16] Features associated with risk of rapid progression to fibrosis in NAFLD patients include diabetes, metabolic syndrome, magnitude of ALT elevation, and extent of insulin resistance.[17] In NASH patients, risk of progression to severe fibrosis is associated with older age (>45–50 years) and T2D.[18,19] There is a small genetic component, with predisposing polymorphisms in *PNPLA3* and *TM6SF2*,[20,21] as well as associations with obesity, arterial hypertension, hypertriglyceridaemia, insulin resistance, and elevated ALT/aspartate aminotransferase.[18,19,22] Despite the progress this information represents, further work is needed to identify biomarkers and particularly to create a non-invasive methodology for assessing risk of progression in NAFLD and NASH.

Improvements in the understanding of NASH pathophysiology have led to the identification of new therapeutic targets. NASH pathophysiology appears to derive from metabolic abnormalities, with insulin resistance — particularly in adipose tissue — likely to be the major predisposing disorder. Free fatty acids, chemokines, and insulin drive further metabolic dysregulation as well as directly causing inflammation and cell death, leading ultimately to fibrogenesis and progression towards cirrhosis. This complex and interconnected pathophysiology results in numerous drug targets but also a need to target multiple pathways to reduce fibrogenesis in the long term.

Foremost amongst the novel therapies targeting NASH are FXR agonists, such as OCA. The SCD1 inhibitor aramchol and the PPAR agonist GFT505 work by reducing liver fat, while the dual CCR2 and CCR5 antagonist cenicriviroc targets inflammation and may also have antifibrotic effects.

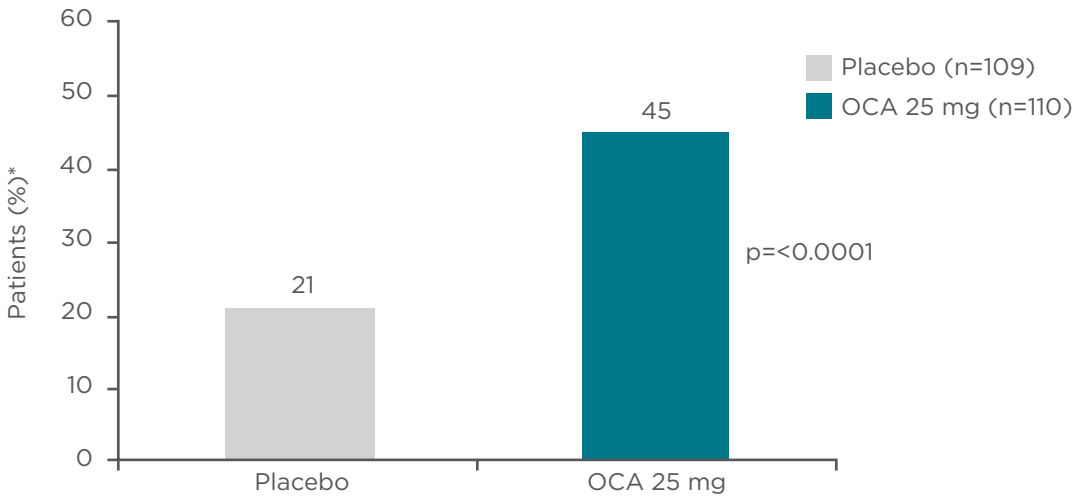

Figure 2: Patients treated with OCA achieving primary outcome measure.
*≥2-point improvement in NAS score without worsening of fibrosis.
OCA: obeticholic acid; NAS: NASH activity score.
Adapted from Neuschwander-Tetri et al.[31]

Other drugs target fibrogenesis by blocking collagen cross-linking (anti-lysyl oxidase-like 2 [LOXL2] monoclonal antibody, simtuzumab [SIM]) or by inhibiting the fibrosis-related protein galectin-3. The urgent need for effective therapies for NASH is recognised by the FDA, as illustrated by the granting of breakthrough status to OCA and fast-track status of the majority of the other novel compounds mentioned.[23-27]

The main mode of action of FXR-agonist therapies, such as OCA, in NASH is through direct cytochrome-modulated blockade of conversion of cholesterol in bile acids. However, as noted above, indirect effects via FGF19 are also present, which may act on metabolic pathways, improving glucose tolerance and insulin sensitivity and reducing lipogenesis and hepatic fat. Direct antifibrotic properties derived from blocking activation of quiescent hepatic stellate cells may also play a role.[28-30] Data are available from a Phase IIb 72-week randomised, double-blind, placebo-controlled trial of OCA 25 mg/day (n=110) versus placebo (n=109) with both clinical and histological endpoints.[31] NASH patients with active disease were eligible. There was a striking difference in the number of patients achieving the primary histological outcome measure (improvement in NASH activity score [NAS] ≥2) between placebo and OCA-treated patients (Figure 2).

There were across-the-board significant improvements in every histological feature that defines NASH (lobular inflammation, steatosis, hepatocellular ballooning, and fibrosis). This represents the first human demonstration of antifibrotic efficacy in NASH, particularly noteworthy given that the trial was not powered for this outcome.[31] Adverse event data showed mild-to-moderate effects in general. As with the above PBC data, pruritis was an issue;[31] however, the PBC data also suggest that it may be addressed via dose adjustment.

As mentioned, the conjugated bile acid-saturated fatty acid aramchol modulates the amount of fat in the liver. It acts via two pathways: inhibition of fatty acid metabolism via blockade of SCD1 enzyme activity, and activation of cholesterol efflux by stimulating the cholesterol pump ABCA1.[32-34] Results from a small (n=57) Phase IIa trial indicate that aramchol dose-dependently reduces liver fat, as measured by non-invasive magnetic resonance spectroscopy. This result is currently being confirmed in a population of NASH patients with active disease and metabolic syndrome in the Phase IIb ARREST trial (n=240). In addition to steatosis, NASH resolution, reduced NAS score, and metabolic improvements will be assessed.

Cenicriviroc is a dual CCR2 and CCR5 antagonist that has shown potential for antifibrotic activity.[35] These two cytokines have overlapping proinflammatory and profibrotic properties, aiding the chemotaxis of inflammatory cells and activating profibrotic stellate cells.[36] The large international Phase II CENTAUR trial[37] (n=252) of cenicriviroc includes patients that have well-defined NASH

either with active disease or progression risk factors. The primary outcome is improvement of NAS score with no worsening of fibrosis, with the main secondary outcome being resolution of NASH with no worsening of fibrosis, which is likely to be important for approval as a NASH therapeutic.

As a selective dual PPAR-α and PPAR-δ agonist with no PPAR-γ activity, GFT505 combines liver-specific (PPAR-α) and multi-organ anti-inflammatory and fat-reducing activity (PPAR-δ).[38] Phase II trials have demonstrated improved lipid metabolism and insulin sensitivity in diabetic and pre-diabetic patients, and animal data suggest the presence of anti-inflammatory and antifibrotic properties in NASH.[39,40] The 1-year Phase IIb GOLDEN trial[41] (n=270) of GFT505 is highly anticipated, with preliminary results suggesting that the primary endpoint of NASH resolution with no worsening of fibrosis has been met.

SIM directly targets fibrosis through highly specific inhibition of LOXL2, the enzyme that promotes the cross-linking of collagen, which is key to the fibrotic process. LOXL2 levels may correlate with clinically relevant NASH endpoints, and blockade of collagen cross-linking has been demonstrated in other conditions.[29,42] Two large 240-week Phase IIb trials are currently underway comparing two doses of SIM (75 mg and 125 mg) with placebo in either cirrhotic (n=259) or non-cirrhotic (n=222) patients. In the cirrhotic patients, the drug is administered intravenously every 2 weeks with a liver biopsy after 1 year, and endpoints are based on hepatic venous pressure gradient and event-free survival. In the non-cirrhotic trial, participants with NASH and bridging fibrosis receive a weekly subcutaneous injection; the primary endpoint is fibrosis and event-free survival (assessed as time-to-progression to cirrhosis).[43]

Challenges remain in the NASH therapeutic pipeline. The multiple pathogenic mechanisms of NASH require therapies that target more than one pathway to achieve histological efficacy. Improved animal models and non-invasive outcomes for proof-of-principle trials are needed to speed up development. The lack of surrogates for hard outcomes, in particular non-histological outcomes, and response on therapy are also issues; however, an effective primary therapy will be required before this can be addressed. Nevertheless, there has been tremendous progress in the field recently. Firstly, the medical need is now being recognised for NASH as a standalone condition related to, but not subsumed by, metabolic disorder. NASH is accepted as an indication for therapy and now has operational pathological definitions. Achievable surrogate endpoints have been set and the regulatory path for approving drugs in NASH is clear, with regulatory bodies behind the push for new therapies.

In conclusion, it is essential to assess liver injury in those with metabolic risk factors such as diabetes or obesity. NAFLD is a cause of liver cirrhosis and primary liver cancer, and prognosis is dependent on the fibrosis stage and also the presence of steatohepatitis (NASH), which ultimately drives fibrosis. There is a need to develop pharmacological agents that target NASH and a number of these are now being tested in large Phase IIb trials, with OCA soon moving into Phase III trials. Once we have demonstrated the efficacy of these drugs, tailoring of therapy to individuals and integrative approaches with diet and lifestyle will be the key concerns.

Concluding Remarks

Professor Michael Trauner

In summary, a significant proportion of patients with PBC have insufficient response to available treatment and require novel therapies. New strategies based around second-line bile acid therapies, particularly OCA, appear to be yielding results, while new immunological approaches targeting symptoms may help address key patient concerns such as fatigue. NASH remains an under-recognised liver disease in clinical practice. New non-invasive detection methods to track progression and measure therapeutic efficacy are needed, although some progress has been made in tracking fibrosis and the inflammatory component of the condition. Treatment of metabolic comorbidities may have a beneficial impact on liver disease but there is an urgent need for novel therapies beyond lifestyle modification. A number of new therapies are at Stage II of testing, including second-line bile acids and others targeting metabolic aspects of the disease alongside inflammation and fibrosis.

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WOULD YOU FIGURE IT OUT? DIFFERENTIAL DIAGNOSES: BEYOND THE USUAL

Summary of Presentations from the Synageva Symposium, held at the International Liver Congress™ 2015, the 50th Annual Meeting of the European Association for the Study of the Liver, Vienna, Austria, 23rd April 2015

Chairperson

Vlad Ratziu[1]

Speakers

Lauren Johansen,[2] Christophe Moreno,[3] Ali Canbay,[4] Mark Bechter[5]

1. Hôpital La Pitié-Salpêtrière, Paris, France
2. Birmingham Children's Hospital, Birmingham, UK
3. CUB Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium
4. Essen University Hospital, Essen, Germany
5. Synageva BioPharma, Lexington, Massachusetts, USA

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MEETING SUMMARY

The Synageva BioPharma-sponsored symposium discussed the differential diagnoses for liver diseases that may be under-recognised in clinical settings, with a focus on lysosomal acid lipase deficiency (LAL D). LAL D is a lysosomal storage disorder caused by deficient activity of the lysosomal acid lipase enzyme, resulting in the accumulation of cholesteryl esters and triglycerides throughout the body, predominantly in the liver, spleen, gastrointestinal tract, and blood vessel walls. LAL D is a progressive, multisystem disease with early mortality and significant morbidity that is characterised by hepatic dysfunction and dyslipidaemia. Evidence suggests that LAL D may be substantially underdiagnosed or misdiagnosed, which is critical given that disease progression can be unpredictable, with liver failure and/or accelerated atherosclerosis potentially contributing to early mortality. However, a definitive diagnosis of LAL D can be made using a LAL enzyme-based biochemical test, thereby allowing for active monitoring of patients to reduce the potential for disease complications. To raise awareness of LAL D, this symposium, chaired by Prof Vlad Ratziu, centered on the presentation of patient cases by Dr Lauren Johansen, Prof Christophe Moreno, and Prof Ali Canbay, who discussed the path to diagnosing LAL D in children and adults. In addition, Dr Mark Bechter of Synageva BioPharma provided an overview of current data from an ongoing Phase III clinical trial assessing the efficacy and safety of sebelipase alfa, a recombinant LAL replacement therapy, in children and adults with LAL D.

Keywords: Lysosomal acid lipase deficiency, lysosomal storage disease, microvesicular steatosis, dyslipidaemia, elevated serum transaminases, enzyme replacement therapy, hepatomegaly, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis.

Overview of Lysosomal Acid Lipase Deficiency

Professor Vlad Ratziu

Prof Ratziu opened the symposium by stating that, although this meeting consists primarily of adult hepatologists, lysosomal acid lipase deficiency (LAL D) is a disease of interest across paediatric and adult specialties. Although LAL D has been historically described as Wolman's disease in infants and cholesteryl ester storage disease in children and adults,[1-3] it is now recognised as a single disease that presents across an age continuum. To this end, Prof Ratziu emphasised that the purpose of the symposium was to raise awareness about the clinical presentation of LAL D and its diagnosis, particularly in adults. Indeed, LAL D can present at any age, and currently many adults with 'silent' disease are being diagnosed.

Clinical Presentation and Diagnosis of LAL D

Professor Ali Canbay and Doctor Mark Bechter

Clinical presentation

LAL D is an autosomal recessive disease resulting from homozygous or compound heterozygous mutations of the *LIPA* gene that markedly reduce or eliminate the activity of the LAL enzyme.[4] Because LAL is important for the lysosomal degradation of cholesteryl esters and triglycerides, these products accumulate in the lysosomes across multiple body tissues, primarily the liver, spleen, gastrointestinal (GI) tract, and blood vessel walls, producing progressive organ damage (Figure 1).[5,6]

Presenting across an age continuum, LAL D is a progressive, multisystem disease with early mortality and significant morbidity affecting infants, children, and adults.[4,5] LAL D is rapidly progressive and fatal in infants, who present with growth failure along with prominent hepatic and GI manifestations, but LAL D in children and adults may have a more variable progression and complex disease presentation, including a combination of dyslipidaemia (elevated low-density lipoprotein [LDL] cholesterol and decreased high-density lipoprotein [HDL] cholesterol) and hepatic dysfunction. In particular, the latter may manifest as hepatomegaly, persistently elevated serum transaminases, microvesicular steatosis (MVS) on liver biopsy, and a high frequency of fibrosis and cirrhosis. Chronic dyslipidaemia may also lead to early atherosclerosis (AS) and increased risk of myocardial infarction, coronary heart disease, stroke, and death.[4,5,7]

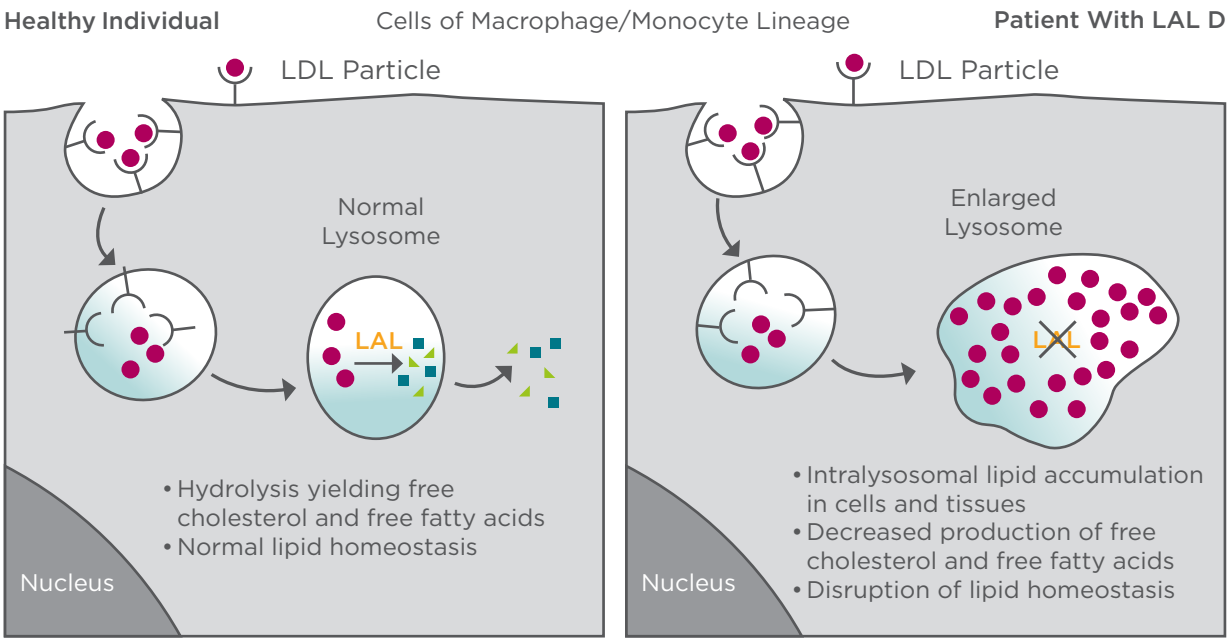

Figure 1: Lysosomal acid lipase plays a key role in lysosomal degradation of cholesteryl esters and triglycerides.

LDL: low-density lipoprotein; LAL D: lysosomal acid lipase deficiency.

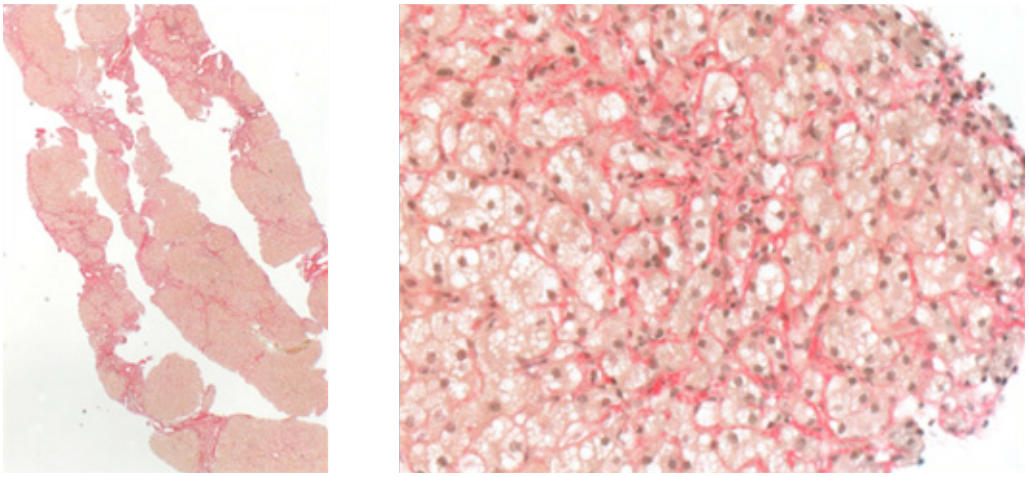

Figure 2: Liver biopsy with haematoxylin/van Gieson stain showing periportal fibrosis as typically seen in lysosomal storage diseases.

The prevalence of disease is difficult to determine due to misdiagnosis and potential underdiagnosis, with estimates ranging between 1:40,000 and 1:300,000.[3,6,8,9] That a small number of cases of LAL D have been reported in the literature suggests that LAL D may be substantially underdiagnosed or misdiagnosed.[7] Given that LAL D shares clinical presentation with other cardiovascular, liver, and metabolic diseases, LAL D might not be readily recognised in clinical practice and many patients may not be accurately diagnosed. This is critical given that the disease may be 'silent' and disease progression can be unpredictable, with liver failure and/or accelerated AS potentially contributing to early mortality.[4,6]

Diagnosis

To date, over 40 loss-of-function *LIPA* mutations have been identified in patients with LAL D, with the most common splice mutation occurring in exon 8 (E8SJM-1).[5] Because the *LIPA* gene is responsible for production of LAL, mutations result in little to no production of LAL. LAL D can be definitively diagnosed using a LAL enzyme-based biochemical test to assess enzyme levels in the peripheral blood of patients with a suspicion of LAL D, with subsequent genetic assays useful from a clinical research perspective.[5,10] LAL enzyme activity levels do not predict disease severity or progression, and there are no genotype/phenotype correlations reported for the presentation of LAL D in children and adults.[5]

LAL D is currently managed with dietary modifications (low-fat diet), lipid-lowering medications, liver transplantation, and haematopoietic stem cell transplantation.[4] There are currently no disease-specific treatments approved for LAL D that address the underlying cause of disease, but studies of potential treatments are ongoing. Existing supportive approaches can help manage disease symptoms; however, they do not treat the underlying cause or alter the course of disease. Early diagnosis is critical to allow for active monitoring of patients to reduce the potential of disease complications. Given the high potential for misdiagnosis or underdiagnosis of LAL D, efforts are increasing to raise awareness of LAL D and make it part of the differential diagnosis for patients with liver dysfunction and/or dyslipidaemia.[5,10]

Keep An Open Mind

Doctor Lauren Johansen

Doctor Johansen discussed a case involving a 7-year-old girl who initially presented to a general practitioner with fatigue, anaemia, and abnormal liver function tests (alanine aminotransferase [ALT]: 166 IU/l; aspartate aminotransferase [AST]: 201 IU/l; gamma-glutamyltransferase [GGT]: 123 IU/l). Investigations at a tertiary center revealed hepatosplenomegaly, prolonged prothrombin time (15 seconds), raised immunoglobulin G (IgG), and a positive antinuclear antibody (ANA) test. Liver biopsy demonstrated chronic hepatitis with moderate fibrosis and fatty change. The steatosis prompted metabolic investigations, which excluded

Wilson's disease (WD). There was also villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis on duodenal biopsy, in keeping with a diagnosis of coeliac disease (CoD).

The patient's symptomatology improved with a gluten-free diet, but her transaminases did not fully normalise. Treating physicians theorised that the patient may have dual pathology, with autoimmune hepatitis (AIH) — as indicated by raised IgG and positive ANA — and CoD. This prompted the initiation of corticosteroids and then azathioprine; however, the patient's persistent lack of response and subsequent development of neutropaenia prompted further evaluation. At 9 years of age, she had a normal body mass index (BMI), but presented with elevated ALT, AST, and GGT despite being compliant with therapy. Magnetic resonance cholangiopancreatography demonstrated that the spleen remained enlarged at 12 cm, but there was no evidence of intra or extra-hepatic bile duct dilatation. Subsequent blood work revealed a profile consistent with dyslipidaemia (elevated total cholesterol [normal range]: 9.9 mmol/l [2.8-4.8], low HDL: 0.9 mmol/l [>0.9], and elevated triglycerides: 1.84 mmol/l [0.38-1.38]). Dr Johansen stressed that dyslipidaemia in conjunction with ongoing signs of inflammation and the development of cirrhosis on liver biopsy (Figure 2) raised the suspicion of LAL D.

LAL D diagnosis was confirmed through the LAL enzyme-based biochemical blood test (BBT), which showed that the patient had low LAL activity (<0.01 nmol/punch/hour [normal 0.07-0.50]). The patient has subsequently received investigational treatment for LAL D in a clinical trial and demonstrated improvement with normalisation of ALT over a 3-month period. She will be re-evaluated for CoD to confirm the dual diagnosis. Given that it took approximately 2 years to arrive at the correct diagnosis of LAL D, Dr Johansen emphasised the following key learning points:

- A lipid profile is mandatory in paediatric patients with liver disease
- It is critical to revisit the initial diagnosis if the patient's profile does not fit the disease
- Do not discontinue diagnostic exploration even if the patient appears to have some response to treatment
- LAL D can now be easily diagnosed with a BBT

This case uniquely highlights that there may be more than one underlying cause for a patient's symptoms, making the process of diagnosing LAL D perhaps complex but not complicated. Dr Johansen's concluding statements further suggest LAL D should be part of the differential diagnosis in paediatric patients with:

- Liver disease, including unexplained persistently raised liver transaminases and/or unexplained hepatomegaly, cryptogenic cirrhosis, and those with predominantly MVS or mixed macro/MVS on biopsy
- Lipid abnormalities, including dyslipidaemia (LDL ≥4.1 mmol/l, HDL ≤1 mmol/l in males or ≤1.3 mmol/l in females), or presumed diagnosis of familial hypercholesterolaemia, and those with an unclear family history or lacking the common genetic mutations LDLR/APO B/PCSK9

Overweight and Elevated Liver Enzymes: Not Always NAFLD!

Professor Christophe Moreno

Prof Moreno described a case of a 33-year-old woman with elevated liver enzymes who was referred by her general practitioner to the outpatient clinic at Erasme Hospital. She was suspected of having Epstein-Barr virus (EBV) infection with current symptoms of fatigue and recent gastroenteritis. The patient had a medical history of hypercholesterolaemia and urticaria; in addition, she did not drink alcohol but smoked two packets of cigarettes per month and was currently taking simvastatin 20 mg, and ibuprofen and ebastine as needed.

A physical examination revealed that the patient was overweight but with normal blood pressure (BP) (weight: 80 kg, height: 170 cm, BMI: 27.7 kg/m^2, BP: 110/60 mmHg). Outside of reddish maculae on her lower extremities, the physical examination was normal. The patient's laboratory workup indicated normal haematologic, renal, and thyroid functioning, no evidence of inflammation, a fasting blood sugar of 86 mg/dl, and ongoing elevated transaminases (AST: 36 U/l; ALT: 58 U/l; GGT: 42 U/l; alkaline phosphatase: 52 U/l; bilirubin total: 20.5 µmol/l); an ultrasound revealed moderate steatosis as well as an ovarian cyst.

Based on these findings, Prof Moreno queried whether the attendees would suspect

non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), EBV reactivation, AIH, or another diagnosis. The treating physicians conducted a fibroscan, more extensive blood workup, and a liver biopsy. The fibroscan revealed no significant liver fibrosis, but hepatic ultrasound was indicative of severe steatosis.[11,12] Further, laboratory evaluation showed hypertriglyceridaemia (1.77 mmol/l) and reduced HDL cholesterol (<1.30 mmol/l), which in conjunction with the patient's central obesity (waist circumference: 94 cm) was indicative of metabolic syndrome. Having excluded WD and other fatty liver diseases, NAFLD/NASH was proposed as the likely diagnosis. Of note, IgG levels were elevated and ANAs were positive (1/160).

After 3 months during which the patient stopped taking statin therapy and modified her diet, a follow-up liver biopsy revealed signs of chronic hepatitis of unknown origin, septal fibrosis, rare hepatocyte ballooning, MVS, and slight lobular inflammation (Figure 3). At this point, AIH was excluded[13,14] and an alternative cause was suspected, with the patient's condition considered borderline NASH. Investigators suspected alternative causes of MVS (Reye's syndrome, medication use [valproate, antiretroviral medicines], acute fatty liver of pregnancy, HELLP syndrome, and inborn errors of metabolism [e.g. LAL D]).[15]

At this point, LAL D was raised as a differential diagnosis. The LAL enzyme-based BBT confirmed that there were undetectable LAL activity levels (<0.02 nmol/punch/hour; reference range: 0.37-2.30), making this the first adult case of LAL D to be officially diagnosed in Belgium. Based on this patient case, Prof Moreno concluded that LAL D is not only a paediatric disease, but should be considered in young adult patients — even those who are overweight — based on their family history and in the presence of dyslipidaemia and MVS.

Lysosomal Acid Lipase Deficiency — Differential Diagnosis Versus NASH or NAFLD

Professor Ali Canbay

Prof Canbay introduced his presentation by discussing common causes of steatosis and steatohepatitis, which include alcohol-induced steatosis, NAFLD/NASH, and chemotherapy-induced steatosis, as well as CoD, Gaucher's disease, and LAL D. He then focussed on LAL D as part of the differential diagnosis versus NASH or NAFLD. As obesity rates increase — approximately 50% of the European population is now considered overweight (BMI >25)[16,17] — NAFLD/NASH has become the primary cause of liver disease in Western countries, and the prevalence has doubled worldwide even though other chronic liver diseases have been stable or decreasing.[18] NAFLD is primarily defined by macrovesicular steatosis; NASH is a subgroup of NAFLD characterised by liver cell injury, inflammation, and the possible presence of fibrosis in addition to steatohepatitis.[18]

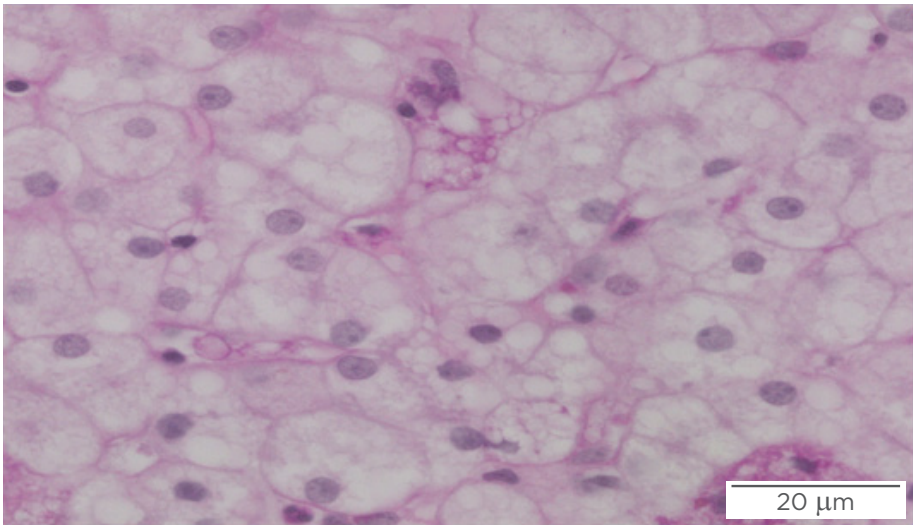

Figure 3: Liver biopsy showing signs of chronic hepatitis of unknown origin, septal fibrosis, rare hepatocyte ballooning, microvesicular steatosis, and slight lobular inflammation.

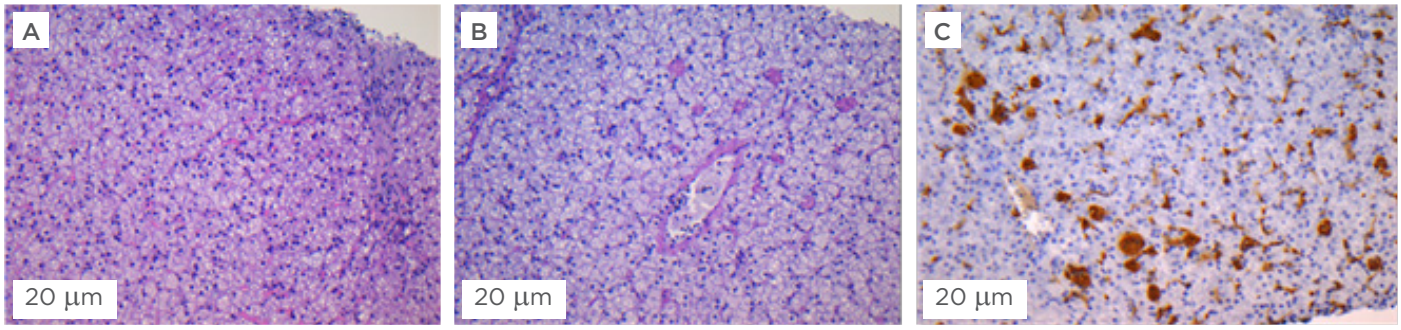

Figure 4: Liver biopsy showing A) periportal fibrosis and microvesicular steatosis, B) D-PAS-positive Kupffer cells, and C) intrahepatic CD68-positive cells.

Generally, NAFLD/NASH does not cause specific symptoms: patients potentially experience fatigue, malaise, and abdominal discomfort; however, accurately diagnosing NAFLD/NASH is critical given that disease progression to cirrhosis/liver failure and hepatocellular carcinoma can occur.[15,18,19]

Other less common diseases such as LAL D should be in the differential diagnosis for NAFLD/NASH. To illustrate this, Prof Canbay described a case of a 39-year-old woman with a normal weight (BMI: 24) who presented with hepatomegaly and splenomegaly of unknown origin as well as thrombocytopaenia and a suspicion of portal hypertension. Following a liver biopsy, periportal fibrosis with 20% MVS and Kupffer cells with positive lysosomes were identified as well as intrahepatic CD68-positive cells (Figure 4).

Dr Canbay stated that these histologic findings raised a suspicion of LAL D and prompted treating physicians to assess LAL and chitotriosidase activity levels, the latter of which is elevated in various lysosomal storage disorders. Tests returned with practically undetectable LAL enzyme activity levels (<0.01 nmol/punch/hour; reference range: 0.10-2.0) and elevated chitotriosidase activity in plasma (12.3 nmol/min/ml; reference range: <1.5). These findings provided the definitive diagnosis of LAL D. Genetic testing identified homozygote mutation G934->A in exon 8 of the *LIPA* gene.

Guidelines currently recommend that a diagnosis of NAFLD requires the presence of steatosis (with the exception of cirrhotic disease) and no other causes for secondary hepatic steatosis.[15,20] Based on this patient case, Prof Canbay urged that it is critical to revisit NAFLD/NASH in all patients whose symptoms may not exactly match this diagnosis. When evaluating a patient with suspected NAFLD, it is essential that all aetiologies of steatosis be considered, and any patient with MVS should be evaluated for LAL D using the biochemical enzyme-based assay.

He further indicated that although clinical and biochemical abnormalities associated with LAL D are subtle and may not trigger medical concerns, LAL D is progressive and unpredictable with the potential for rapid clinical decline.[4,7] He stated that because diagnosis requires only a simple enzymatic BBT, it will be important to identify LAL D patients so that medical follow-up can be tailored to known risks.[5,10]

Silent Fibrosis and Cirrhosis — Acid Lipase Replacement Investigating Safety and Efficacy (ARISE) Trial

Doctor Mark Bechter

LAL D is currently managed through dietary modifications (low-fat diet), lipid-lowering medications, liver transplantation, and haematopoietic stem cell transplantation.[4] Although these supportive approaches can help to manage disease symptoms, they do not treat the underlying cause or notably alter disease progression. Sebelipase alfa (SA) is an enzyme replacement therapy currently in clinical development. It is an exogenous replacement of the deficient LAL enzyme with a recombinant human enzyme that catalyses the hydrolysis of cholesteryl esters and triglycerides. In pre-clinical studies and studies in patients with LAL D, SA has been shown to improve markers of liver damage and lipid abnormalities.[21-23] In a Phase I/II open-label, dose-escalation trial to evaluate the

long-term safety of SA in adults with LAL D, patients (n=8) received intravenous infusions of SA for up to 104 weeks. Improvements in serum transaminases and lipid levels were sustained through the entire 104-week treatment period. Further, SA was not associated with drug-related serious adverse events (SAEs), although one patient had one unrelated SAE (cholecystitis and cholelithiasis) and continued on the study. Most infusion-associated reactions were mild and primarily gastrointestinal, and there was no evidence of anti-drug antibodies in patients tested to date in this study.[21]

At this symposium, Dr Bechter presented current data from an ongoing Phase III randomised, placebo-controlled clinical trial that is assessing the efficacy and safety of intravenous infusions of SA in children and adults with LAL D.[24,25] In the Acid Lipase Replacement Investigating Safety and Efficacy (ARISE) trial, the study population includes patients with LAL D who were a minimum of 4 years of age with ALT levels ≥1.5 times the upper limit of normal and who could be taking a stable dose of lipid-lowering medications. Patients who had undergone a previous liver or haematopoietic stem cell transplant, or who had severe hepatic dysfunction (Child-Pugh Class C) were excluded. The primary endpoint for ARISE is ALT normalisation; secondary endpoints include LDL cholesterol reduction, non-HDL cholesterol reduction, AST normalisation, triglyceride reduction, HDL cholesterol increase, liver fat content reduction, improvement in steatosis, and liver volume reduction.

In summary, it will be critical to increase our understanding of LAL D. In addition to ongoing studies of new therapies for LAL D, there is currently a LAL D registry available for collecting longitudinal data.[25]

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PROVIDING HEPATITIS-TESTING SERVICES TO PERSONS WITH HIV INFECTION IN LOW AND MIDDLE-INCOME COUNTRIES: THE MÉDECINS SANS FRONTIÈRES EXPERIENCE

***Isabelle Meyer-Andrieux,[1] Krzysztof Herboczek,[2] Dmytro Donchuk,[3] Helen Bygrave,[3] Anita Mesic,[2] Linn Htet,[4] Marcelo Fernandez,[4] Lucas Molfino,[5] Javier Goiri,[1] Susanna Cristofani[1]**

1. Médecins Sans Frontières, Geneva, Switzerland
2. Médecins Sans Frontières, Amsterdam, Netherlands
3. Médecins Sans Frontières, Brussels, Belgium
4. Médecins Sans Frontières, Yangon, Myanmar
5. Médecins Sans Frontières, Maputo, Mozambique
**Correspondence to isabelle.andrieux-meyer@geneva.msf.org*

Objective

Little is known about the magnitude of the viral hepatitis B (HBV) and viral hepatitis C (HCV) disease burden in resource-limited settings. The objective of this study was to estimate the prevalence of HBV and HCV mono-infection, and co-infection with HIV, in several Médecins Sans Frontières (MSF) projects.

Methods

Cross-sectional surveys have been conducted, or are ongoing, in order to estimate the prevalence of HBV and/or HCV infection in MSF HIV cohorts in India, Kenya, Mozambique, and Myanmar (Table 1). The HIV—HCV cascade of screening in Dawei, Myanmar is also detailed, including the patients' baseline characteristics. HBV and HCV screening activities in prisons in Ukraine, slums in Pakistan, pre-natal and delivery care in Mozambique, and among blood donors in the Democratic Republic of the Congo are also reported. The following screening tests have been used: HCV rapid antibody tests (Oraquick®, Orasure®, or HCV spot®), and Determine HBsAg® for HBV screening.

Results

The HIV—HCV screening cascade in Dawei, Myanmar: among the 300 HIV—HCV co-infected patients identified using HCV Oraquick, 81.7% were male, the mean CD4 count was 370 cells/mm^3, HIV viral load was <1,000 copies/ml in 96.4%, 28.3% were migrants, 51.3% were fishermen, 42.3% reported past or present injection drug use, and 62.0% reported transactional sex. A total of 86.2% (207/240) displayed an aspartate-aminotransferase-to-platelet ratio index (APRI) >0.5, including 22.5% with an APRI >1.0 and 14.6% with an APRI >1.5. Patients with a positive Oraquick result and an APRI >0.5 were prioritised for HCV viral load and HCV genotype testing. Among the first 37 samples sent for genotyping and viral load testing, 18 samples were infected with HCV genotype 3, and 17 samples were infected with HCV genotype 1; only two samples had no active HCV infection.

Discussion

The prevalence of HBV and HCV mono-infection and co-infection with HIV in the settings surveyed reveals an unaddressed public health problem. More efforts are needed to better understand the true burden of HBV and HCV epidemics in resource-limited settings, as well as their risk factors, in order to design an adequate package of care and treatment. A combination of APRI and HCV serological testing is helpful to identify individuals most at risk of advanced liver disease, and to prioritise them for HCV viral load and genotype testing and treatment.

Conclusion

Scaling up access to screening, diagnostics, and generic treatments at an affordable price for people living with HBV and HCV infections in resource-limited settings remains a major barrier for mono and co-infected patients.

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Table 1: Prevalence of HIV—HCV and HIV—HBV co-infection, and HCV or HBV mono-infection in Médecins Sans Frontières projects.

HIV cohort	HIV—HCV prevalence, n (%)	HIV—HBV prevalence, n (%)
India, Manipur	325/1,125 (28.9)	60/1,234 (4.9)
Kenya, Kibera	2/650 (0.03)	133/2,543 (5.2)
Mozambique, Maputo*	9/565 (1.6)	112/565 (19.8)
Myanmar, Dawei	300/3,399 (8.8)	355/3,699 (9.6)
Myanmar, Yangon	574/12,281 (4.7)	1,408/14,652 (9.6)
Myanmar, Shan	1,006/3,820 (26.3)	400/3,183 (12.6)
Myanmar, Kachin	1,819/6,243 (29.1)	965/11,107 (8.7)
Myanmar, total	3,699/25,743 (14.4)	3,128/32,641 (9.6)
Other programmes	**HCV mono-infection, n (%)**	**HBV mono-infection, n (%)**
Pre-natal and delivery care Mozambique, Maputo	-	Pre-natal: 60/1,671 (3.6) Delivery: 134/2,801 (4.8)
Slums primary healthcare Pakistan, Karachi	159/522 (30.5)	26/679 (3.8)
Prisons drug-resistant tuberculosis Ukraine, Donetsk	163/268 (60.8)	19/305 (6.2)
Blood donors DRC, Baraka DRC, Kimbi	0/3,457 (0.0) 16/1,683 (1.0)	179/3,457 (5.2) 91/1,683 (5.4)

*Carinemo study[1]
DRC: Democratic Republic of the Congo; HCV: hepatitis C virus; HBV: hepatitis B virus.

WHO PREQUALIFICATION ASSESSMENT OF RAPID DIAGNOSTIC TESTS FOR HEPATITIS C VIRUS AND HEPATITIS B SURFACE ANTIGEN

***Anita Sands**

Diagnostics and Laboratory Technology Team, World Health Organization, Geneva, Switzerland
**Correspondence to sandsa@who.int*

Background

Regulation, especially that of *in vitro* diagnostics (IVDs), is often poorly understood by national authorities, and therefore tends to be poorly enforced. To bridge this gap in pre-market regulatory assessment of IVDs for resource-limited settings, the World Health Organization (WHO) coordinates the WHO Prequalification of IVDs Programme. Based on guidance set down by the Global Harmonization Task Force and its successor, the International Medical Device Regulators Forum, WHO conducts independent assessment of the safety, quality, and performance of IVDs used in resource-limited settings, i.e. rapid diagnostic tests (RDTs), CD4, and quantitative (viral load) and qualitative (early infant and adult diagnosis) nucleic acid tests for HIV, hepatitis C virus (HCV), hepatitis B virus (HBV), and malaria.

Challenges And Their Implications

The use of RDTs provides an opportunity to take testing for HCV and HBV outside of the traditional laboratory and into other types of health facilities and community settings. This is particularly

practical for certain key affected populations that might not access traditional health/medical services, and therefore testing services, and who might be better served within their community. Efforts to expand access to testing for HCV and HBV into non-laboratory settings have been hampered by the marketing of RDTs that are poorly adapted for use in these non-laboratory settings. Factors such as restricted stability in adverse environmental conditions (high/low temperature, high/low humidity) mean that many commercially available RDTs require cold chain storage. This is not technically surmountable as many HIV RDTs are stable at 2-30 °C. Therefore, manufacturers should be encouraged to undertake this additional design step, with adequate validation.

Furthermore, many RDTs are not suitable for use with capillary (fingerstick) whole blood and thus require phlebotomy and centrifugation of whole blood within 6 hours in order to generate serum/plasma. Again, manufacturers should be encouraged to adapt and validate their RDTs for use with capillary whole blood. For certain RDTs capable of measuring hepatitis B surface antigen (HBsAg), this cannot be circumnavigated because a large specimen volume is required, which cannot be feasibly collected by fingerstick. Moreover, poor analytical sensitivity of HBsAg RDTs undermines the usefulness of these products in screening (of asymptomatic individuals) such as in antenatal care services and blood transfusion services. However, these RDTs may provide some benefit once an individual is showing signs and symptoms consistent with viral hepatitis.

Way Forward

Manufacturers should be encouraged to adapt and validate RDTs for detecting anti-HCV antibodies and HBsAg for use in non-laboratory settings, including in resource-limited settings; this means RDTs that are simple and easy to use, RDTs that do not require cold chain storage, and RDTs that can be used with capillary whole blood. Furthermore, manufacturers should submit their products to the WHO Prequalification of IVDs Programme so that they may be independently evaluated for their safety, quality, and performance. These technical data are made available to end-users and programme managers.

Further Reading

World Health Organization. Prequalification of *in vitro* diagnostics. Available at: http://www.who.int/diagnostics_laboratory/evaluations/en. Last accessed: 12 May 2015.

WHO APPROACH TO DEVELOPING GUIDELINES ON TESTING FOR CHRONIC HEPATITIS B AND C INFECTION

***Philippa Easterbrook**

Global Hepatitis Programme, WHO, Geneva, Switzerland
**Correspondence to easterbrookp@who.int*

A key role of the World Health Organization (WHO) is the provision of normative guidance, particularly for low and middle-income countries. Over the last year, guidelines on the prevention, care, and treatment of persons with chronic hepatitis C virus (HCV) infection,[1] and for hepatitis B virus (HBV)[2] infection have been published. Distinctive features of these WHO guidelines, in comparison with guidance issued from other international or professional associations, include: (i) a target audience of national programme managers in low and middle-income settings, as compared with treating clinicians in high-income settings; (ii) adoption of a public health approach to treatment access that seeks to ensure the widest possible access to high-quality services at the population level, based on simplified and standardised approaches, as compared with an individualised treatment approach; and (iii) particular consideration of the other domains of the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) method and framework in formulating recommendations, such as feasibility, resource use, and cost-effectiveness, in addition to the evidence base.

A key barrier for the implementation of recommendations in these guidelines on treatment and care is that, at present, very few people in low and middle-income countries have access to HBV and HCV testing, as laboratory capacity is poor and testing facilities are few. As a result, most people infected with HBV and/or HCV are unaware of their infection, and generally only present when the disease is already advanced. This is further compounded by the lack of guidance on hepatitis testing in low-income settings. Earlier identification of persons with chronic HBV (or HCV) infection would enable infected persons to receive the necessary treatment required to prevent or delay the onset of liver disease, and also enables other preventative measures including HBV vaccination of susceptible household contacts and sex partners, to help interrupt ongoing transmission.

The WHO Global Hepatitis Programme is currently planning guidelines on testing for chronic HBV and HCV infection to complement the existing treatment guidance. The guidelines will address the following key issues: (i) Guiding principles of hepatitis testing and minimum standards: the '5 Cs' (consent, confidentiality, counselling, correct test results, linkage to care) and quality assurance of testing and of the assays/testing technologies used; (ii) How to screen, including testing strategies and validation of testing algorithms for HBV and HCV, and the principles for selection and use of assays; (iii) Who to screen, evidence base from modelling of impact and cost-effectiveness of different screening strategies using different prevalence thresholds, and recommendations for priority populations for HBV and HCV screening; (iv) Where and when to test, including testing in special settings and populations (drug treatment programmes or harm-reduction services, HIV-infected populations and HIV/antiretroviral therapy clinics, tuberculosis clinics, health-care workers, antenatal clinics, prisons, child health services, family planning clinics, community settings, and home-based or self-testing). This guidance will also build on the strong foundation and experience of more than two decades of HIV-testing policy and service-delivery experience with both provider-initiated and community-based testing and counselling.

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SOFOSBUVIR-LEDIPASVIR ACHIEVES 96% HCV CURE RATES IN HIV—HCV CO-INFECTION

***Curtis Cooper**

Division of Infectious Diseases, The Ottawa Hospital, Ottawa, Canada
**Correspondence to ccooper@toh.on.ca*

Historically, the most challenging aspect in curing hepatitis C virus (HCV)-infected populations with antiviral therapy was treating those individuals co-infected with HIV. Most co-infected patients were not eligible for interferon-based regimens; in those who were, the side-effect profile was severe and the sustained virological response (SVR) rate was <30% with pegylated interferon and ribavirin. The addition of a protease inhibitor increased SVR rates to the 60-70% range but with additional toxicity. The ION-4 study evaluated 335 HIV—HCV co-infected patients who received a 12-week course of sofosbuvir (SOF) and ledipasvir (LDV) co-formulated in a single tablet dosed once daily. All study participants were on antiretroviral therapy with fully suppressed HIV viral loads. HIV antiretroviral regimens consisted of efavirenz (EFV), raltegravir, or rilpivirine plus tenofovir (TDF) and emtricitabine. SOF/LDV was well tolerated without any serious adverse event attributable to the HCV antiviral medication. HIV viral load remained fully suppressed and no HIV-specific issues were identified. The overall SVR rate was 96% and was consistently within this range irrespective of prior HCV antiviral exposure or the presence or absence of cirrhosis. The SVR

rate was slightly lower in black patients (90%). The explanation for this observation continues to be explored. Of note, there was no evidence of reduced LDV levels in EFV recipients. There was no drug-attributable renal toxicity identified, despite the known interaction between LDV and TDF. Overall, this study demonstrated that a 12-week course of SOF/LDV is safe, well tolerated, and achieves very high cure rates in HIV—HCV co-infection.

T AND B CELL RESPONSES AND PREVIOUS EXPOSURE TO HEPATITIS B VIRUS IN 'ANTI-HBC ALONE' PATIENTS

Qixia Wang,[1,2] Philip Sachse,[1] Mariam Semmo,[3] Megha Lokhande,[1,4] Matteo Montani,[5] Jean-François Dufour,[1,4] Fabien Zoulim,[6] Paul Klenerman,[7] *Nasser Semmo[1,4]

1. Hepatology, Department of Clinical Research, Inselspital, University of Bern, Bern, Switzerland
2. State Key Laboratory for Oncogenes and Related Genes, Key Laboratory of Gastroenterology & Hepatology, Ministry of Health, Division of Gastroenterology and Hepatology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Institute of Digestive Disease, Shanghai, China
3. Department of Nephrology, Inselspital, University of Bern, Bern, Switzerland
4. Department of Hepatology, University Clinic of Visceral Surgery and Medicine, Inselspital, Bern, Switzerland
5. Institute of Pathology, Inselspital, University of Bern, Bern, Switzerland
6. Inserm, U1052, UMR CNRS 5268, Centre de Recherche en Cancérologie de Lyon, Lyon, France
7. Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, UK
**Correspondence to nasser.semmo@insel.ch*

Background and Aim

A serological response to the hepatitis B virus (HBV), defined as 'anti-HBc alone', is commonly observed but its significance remains unclear. This study aimed to define the relationship between anti-HBc alone serostatus and HBV infection, including HBV-specific T and B cell memory responses.

Methods

A total of 31 anti-HBc alone patients were enrolled. Total HBV DNA and covalently closed circular DNA (cccDNA) were tested by nested polymerase chain reaction analysis in liver samples from 22 anti-HBc alone patients versus controls (chronic or resolved HBV infection), followed by hepatitis B surface antigen (HBsAg)/hepatitis B core antigen (HBcAg) immunohistochemical (IHC) staining. Interferon gamma secretion by HBV-specific T cells was compared in individuals who were anti-HBc alone (n=27), resolved HBV (n=21), chronic HBV (n=24), and 12 healthy controls using enzyme-linked immunospot (ELISpot) assays. An HBsAg-IgG B-cell ELISpot assay was performed in anti-HBc alone patients before and after one dose of recombinant HBsAg vaccine.

Results

The majority (23/31, 74.2%) of the anti-HBc alone individuals were co-infected with hepatitis C virus (HCV). Infrequent intrahepatic total HBV DNA (2/22, 9.1%) and cccDNA (1/22, 4.5%) were detected in biopsies; HBsAg and HBcAg IHC staining was negative. HBV-specific T cell responses were similar between anti-HBc alone individuals and HBV resolvers. Circulating HBV-memory B cell responses were detected in all anti-HBc alone individuals, consistent with an HBsAg-specific memory pool. After one HBV vaccine dose, increased anti-HBs antibody levels were observed, accompanied by an expansion of HBsAg-specific memory B cells (p=0.0226).

Conclusion

Anti-HBc alone individuals showed HBV-specific T cell and memory B cell responses typical of previous viral exposure and protective memory, suggesting a resolved infection. Measuring memory B cells in particular may provide a helpful insight into the Ag-specific memory B cell pool available for long-term protection and for patient stratification in the face of immune-suppression

and biological therapy. One question at the ILC 2015 meeting in Vienna was whether there was a difference in the anti-HBc alone group with HCV co-infection and without HCV co-infection. We subsequently carried out a subanalysis with respect to the HBV-specific T cell responses and could not see any significant difference regarding this question.

ENGINEERED HBV-SPECIFIC T CELLS: DISENTANGLING ANTIVIRAL FROM KILLING CAPACITY

***Sarene Koh,[1] Christine Y. L. Tham,[2] Anthony T. Tan,[2] Andrea Pavesi,[3] Adeline Chia,[2] Roger Kamm,[4] Dario Campana,[5] Antonio Bertoletti[1,2]**

*1. Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore*
2. Emerging Infectious Diseases Program, Duke-NUS Graduate Medical School, Singapore
3. Singapore MIT Alliance – SMART, Singapore
4. Mechanobiology Laboratory, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
5. Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
**Correspondence to sarene_koh@sics.a-star.edu.sg*

Hepatitis B virus (HBV)-specific T cells are essential for the control of HBV infection. However, they are often deleted or functionally exhausted in patients with chronic HBV infection. Genetic engineering of T-cell receptors (TCRs) expressed by peripheral blood T cells can rapidly endow these cells with a defined antigen specificity[1,2] and represents an attractive approach to cellular therapy for persistent viral infections or tumours expressing viral peptides, such as chronic HBV infection and HBV-related hepatocellular carcinoma (HCC). Indeed, we recently demonstrated that adoptive transfer of engineered HBV-specific T cells in a patient with hepatitis B surface antigen (HBsAg)-productive HCC caused a profound inhibition of HBsAg production.[3]

The traditional method used to genetically engineer T cells, viral transduction, requires pre-activation of the T cells and produces T cells that permanently display anti-HBV specificity,[4] which may be difficult to eliminate once infused into patients. Moreover, the difficulty in disentangling the antiviral effect of HBV-specific T cells from their killing ability constitutes a barrier to the application of this immunotherapeutic approach in patients with chronic HBV infection. Therefore, our aim was to produce HBV-specific T cells that can inhibit viral replication without hepatotoxicity, and to analyse the antiviral mechanism mediated by these engineered T cells.

Using mRNA electroporation as a tool for HBV-specific TCR gene transfer,[5] we were able to express HBV envelope-specific TCRs in classical pre-activated T cells and unstimulated resting T cells. These resting electroporated T cells, unlike classical activated T cells, expressed low levels of perforin and granzymes. Analysis of their killing capacity in a 3-dimensional microfluidics device seeded with HepG2 target cells expressing HBV-envelope protein revealed that while activated electroporated T cells lysed targets efficiently, resting electroporated T cells did not lyse targets.

Furthermore, these perforin/granzyme-low HBV-specific T cells induced a 50% reduction in HBV viral load in HepG2.2.15 cells without causing detectable hepatotoxicity at a 1:3 effector-to-target ratio. After HBV-specific recognition, these resting electroporated T cells produced a large range of cytokines, such as interferon gamma, tumour necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, and lymphotoxin alpha and beta. Furthermore, blocking the activation of the lymphotoxin beta receptor (LTBR) reduced antiviral activity mediated by resting electroporated T cells, which shows that a specific synergy between classical antiviral cytokines and activation of the LTBR pathway mediates the antiviral but non-cytopathic effect.

In conclusion, we demonstrate that it is possible to produce HBV-specific T cells able to efficiently inhibit HBV replication without causing direct hepatocyte death. The prospects of using engineered T cells for treatment of chronic HBV infection are promising and, if successful, it might be possible to reconstitute an HBV-specific T cell repertoire that can achieve sustained control of HBV infection without overt lysis of HBV-infected hepatocytes (please also see the summary from Prof Antonio Bertoletti in this issue of *EMJ Hepatology*).

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CAN TREATMENT FOR HEPATITIS C VIRUS INFECTION BE DELAYED SAFELY? EVIDENCE FROM THE VETERANS ADMINISTRATION HEALTHCARE SYSTEM

***Jeff McCombs,[1,2] Tara Matsuda,[1] Ivy Tonnu-Mihara,[3] Steve Fox,[2,4]**

1. University of Southern California (USC) School of Pharmacy, Los Angeles, California, USA
2. Leonard D. Schaeffer Center for Health Policy and Economics, USC, Los Angeles, California, USA
3. Veterans Administration Health Care System, Long Beach, California, USA
4. Keck School of Medicine, USC, Los Angeles, California, USA
**Correspondence to jmccombs@healthpolicy.usc.edu*

Recent research by the University of Southern California (USC) and the Veterans Administration (VA) has produced a publication and three additional manuscripts that provide significant insight into how effective the previous standard treatment for hepatitis C virus (HCV) infection was in VA patients during the period 1999-2010. While most of the results from the USC/VA analysis of historical data are not surprising to clinicians, these studies provide the evidence needed to manage the use of the newer, more effective, and more extensive alternatives now available on the market.

The first paper, published early last year, found that suppression of viral load significantly reduces the risk of future liver events (hazard ratio [HR]: 0.73, 95% confidence interval [CI]: 0.66-0.82) and death (HR: 0.55, 95% CI: 0.47-0.64). Other results include data on the impact of HCV genotype (GT). Specifically, patients infected with GT2 experience lower risk relative to those infected with GT1, while GT3 patients experience higher risk for future events and death relative to GT1. Black patients are at lower risk of future liver events (HR: 0.72, 95% CI: 0.71-0.74]) and death (HR: 0.65, 95% CI 0.62-0.67) than white patients.

The second study identified five common laboratory tests whose results were correlated with changes in the risk of liver-related events and death over time: albumin, aspartate transaminase: alanine transaminase ratio, platelet count, gamma-glutamyl transferase, and alpha fetoprotein. More importantly, the effectiveness of the treatment in reducing the risk of adverse events decreased significantly if treatment was initiated after the results from these laboratory tests became 'abnormal'. Treatment initiated prior to any one laboratory test result becoming abnormal decreased the risk of the composite adverse clinical event (HR: 0.80, 95% CI: 0.70-0.92) and death (HR: 0.78, 95% CI: 0.65-0.93). Initiating treatment late reduced the reductions in risk for the composite adverse clinical event (HR: 0.96, 95% CI: 0.72-1.28) and death (HR: 0.83, 95% CI: 0.57-1.20).

The third study considered whether or not the progression of hepatic fibrosis (as assessed using FIB-4 scores) can be used to monitor the increasing risk of adverse liver-related events and death in HCV patients over time. Patients with a FIB-4 score >1.45 exhibit significantly increased risk of the composite event (HR: 2.22, 95% CI: 2.18-2.28) and death (HR: 2.27, 95% CI: 2.21-2.33). Patients with a FIB-4 score >3.25 exhibit an even greater risk of the composite clinical event (HR: 4.01, 95% CI: 3.92 4.10]) and death (HR: 3.56, 95% CI: 3.47-3.65).

The final piece of the puzzle was the study presented at EASL in April 2015. This study investigated whether or not the effectiveness of initiating treatment was affected by the patient's FIB-4 score at the time of treatment. Three FIB-4 scores were used to define 'early' and 'late' treatment: 1.00, 1.45, and 3.25. This study found that treatment was effective if initiated late as defined by these three FIB-4 scores, but delays in the initiation of treatment do have adverse effects on effectiveness, and the scale of the impact of the delay on effectiveness increased with the severity of the illness.

The take-away point from all of the above research on how well the older medication worked before 2010 is that waiting to initiate treatment during the early stages of an HCV infection does not increase risk or decrease the effectiveness of treatment beyond tolerable levels. Clinicians understood this already when they advised their patients to delay treatment pending the approval of the new medications that are now available. The patient's FIB-4 score works well as an indicator to watch while waiting to initiate therapy. More work is needed to draw the 'line in the sand' regarding the point at which treatment should be started.

SOX9 IS A NOVEL CANCER STEM CELL MARKER SURROGATED BY OSTEOPONTIN IN HUMAN HEPATOCELLULAR CARCINOMA

Takayuki Kawai,[1] *Kentaro Yasuchika,[1] Takamichi Ishii,[1] Hokahiro Katayama,[1] Elena Yukie Yoshitoshi,[1,2] Satoshi Ogiso,[1] Sadahiko Kita,[1] Katsutaro Yasuda,[1,2] Ken Fukumitsu,[1] Etsuro Hatano,[1] Yoshiya Kawaguchi,[2] Shinji Uemoto[1]

1. Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
2. Department of Clinical Application, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan
**Correspondence to kent@kuhp.kyoto-u.ac.jp*

Developments in stem cell biology have revealed the existence of cancer stem cells (CSCs) in various malignancies, including hepatocellular carcinoma (HCC).[1-5] CSCs are reported to be involved in many malignant processes, including tumour recurrence/metastasis through epithelial–mesenchymal transition (EMT). Therefore, CSCs have been receiving considerable attention as a key player during the establishment of new therapeutic strategies.[6] CSCs have similar properties to normal tissue stem cells. During normal liver development, sex-determining region Y-box 9 (SOX9) is known to be an important marker.[7-8] During liver fibrosis, SOX9 is reported to be surrogated by osteopontin (OPN), which is an important component of the extracellular matrix[9] and is also reported to be an important marker of early HCC.[10] However, the relationship between SOX9 and HCC-CSCs, and between SOX9 and OPN in HCC is not understood. From this background, we hypothesised that SOX9 can be used as a new CSC marker in HCC.

In this study, we used transgenic HCC cell lines that were transfected with a SOX9 promoter-driven, enhanced green fluorescence protein gene. CSC characteristics, involvement in EMT, and association with OPN were examined in fluorescence-activated cell sorting (FACS)-isolated SOX9+/SOX9− cells. Additionally, we investigated the expression of SOX9 and OPN in 166 human HCC surgical specimens and the serum level of OPN in SOX9+/SOX9− HCC patients.

FACS-isolated, single SOX9+ cells showed the ability to self-renew and differentiate into SOX9− cells, while single SOX9− cells could only self-renew in single-cell culture analyses. SOX9+ cells also displayed significantly greater proliferation capacity, higher colony/sphere-forming capability, and stronger 5-fluorouracil resistance based on higher expression of multidrug-resistant protein 5. Moreover, xenotransplantation into immunodeficient mice revealed that SOX9+ cells could generate larger tumours at a higher frequency than SOX9− cells. The tumours generated from SOX9+ cells contained SOX9+ and SOX9− cell fractions, while the tumours generated from SOX9− cells contained only SOX9− cell fractions. These results showed that SOX9+ cells have CSC properties *in vitro* and *in vivo*.

In addition, we found that, following stimulation with transforming growth factor beta (TGF-β), SOX9+ cells showed greater motility than SOX9− cells in migration and wound-healing assays. SOX9+ cells stimulated with TGF-β developed an EMT expression profile. In contrast, these changes were not observed in SOX9− cells stimulated with TGF-β. Our study also revealed that SOX9+ cells displayed higher levels of OPN expression than SOX9− cells, and that OPN expression was suppressed by SOX9 knockdown and rescued by SOX9 overexpression.

Immunohistochemistry of HCC specimens revealed that SOX9+ HCC patients had significantly poorer recurrence-free survival and stronger venous invasion. SOX9 expression was strongly correlated with OPN expression in both primary HCC and metastatic HCC regions. Receiver operating characteristics analysis showed that pre-operative serum OPN levels could be useful in identifying SOX9 expression in HCC patients.

In conclusion, SOX9+ cells possess CSC properties correlated with EMT in human HCC. Measurement of serum OPN levels may serve as a useful surrogate marker for SOX9 expression and be suitable for clinical application.

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NOVEL CONCEPTS IN IMMUNE THERAPY OF CHRONIC HBV INFECTION

***Antonio Bertoletti[1,2]**

1. Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore, Singapore
*2. Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*
**Correspondence to antonio@duke-nus.edu.sg*

Approved antiviral therapies for chronic hepatitis B virus (HBV) infection display only limited efficacy. Treatment with peginterferon alpha leads to sustained virological response in only one-third of patients, and is frequently associated with adverse side-effects. Nucleos(t)ide analogues effectively suppress HBV replication but do not eliminate the covalently closed circular DNA of HBV within hepatocytes, and thus most treated patients experience HBV reactivation after cessation of antiviral therapy. The failure to achieve sustained control of HBV infection is linked with an inability to mount an effective immune response similar to that which is present

in patients who have resolved acute HBV infection. Immunological therapeutic strategies designed to cure HBV infection aim to restore a level of antiviral immunity similar to that present in these patients. The goals of these strategies are to elicit a functional and efficient HBV-specific T or B cell immunity and trigger, within the intrahepatic compartment, the production of antiviral cytokines able to stably suppress HBV replication.[1] Indirect proof that such approaches work came from results obtained in chronic hepatitis B (CHB) patients who cleared HBV infection after receiving bone marrow transplantation from donors with natural HBV immunity.[2]

These immunological therapeutic strategies can be divided into two groups: therapies that increase the delivery (T cell receptor-like antibodies) or the production (Toll-like receptor [TLR] agonists) of antiviral cytokines within the liver; and therapies that aim to restore HBV-specific T cell immunity (checkpoint inhibitors, vaccine therapy, and T cell engineering).[3] Some of these approaches have already shown their potential. Results obtained in animal models and in patients have shown the ability of TLR agonists,[4] vaccines,[5] and TCR-redirected T cells[6,7] to increase intrahepatic cytokine production or restore a functional HBV-specific T cell response and achieve control of HBV replication.

One of the current challenges concerning the use of these new immunological therapeutic approaches is to define which categories of CHB patients should be targeted. Therapeutic guidelines for antiviral therapies recommend treating adult CHB patients categorised as 'immune active'. These CHB patients are characterised by elevated levels of alanine transaminase (ALT), an enzyme released by dying hepatocytes, and fluctuating levels of HBV DNA. However, such categorisation is, in reality, based on clinical and virological features that do not have any immunological meaning. The levels of ALT are an indicator of generic liver inflammation and not of the strength of the immune system.[8] For example, it is has been demonstrated that young CHB patients with a normal level of ALT (who are considered immunotolerant) harbour a superior level of residual HBV-specific T cell responsiveness than adult CHB patients categorised as 'immune active'.[9] It is likely that immunological therapies might be more active and less dangerous in patients with residual HBV-specific T cells and less active in those with pre-existing inflammatory liver conditions. For example, therapies involving checkpoint inhibitors such as anti-PD-1 or therapeutic vaccines should theoretically be efficacious in patients harbouring residual T cell responsiveness that can be boosted by antibodies or vaccines. In contrast, T cell therapy with engineered T cells should be targeted mainly towards patients who have a complete deletion of HBV-specific T cells.

The challenge of translating these new approaches into treatments for CHB patients will not only be the design of novel strategies with more 'immune activation potential'. A deeper understanding of how to individually tailor immunological therapies in the correct patient category and how to combine them with therapies directly targeting HBV replication (antisense, CRISPRs, nucleoside assembly inhibitors, infection inhibitors) is likely to be necessary to achieve functional control of HBV infection.

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HEPATITIS C VIRUS TRIGGERS WNT PARACRINE SIGNALLING THAT MODULATES METABOLIC LIVER ZONATION

Marie Moreau,[1] Benjamin Rivière,[2] Serena Vegna,[1] Jeanne Ramos,[2] Eric Assenat,[1] *Urszula Hibner[1]

1. Institut de Génétique Moléculaire, Montpellier, France
2. Département de Biopathologie Cellulaire et Tissulaire des Tumeurs, CHU Montpellier, Montpellier, France
**Correspondence to ula.hibner@igmm.cnrs.fr*

The physiological function of a healthy liver critically depends on a division of labour between hepatocytes whose metabolic identity is determined by their localisation along the centro-portal axis of the hepatic lobule.[1] This phenomenon, referred to as 'metabolic liver zonation', is controlled by paracrine signalling, including signals stimulating the Wnt/β-catenin pathway.[2] While the number of infected cells in chronically infected patients is variable, even a small minority can apparently give rise to major metabolic changes affecting the whole organ, which highlights the importance of inter-cellular crosstalk between the infected and the healthy parenchymal cells.

Hepatic steatosis, which is due largely to increased *de novo* lipogenesis, is a well-documented early outcome of hepatitis C virus (HCV) infection.[3] This outcome confers an evolutionary advantage on HCV because the virus critically depends on lipid droplets for its replication and spread.[4]

Transgenic mice with hepatocyte-targeted expression of all HCV proteins (FL-N/35 line) recapitulate several important aspects of human disease, including steatosis.[5-9] Using this transgenic model maintained against a genetic background that favours HCV-driven tumourigenesis (F1 C57BL/6 × C3H), we have discovered that the pattern of lipid droplet accumulation, which precedes tumour formation by several months, is exquisitely zonated (Figure 1, Panels A and B). Zonated steatosis is accompanied by an altered pattern of expression of fatty acid synthase, a key enzyme involved in lipogenesis, the expression of which coincides with steatotic cells in HCV-transgenic livers (Figure 1, Panels C and D).

Changes in metabolic zonation under the influence of viral proteins are not limited to lipid-metabolising enzymes. Immunohistochemical analysis of glutamine synthetase, a hepatospecific target of β-catenin, revealed a dramatic alteration in its pattern of expression within the livers of transgenic animals (Figure 1, Panels E and F).

To ascertain the relevance of our data to human pathology, we next analysed a large collection of needle biopsies from non-cirrhotic HCV patients in the early stages of the disease and infected with genotype 1 virus. Since the metabolic syndrome, which frequently complicates HCV infection, could be a confounding factor in the interpretation of our results, we excluded patients who were diabetic, obese or overweight, or had a known excessive alcohol consumption. These data fully confirmed the results obtained in the transgenic mouse model. Indeed, HCV-infected livers displayed a striking alteration in the pattern of expression of both the lipogenic and the glutamine metabolic enzymes. Interestingly, this phenotype preceded steatosis, although it was aggravated in fatty livers.

Finally, we investigated molecular mechanisms involved in HCV-driven alterations of metabolic liver zonation. We showed increased levels of expression of Wnt4, an activating ligand of the β-catenin pathway, in both transgenic mouse and infected human livers. We also demonstrated increased signalling of the pathway, which strongly argues for the importance of this 'master regulator' of liver zonation in the pathological consequences of HCV infection.

Our results shed new light on an early manifestation of pathology triggered by HCV infection, and they open novel perspectives for investigation of HCV-driven metabolic changes. It is noteworthy that perturbations of glutamine metabolism might impact a wide range of liver functions, including glutathione-dependent detoxification, acid–base balance, and susceptibility to tumourigenesis.

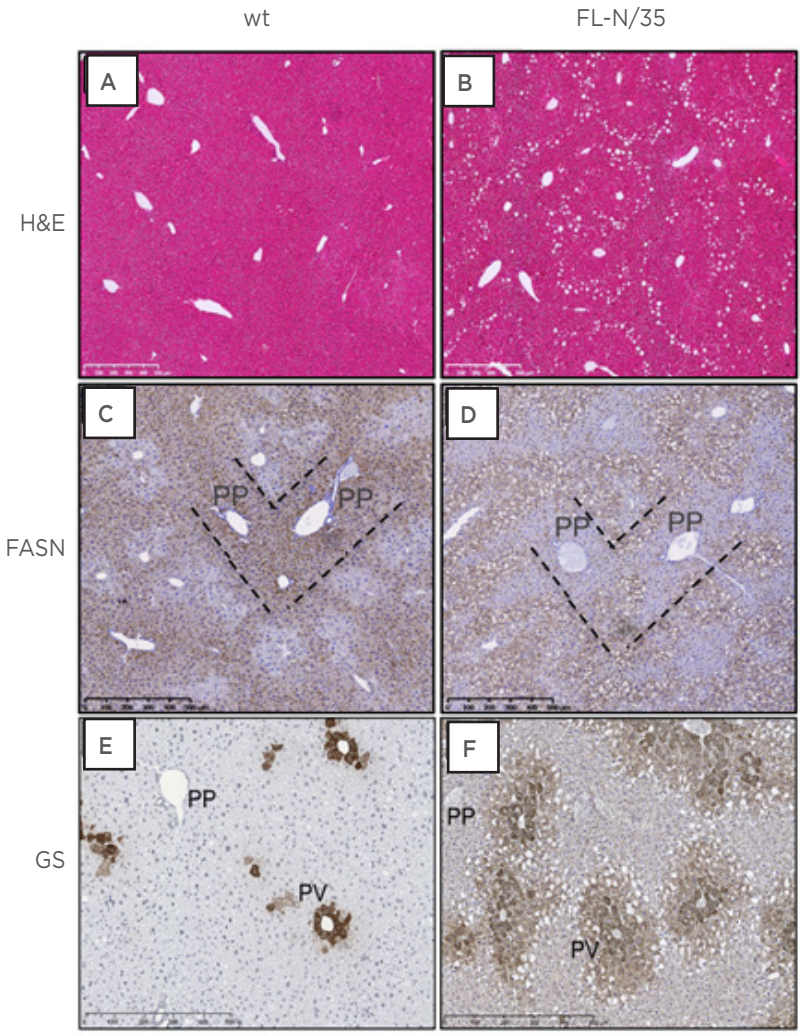

Figure 1: Immunohistological analysis of liver sections from wild-type (wt) and HCV-transgenic (FL-N/35) mice.
H&E staining allows visualisation of steatosis (Panels A and B). FASN (Panels C and D) and GS (Panels E and F) expression is altered in transgenic animals. PP and PV (centrilobular) regions are indicated. FASN: fatty acid synthase; GS: glutamine synthetase; H&E: haematoxylin and eosin; PP: periportal; PV: perivenous.

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EARLY CLINICAL FEATURES ASSOCIATED WITH LONG-TERM RISK OF TRANSPLANTATION IN PRIMARY SCLEROSING CHOLANGITIS: RESULTS FROM THE UK-PSC CONSORTIUM

***Elizabeth C. Goode,[1,4] Brijesh Srivastava,[2] Allan B. Clark,[3] Kelly Spiess,[4] Gideon M. Hirschfield,[5] William T.H. Gelson,[6] Palak J. Trivedi,[5] George F. Mells,[4] Richard N. Sandford,[4] Graeme J. Alexander,[2] Roger W. Chapman,[7] Simon M. Rushbrook,[1] and the UK-PSC Consortium**

1. Department of Gastroenterology, Norfolk and Norwich University Hospital, Norwich, UK
2. Division of Gastroenterology and Hepatology, Department of Medicine, University of Cambridge, Cambridge, UK
3. Norwich Medical School, University of East Anglia, Norwich, UK
4. Academic Department of Medical Genetics, University of Cambridge, Cambridge, UK
5. Centre for Liver Research and NIHR Biomedical Research Unit, University of Birmingham, Birmingham, UK
6. Department of Hepatology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
7. Department of Gastroenterology, John Radcliffe Hospital, Oxford, UK
**Correspondence to elizabeth.goode@nnuh.nhs.uk*

Background and Aim

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease that leads to end-stage liver disease, which necessitates transplantation. There is no effective medical treatment and transplantation does not guarantee a cure – there is a 20% recurrence rate in the graft. Recent evidence suggests that PSC is a heterogeneous disease with some groups conferring a particularly poor prognosis. It is important to identify those patients at an increased risk of adverse outcome early in their disease course so that they can be targeted for trials of new and emerging therapeutic agents. We therefore need to identify the early factors predicting adverse disease outcome and establish surrogate markers of disease outcome for use in clinical trials. Therefore, we sought to determine the baseline and follow-up factors associated with transplantation in a representative UK cohort.

Methods

Detailed phenotypic data regarding patients recruited to the UK-PSC national cohort (August 2008 to July 2014) were collated. Unadjusted hazard ratios (HRs) were estimated using the proportional hazards model; adjusted HRs were estimated using forward selection to identify variables associated with transplantation (Stata 11.2/SE).

Results

From a cohort of 1,700 patients, detailed phenotypic data from 500 patients (77 hospitals; 5 transplant centres; 59.4% male) were analysed. Median age at diagnosis was 49.5 years, median follow-up was 14 years, and median time-to-event was 5.6 years. Inflammatory bowel disease (IBD) was present in 68.4% of patients (75.1% ulcerative colitis) and 13.4% had another autoimmune disease. Ursodeoxycholic acid (UDCA; mean dose: 12.8 mg/kg/day) was prescribed to 87% of patients. A total of 100 patients (20%) received a liver transplant (median age: 51 years) and the need for transplantation was associated with cholangiographic disease burden, with only 9.4% of patients without cholangiographic changes at baseline undergoing transplantation.

Comparing sexes, 14% of females received a transplant compared with 23.9% of males; 9.2% of all patients died without receiving a transplant: 4.4% due to PSC. Gastrointestinal cancer, most commonly colorectal (4.2%) and cholangiocarcinoma (3%), developed in 8.8% of patients. According to univariate analysis, factors associated with an increased need for transplantation were baseline alkaline phosphatase (ALP) >2 times the upper limit of normal (ULN) (HR: 1.87, 95% confidence interval [CI]: 1.21-2.89; p=0.005) and male sex (HR: 1.63, 95% CI: 1.05-2.53; p=0.03). At 1 and

2 years post-diagnosis, ALP >1.5×ULN and >2×ULN were associated with transplantation need (p<0.001). The absence of cholangiographic changes at baseline was protective (HR: 0.38, 95% CI: 0.16-0.87; p=0.023). After multivariate analysis, ALP >2×ULN at baseline (HR: 2.32, 95% CI: 1.43-3.77; p=0.001) and Year 2 (HR: 2.96, 95% CI: 1.23-7.13; p=0.015) and ALP >1.5×ULN at Year 1 (HR: 2.92, 95% CI: 1.85-4.59; p<0.001) remained associated with the need for transplantation. Absence of cholangiographic changes (HR: 0.28, 95% CI: 0.11-0.72; p=0.008) and older age at diagnosis (HR: 0.98, 95% CI: 0.97-1.0; p=0.01) appeared protective. Notably, UDCA use and IBD had no effect on transplantation need.

Conclusion

Long-term outcome in patients with PSC is associated with cholangiographic disease burden and ALP at baseline and follow-up, with no effect of UDCA use. These emerging data from a representative UK cohort help inform models for disease stratification and the choice of early exploratory endpoints for clinical trials.

Discussion

Discussion centred on UDCA-prescribing practice in the UK. In our study, 87% of patients were prescribed UDCA, which reflects UK prescribing practices based upon European Association for the Study of the Liver guidance. However, similar to other studies, there was no effect of UDCA on transplantation rates. Further discussion included the use of transplantation as the primary outcome. Ultimately, the aim of emerging therapies in PSC will be to delay or prevent the need for transplantation. Thus, the consensus was that transplantation is the most appropriate and useful outcome measure. However, this study also suggests ALP may be a useful surrogate marker of disease outcome for use in clinical trials.

HIGH-THROUGHPUT SEQUENCING OF THE HUMAN HEPATIC PROGENITOR CELL NICHE REVEALS DIFFERENT SIGNALLING PATHWAYS DEPENDING ON THE UNDERLYING CHRONIC LIVER DISEASE

***Olivier Govaere**

Department of Imaging and Pathology, KU Leuven and University Hospitals Leuven, Leuven, Belgium
**Correspondence to Olivier.Govaere@med.kuleuven.be*

Under normal/healthy circumstances, the epithelial cells of the liver (hepatocytes and/or cholangiocytes) respond to injury by proliferating. When the damage is too severe, or when the epithelial cells become senescent (as the result of ongoing proliferation during 20-30 years of chronic liver disease [CLD]), the hepatic progenitor cells (HPCs) try to restore liver homeostasis by differentiating towards cholangiocytes or hepatocytes, depending on the damaged cell compartment and the underlying liver disease. The HPCs have typical biliary features that they gradually lose upon differentiating towards hepatocytes, whereas they gain mature biliary markers when they differentiate towards cholangiocytes.

Several signalling pathways, such as Hippo, Sonic Hedgehog, and Notch, have been reported to play an essential role in the process of differentiation, although the microenvironment of the HPCs is highly dynamic and complex, and encompasses a multitude of cellular interactions.[1-3] In this study we aimed to investigate the human HPC microenvironment, the so-called 'niche', by high-throughput sequencing in order to identify novel mechanisms that control activation and differentiation of HPCs. Using laser microdissection, the HPC niche was isolated from freshly frozen human liver samples obtained from patients diagnosed with primary sclerosing cholangitis (PSC; n=6) or infection with hepatitis C virus (HCV; n=5), as models of biliary or hepatocellular regeneration, respectively. Isolated mRNA was amplified and processed for Illumina HiSeq™

sequencing. Differentially expressed genes were integrated with Ingenuity® Pathway analysis and a selection of genes were validated at the protein level by immunohistochemistry on end-stage cirrhotic and early-stage liver samples (PSC and HCV).

A total of 304 genes were significantly differentially expressed between the HPCs in PSC and HCV samples. The recruitment and homing of inflammatory cells was distinctly different. The HPC niche in PSC was characterised by neutrophil-attractant chemokines (CXCL5, CXCL6, interleukin-8) together with CCL28, whereas HCV was characterised by T and B-lymphocyte infiltration and a strong interaction with macrophages (HPCs in HCV samples expressed the macrophage receptor MARCO). In addition, the composition of the niche's extracellular matrix differed depending on the disease (e.g. *FN1* and *LAMC2* were upregulated in PSC samples and *COL17A1* was upregulated in HCV samples) but also reflected the stage of the disease. The neighbouring endothelial cells also proved to have a different phenotype, and transcriptional regulators and growth factors were also differentially expressed between the diseases. Our data shed light on the different signalling pathways involved in the HPC niche of human biliary and hepatitis CLD. Not only do the HPCs display a different phenotype, but the composition of their niche varies depending on the underlying disease.

After the presentation at the congress, the Chair inquired about how our group would discern aetiology-dependent signals from signals that directly influence HPC behaviour, and how we would proceed with our current research. Validating markers of interest in another CLD is one possibility, such as in alcoholic steatohepatitis where there is not only hepatocyte damage but also cholangitis. In the future we aim to further investigate the functional role of different proteins on the behaviour of HPCs using *in vitro* models, as well as *in vivo* mouse models (for example, focussing on the role of the extracellular matrix).

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THE UK-PBC RISK SCORES: DERIVATION AND VALIDATION OF A SCORING SYSTEM TO PREDICT THE LONG-TERM RISK OF END-STAGE LIVER DISEASE IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

***Marco Carbone,[1,2,3] Stephen J. Sharp,[3] Steven Flack,[1] Dimitrios Paximadas,[1] Kelly Spiess,[1] Carolyn Adgey,[4] Laura Griffiths,[5] Reina Lim,[6] Paul Trembling,[7] Kate D. Williamson,[8] UK-PBC Consortium, Nick J. Wareham,[3] Mark Aldersley,[9] Andrew Bathgate,[10] Andrew K. Burroughs,[11] Michael A. Heneghan,[12] James M. Neuberger,[6] Douglas Thorburn,[11] Gideon M. Hirschfield,[13] Heather J. Cordell,[14] Graeme J. Alexander,[2] David E. Jones,[5] Richard N. Sandford,[1] and George F. Mells[1,2]**

1. Academic Department of Medical Genetics, University of Cambridge, Cambridge, UK
2. Division of Gastroenterology and Hepatology, Addenbrooke's Hospital, Cambridge, UK
3. MRC Epidemiology Unit, University of Cambridge, Cambridge, UK
4. Liver Unit, Royal Victoria Hospital, Belfast, UK
5. Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
6. Liver Unit, Queen Elizabeth Hospital, Birmingham, UK

7. Liver Unit, Barts and the London NHS Trust, London, UK
8. Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford, UK
9. Liver Unit, St James's University Hospital, Leeds, UK
10. Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, UK
11. Sheila Sherlock Liver Centre, The Royal Free London NHS Foundation Trust, London, UK
12. Institute of Liver Studies, King's College Hospital NHS Foundation Trust, London, UK
13. Centre for Liver Research and NIHR Biomedical Research Unit, University of Birmingham, Birmingham, UK
14. Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK
**Correspondence to mc767@medschl.cam.ac.uk*

Using the largest ever prospective cohort of patients with primary biliary cirrhosis (PBC) - the UK-PBC Research Cohort, which included more than 4,000 patients with PBC - we developed and validated a scoring system to predict the medium and long-term risk of end-stage liver disease in patients with PBC. We modelled variables by using the Cox proportional hazards regression model and the multivariable fractional polynomial approach in order to detect any non-linearity in the relationship between the hazard for an event and each variable within the best-fitting model. We developed and internally validated three scores that accurately quantify the risk of liver failure occurring within 5, 10, and 15 years in patients treated with ursodeoxycholic acid.

This scoring system can be used in clinical practice as a disease management tool to identify high-risk patients for closer monitoring and second-line therapies, as well as low-risk patients who require infrequent monitoring and might even be followed up in primary care. This can certainly lead to a better distribution of healthcare resources, reduction of the health costs, and improvement of care delivery. However, before clinical application, the validation of the scoring system in an external cohort is needed.

SILYMARIN FOR THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS: INTERIM ANALYSIS OF A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

***Wah-Kheong Chan,[1] Nik Raihan Nik Mustapha,[2] Sanjiv Mahadeva[1]**

1. Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
2. Department of Pathology, Hospital Sultanah Bahiyah, Alor Setar, Malaysia
**Correspondence to wahkheong2003@hotmail.com*

Silymarin, which is derived from the milk thistle plant *Silybum marianum,* has been used for centuries as a herbal remedy for liver diseases.[1] There have been several *in vitro* and animal studies demonstrating the anti-oxidant, anti-inflammatory, and anti-fibrotic properties of this product.[2-5] Several human studies have also suggested that silymarin may be useful for the treatment of non-alcoholic fatty liver disease (NAFLD).[6-8]

We initiated a randomised, double-blind, placebo-controlled study of silymarin 700 mg t.i.d. for the treatment of non-alcoholic steatohepatitis (NASH) in November 2012. All included patients had biopsy-proven NASH, were given lifestyle advice, and received either silymarin or placebo for 48 weeks. A repeat liver biopsy was performed at the end of the study.

An interim analysis was performed in February 2015 and included 64 patients who had completed the study. The results were presented at The International Liver Congress™ 2015 in Vienna, Austria.[9] The changes in liver histology that occurred in the silymarin and placebo groups after 48 weeks are summarised in Table 1. The most important finding was that silymarin treatment was associated with a significantly higher percentage of patients with NASH resolution and fibrosis

improvement compared with placebo. While patients in both groups experienced significant improvement in the NAFLD activity score, only patients in the silymarin group demonstrated significant improvement in the stage of fibrosis. It is interesting to note that none of the patients in the silymarin group developed cirrhosis while four patients in the placebo group did.

This is the first ever study of silymarin for NASH that utilised paired liver biopsies and will provide histological confirmation of previous observations that silymarin is beneficial for the treatment of NAFLD. Furthermore, the randomised, double-blind, placebo-controlled design eliminates the potential for confounding due to lifestyle modifications and potential biases that may be seen in open-labelled, uncontrolled studies. The percentage of patients with fibrosis improvement was comparable to that seen following treatment with obeticholic acid 25 mg for 72 weeks in the FLINT trial.[10] This remarkable preliminary finding should be confirmed by completion of the study and by further studies with a larger number of patients.

Table 1: Changes in liver histology in the silymarin and placebo groups after 48 weeks.

	Silymarin (n=30)	Placebo (n=34)	p for difference between groups
Primary endpoint*, n (%)	10 (33.3)	7 (20.6)	0.517
Histological improvement**, n (%)	20 (66.7)	19 (55.9)	0.378
Steatosis Improvement, n (%) Mean change p for change within group	 7 (23.3) −0.133 0.211	 8 (23.5) −0.088 0.414	 0.985 0.765
Inflammation Improvement, n (%) Mean change p for change within group	 8 (26.7) −0.167 0.134	 14 (41.2) −0.353 0.002	 0.223 0.216
Ballooning Improvement, n (%) Mean change p for change within group	 15 (50.0) −0.433 0.003	 12 (35.3) −0.265 0.037	 0.235 0.352
NAS Improvement, n (%) Mean change p for change within group	 18 (60.0) −0.733 0.003	 20 (58.8) −0.706 0.006	 0.924 0.934
NASH resolution***, n (%)	4 (13.3)	0 (0)	0.043
Fibrosis Improvement, n (%) Mean change p for change within group	 11 (36.7) −0.367 0.019	 5 (14.7) +0.147 0.282	 0.043 0.012
Development of cirrhosis, n (%)	0 (0)	4 (11.8)	0.116

*Primary endpoint was defined as at least 30% improvement in NAS.
**Histological improvement was defined as improvement in fibrosis stage regardless of changes in NAS, or improvement in NAS without any change in fibrosis stage.
***NASH resolution was defined as NAS <3.
For continuous variables the p values were calculated using paired t-tests for changes within a group and independent t-tests for changes between groups; chi-squared tests or Fisher exact tests, where appropriate, were used for categorical variables.
NAS: non-alcoholic fatty liver disease activity score; NASH: non-alcoholic steatohepatitis.

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CARDIOVASCULAR DISEASE AND MORTALITY ACROSS THE NON-ALCOHOLIC FATTY LIVER DISEASE SPECTRUM IN THE UK

***Jake P. Mann,[1] Matthew J. Armstrong,[2] Hardeep Uppal,[3] Suresh Chandran,[4] Philip N. Newsome,[2] Rahul Potluri[3]**

1. Department of Paediatrics, University of Cambridge, Cambridge, UK
2. NIHR Centre for Liver Research, University of Birmingham, Birmingham, UK
3. ACALM Study Unit in collaboration with Aston Medical School, School of Medical Sciences, Aston University, Birmingham, UK
4. Department of Acute Medicine, North Western Deanery, Manchester, UK
**Correspondence to jakemann@doctors.org.uk*

Background

Non-alcoholic fatty liver disease (NAFLD) is recognised as an important risk factor for cardiovascular disease (CVD), and CVD represents the main cause of death in NAFLD. However, it is not entirely clear whether there is a step-wise increase in risk during progression from simple steatosis (non-alcoholic fatty liver [NAFL]) to non-alcoholic steatohepatitis (NASH) and then to NASH-cirrhosis.

The association between NAFLD and CVD is thought to be partly due to shared aetiological factors, as well as the acceleration of atherosclerosis by NAFLD.[1] Central adiposity and insulin resistance conspire to generate elevated levels of circulating non-esterified fatty acids, which are deposited in the sub-endothelium and hepatic parenchyma. Elevated insulin with hepatic insulin resistance triggers *de novo* hepatic lipogenesis, which exacerbates the circulating dyslipidaemia and hepatic steatosis. NAFLD is also strongly associated with overt Type 2 diabetes, hypertension, and hypertriglyceridaemia.

There is also evidence to support increased atherosclerosis in NASH compared with NAFL.[2] The hepatic inflammation may contribute to: systemically elevated inflammatory cytokines (e.g. C-reactive protein and interleukin 6), reduced fibrinolysis (due to elevated plasminogen activator inhibitor 1), and hypercoagulability (from increased Factor VIII and fibrinogen). However, this is not entirely consistent with epidemiological findings. A meta-analysis demonstrated an increase in overall mortality between NAFL and NASH, although no increase in cardiovascular deaths.[3]

Summary of Study

In the current study, we used the Algorithm for Comorbidities, Associations, Length of stay and Mortality (ACALM) study database to identify patients with NAFLD-spectrum disease using

International Classification of Disease (ICD)-10 codes.[4,5] This database contains completely anonymous information from 1 million patients in England and was compiled by applying the ACALM method to hospital admissions data. We then assessed over 2,700 patients with NAFLD-spectrum diagnoses (48% with simple NAFL[D], 5% with NASH, and 47% with NASH-cirrhosis) for cardiovascular comorbidities and risk factors using ICD-10 codes. We also calculated crude, all-cause mortality over 14 years of follow-up.

Here, we report a trend towards increasing association of CVD with increasing severity of the NAFLD spectrum. For example, atrial fibrillation was found in 5% with NAFL(D), 8% with NASH, and 8% with NASH-cirrhosis. Type 2 diabetes increased from 21% in NAFL(D) to 25% in NASH and to 31% in NASH-cirrhosis. There was also a step-wise increase in crude, all-cause mortality across 14 years: 15% for NAFL(D), 22% for NASH, and 53% for NASH-cirrhosis.

Conclusion

These represent the first UK data regarding the burden of CVD across a large cohort of NAFLD patients. There is a trend of increasing CVD and mortality as severity of NAFLD progresses. Clinicians should screen NAFLD patients for CVD and NAFLD, and have a higher index of suspicion in more advanced disease.

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THE USE OF A POCKET-SIZED ULTRASOUND DEVICE CAN REDUCE THE NEED FOR FURTHER TESTS IN A VARIETY OF CLINICAL SETTINGS

***Agostino Colli**

Department of Internal Medicine, Ospedale A Manzoni, Lecco, Italy
**Correspondence to a.colli@ospedale.lecco.it*

Pocket-sized ultrasound devices (PUDs) offer a comparable diagnostic accuracy to standard ultrasonography, and the reproducibility and accuracy of a physical examination is often poor, meaning that further tests are required. This study assessed whether adding the use of a PUD to physical examination could lead to a reduction in the rate of additional tests. After a short training period, 135 physicians (90 general practitioners, 30 specialists in internal medicine, and 15 hepatologists) evaluated the effectiveness of the PUD when testing for the following conditions (diagnostic hypotheses): ascites, pleural effusion, pericardial effusion, gallstones, biliary-duct dilation, urinary retention, urinary stones, splenomegaly, abdominal mass, and aortic aneurysm. The physicians had to decide whether PUD examination was able to exclude or confirm the diagnostic hypothesis and whether any additional tests were required. Their decision was based on their confidence in the PUD results and their clinical judgment.

Of the 1,962 patients included in the study:

- 726 (37%) were inpatients, 510 (26%) were hepatology outpatients, and 726 (37%) were recruited from general practitioners.
- Gallstones (37%), ascites (17%), pleural effusion (13%), urinary stones (13%), and urinary retention (12%) accounted for more than 90% of the clinical questions. PUDs were used to confirm 66% of the clinical questions, which suggests a prevailing use of the PUD to 'rule in' the diagnostic hypotheses.

- The overall frequency of further tests needed after use of a PUD was 37%, i.e. the physicians were confident in the PUD results and did not order additional tests (standard ultrasound, computed tomography, and magnetic resonance imaging) in 63% of cases. More than 1,000 additional tests were spared.
- The diagnostic accuracy of the PUD was estimated in the 645 patients who underwent additional tests. Considering these tests as the reference standard, the sensitivity of the PUD was 90.8% (95% confidence interval [CI]: 88.3-93.4%), the specificity was 83.2% (95% CI: 77.3-89.1%), with likelihood ratios (LRs) of: LR+ 5.4 (95% CI: 3.8-7.70) and LR− 0.11 (95% CI: 0.08-0.15). These performance characteristics allow the PUD to play a role as a triage test or even to replace existing tests in some cases.

In the 1,089 patients who did not undergo further testing, the concordance of PUD results with the final diagnosis, i.e. the diagnosis at discharge or at the end of a 3-month follow-up, was assessed. The PUD results were not confirmed by the final diagnosis in 10% of positive PUD results and not confirmed in only 5% of negative results.

In conclusion, after a brief period of simple training, a PUD examination can be successfully used by different physicians in different settings as a means of considerably reducing the number of further diagnostic tests for 10 common clinical indications. Adding a PUD examination to a physical examination is therefore a promisingapproach to reducing waiting times and healthcare costs.

NOVEL TREATMENT OPTIONS TO IMPROVE SPLICING AND PROTEIN FOLDING IN ATP8B1 DEFICIENCY

***Wendy van der Woerd,[1,2] Johanna Mulder,[1] Franco Pagani,[3] Ulrich Beuers,[1,4] Roderick Houwen,[2] Stan van de Graaf[1,4]**

1. Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, Netherlands
2. Department of Pediatric Gastroenterology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, Netherlands
3. Human Molecular Genetics, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy
4. Department of Gastroenterology & Hepatology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands
**Correspondence to wwoerd@umcutrecht.nl*

ATP8B1 deficiency is a severe, autosomal recessive liver disease caused by mutations in the *ATP8B1* gene and characterised by intrahepatic cholestasis. The clinical spectrum ranges from only episodic symptoms (benign recurrent intrahepatic cholestasis [BRIC]) to progressive familial intrahepatic cholestasis. Current therapeutic options are generally invasive and often insufficient. Therefore, the aim of our study was to explore new treatment possibilities, specifically mutation-specific therapies, and we focussed on mutations expected to affect pre-messenger RNA splicing and mutations associated with protein misfolding.

Firstly, we decided to elucidate the molecular consequences of 14 *ATP8B1* mutations located at exon—intron boundaries and associated with ATP8B1 deficiency. Their effect on pre-messenger RNA splicing was analysed using an *in vitro* minigene system, and the resultant splicing products were evaluated by reverse transcription polymerase chain reaction. Eleven mutations resulted in the complete absence of correctly spliced product and three mutations led to partially incorrect splicing. Interestingly, patients harbouring mutations associated with residual, correctly spliced mRNA generally exhibited a less severe BRIC phenotype. To explore the possibility of rescuing aberrant splicing, mutation-adapted versions of U1 small nuclear RNA (snRNA) were created. Expression of modified U1 snRNA complementary to the splice donor sites strongly improved or completely rescued splicing for several *ATP8B1* mutations located at donor as

well as acceptor splice sites. Furthermore, exon-specific U1 snRNA variants targeting non-conserved intronic sequences downstream of the exon were also very effective in correcting exon skipping.

It was previously shown that the missense mutation p.I661T, which is most frequently identified in European patients, led to protein misfolding and disturbed protein homeostasis (proteostasis). Cystic fibrosis (CF), which is caused by mutations in the CF transmembrane conductance regulator gene (*CFTR*), is a more common protein-folding disease. Previous screening identified several compounds called 'CFTR correctors' that were able to rescue the misfolded CFTR protein. The potential of 14 of these correctors to restore ATP8B1 p.I661T plasma membrane expression was evaluated by cell surface biotinylation. Six compounds resulted in significant upregulation of ATP8B1 p.I661T plasma membrane expression, which suggests a more general function as proteostasis regulators.

In conclusion, we found that the majority of *ATP8B1* mutations at exon—intron boundaries resulted in total exon skipping. The amount of correctly spliced product was inversely correlated with disease severity. Treatment with modified U1 snRNAs could be applied successfully to correct splice defects of *ATP8B1* transcripts in a minigene system. In addition, we showed that CFTR correctors, functioning as proteostasis regulators, were able to improve *ATP8B1* p.I661T plasma membrane expression. Therefore, modified U1 snRNAs and proteostasis regulators are promising strategies for future personalised treatment of ATP8B1 deficiency, as well as numerous other genetic diseases.

THE USE OF DIRECT ORAL ANTICOAGULANTS IN PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS AND/OR CIRRHOSIS: SUMMARY FROM ILC 2015

***Andrea De Gottardi[1,2]**

1. On behalf of the EASL-VALDIG investigators
2. Department of Clinical Research and Clinic of Visceral Surgery and Medicine, Inselspital, University of Bern, Bern, Switzerland
**Correspondence to andrea.degottardi@insel.ch*

Direct oral anticoagulants (DOACs) are increasingly used off-label in patients with splanchnic vein thrombosis (SVT) and/or cirrhosis. However, data regarding the safety and efficacy of these new drugs in patients with cirrhosis are limited to case reports or small series and their use cannot yet be recommended in this setting.[1] In addition, drug-induced liver injury has been increasingly described in patients treated with DOACs.[2] Therefore, the aims of this study were to provide an overview of the use of DOACs in this group of patients, to identify the indications and the reasons for starting or switching from other anticoagulants, and to collect information regarding safety issues by describing possible adverse effects (AEs).[3]

Collection of data including demographic information, clinical and laboratory data, DOAC treatment characteristics, and complications was performed using an electronic survey, which was sent to all centres of the VALDIG Consortium.[4] Between June and December 2015, 45 centres (90%) answered the questionnaire and the use of DOACs in the above-mentioned indications was reported in 94 patients from 17 centres (38%). In the 58 patients (62%) who did not have cirrhosis (mean age: 50 years; equal gender distribution), the most frequent indication for anticoagulation was portal vein thrombosis (PVT) (65%), followed by atrial fibrillation and Budd-Chiari syndrome (BCS). Over a median treatment duration of 13 months, rivaroxaban was the most commonly used DOAC (84%), followed by dabigatran and apixaban. These drugs were administered at full anticoagulant dose *de novo* (33%) or to replace another anticoagulant treatment (low-molecular-weight heparin in 45% of cases). DOACs were chosen in order to avoid the need for international normalisation ratio (INR) monitoring in 33% of cases, or due to complications with other anticoagulants in 15% of cases. Bleeding

complications occurred in 9/58 patients, but only in two cases, after variceal band ligation and after hysterectomy, was it classified as major.

Another 36 patients (38%) had cirrhosis (21 men and 14 women; mean age: 52 years; mean Child-Pugh (CP) score: 5.7 points; Model for End-Stage Liver Disease score: 11). Despite the fact that indications for anticoagulation were identical to those in patients without cirrhosis, the daily dose of DOACs in this group was 25% lower. The main reason for choosing DOACs was the inadequacy of INR to monitor anticoagulation in patients with cirrhosis treated with vitamin K antagonists. Bleedings were reported in 6/36 patients, but in only one case (lower gastrointestinal bleeding) was it considered as a serious AE. PVT recurred in one patient during treatment with rivaroxaban. In both groups of patients, with or without cirrhosis, no significant changes in kidney or liver function tests were reported.

In conclusion, DOACs were used off-label in patients with portal vein thrombosis, BCS, or cirrhosis (CP Class A-B, but not C) in approximately 40% of the participating centres. Complications of DOACs included not only bleeding but also re-thrombosis. Although DOACs cannot currently be recommended either for the treatment of splanchnic thrombosis or in cirrhotic patients, they are regularly used off-label in several centres.

At ILC 2015, the participants in the discussion following the presentation of these data, and in the early-morning workshop on the use of DOACs in patients with liver disease, underlined the potential advantages of these new anticoagulants in patients with mild hepatic impairment, but also expressed their concerns due to the lack of validated tests to monitor the coagulation function in cirrhotic patients. This means that, although complications seem to occur rarely, caution is needed when prescribing DOACs in patients with SVT and/or cirrhosis until more data regarding the safety and efficacy of DOACs in these patients are available.

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