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# HEPATOLOGY

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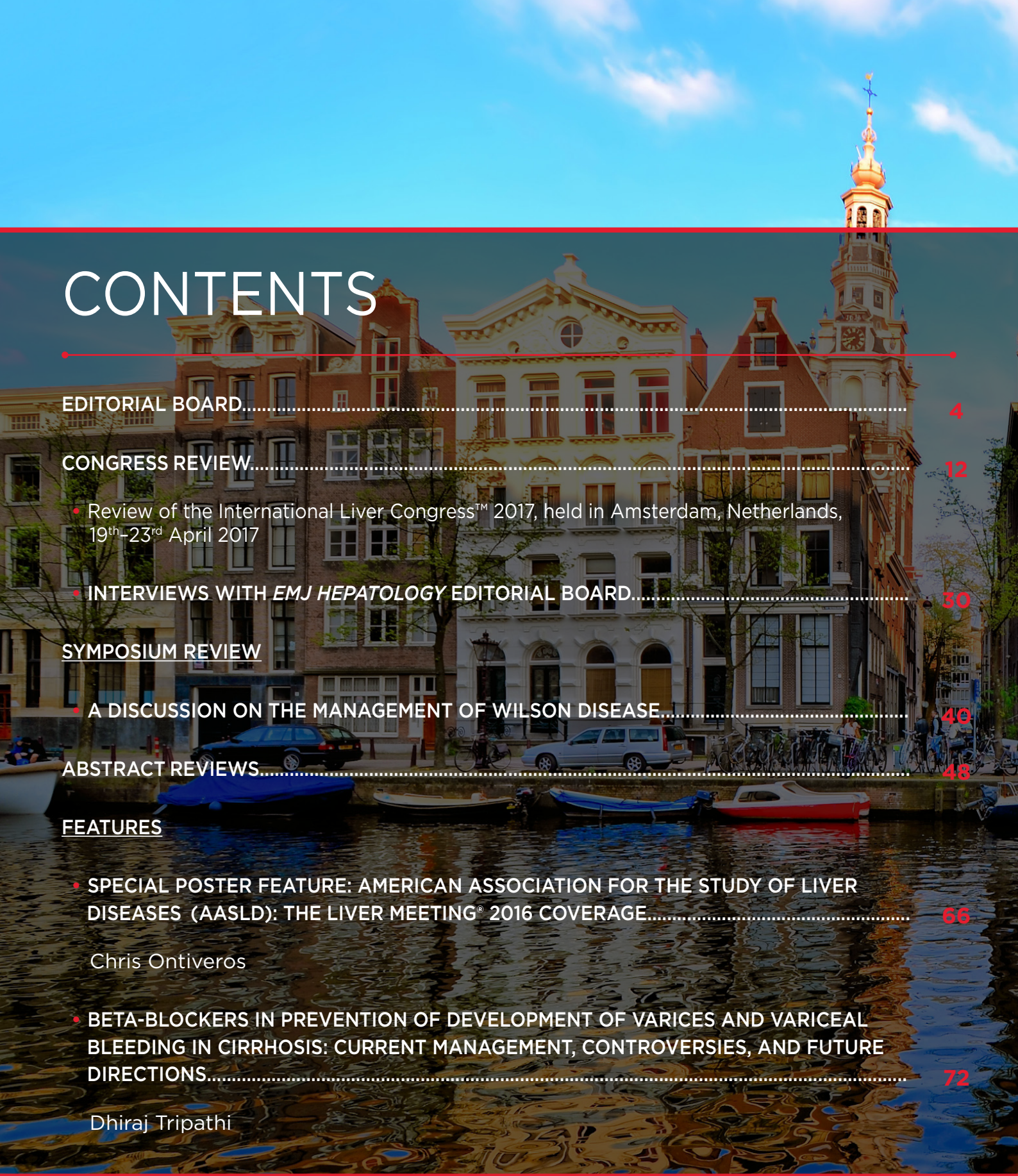
## INSIDE

Review of

**ILC 2017**

Amsterdam, Netherlands



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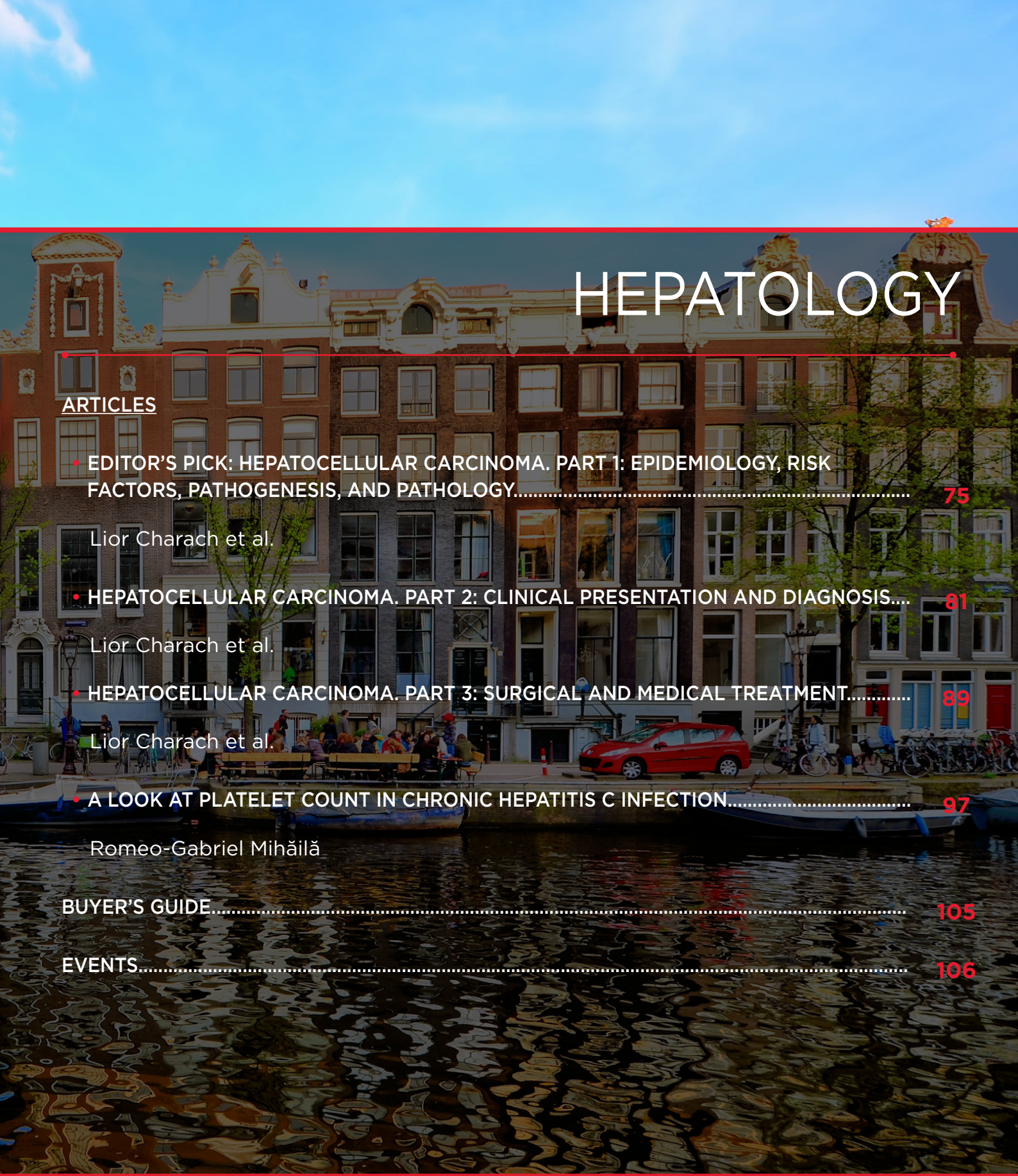

# HEPATOLOGY

## ARTICLES

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*Front cover and contents photograph: Amsterdam, Netherlands, home of ILC 2017. © Jennifer Barrow / 123rf.com*

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**HEPATOLOGY** 5.1
JUNE 2017



Staff members attend Medical Congresses as reporters when required.

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# Welcome

I would like to bid everyone a warm welcome to *EMJ Hepatology 5.1*. In this edition, you will find a selection of peer-reviewed articles, entertaining interviews with members of our *EMJ Hepatology* Editorial Board, and coverage of this year's International Liver Congress™ (ILC). The wide variety of content is sure to feature something of interest for all who specialise in hepatology.

This year, the EMJ team visited the historic city of Amsterdam, Netherlands, to attend the ILC. The event saw >10,000 delegates and 250 media representatives from the 127 countries that attended. In our congress review section, we have provided summaries of some of the breaking news discussed at the congress, a selection of the abstracts presented, and further information about the meeting. Alongside our coverage of the ILC, we are excited to include a special feature giving insight into the American Association for the Study of Liver Diseases (AASLD) Liver Meeting®. This article summarises poster presentations focussed on viral hepatitis and orthotopic liver transplantation, as well as oral presentations discussing chronic hepatitis infection.

We are proud to offer interviews with four members of our Editorial Board. These are, as always, highly interesting. The interviewees discuss a wide range of topics, such as their personal inspirations, the major challenges for hepatologists, and whether viral hepatitis can be eliminated as a major public health threat by 2030. It is always a privilege to obtain insights from leading hepatologists, and we hope that you too will be able to learn something from our Editorial Board members.

> "We hope you find *EMJ Hepatology* as exciting and interesting as we do, and that it inspires you throughout the year."

In our articles section, Mihăilă has penned a paper examining platelet count in patients with chronic hepatitis C, and Charach et al. have written a tripartite article on hepatocellular carcinoma, which is our Editor's Pick for this edition. This thorough examination details the epidemiology, risk factors, pathogenesis, pathology, clinical presentation, and diagnosis of hepatocellular carcinoma before covering surgical and medical treatment options. We have also included a feature by Tripathi on beta-blockers for the prevention of the development of varices and variceal bleeding in cirrhosis.

We hope you find *EMJ Hepatology* as exciting and interesting as we do, and that it inspires you throughout the year. It has been an absolute pleasure producing this journal and I would like to wish you all a successful year!

Spencer Gore

*Director, European Medical Journal*

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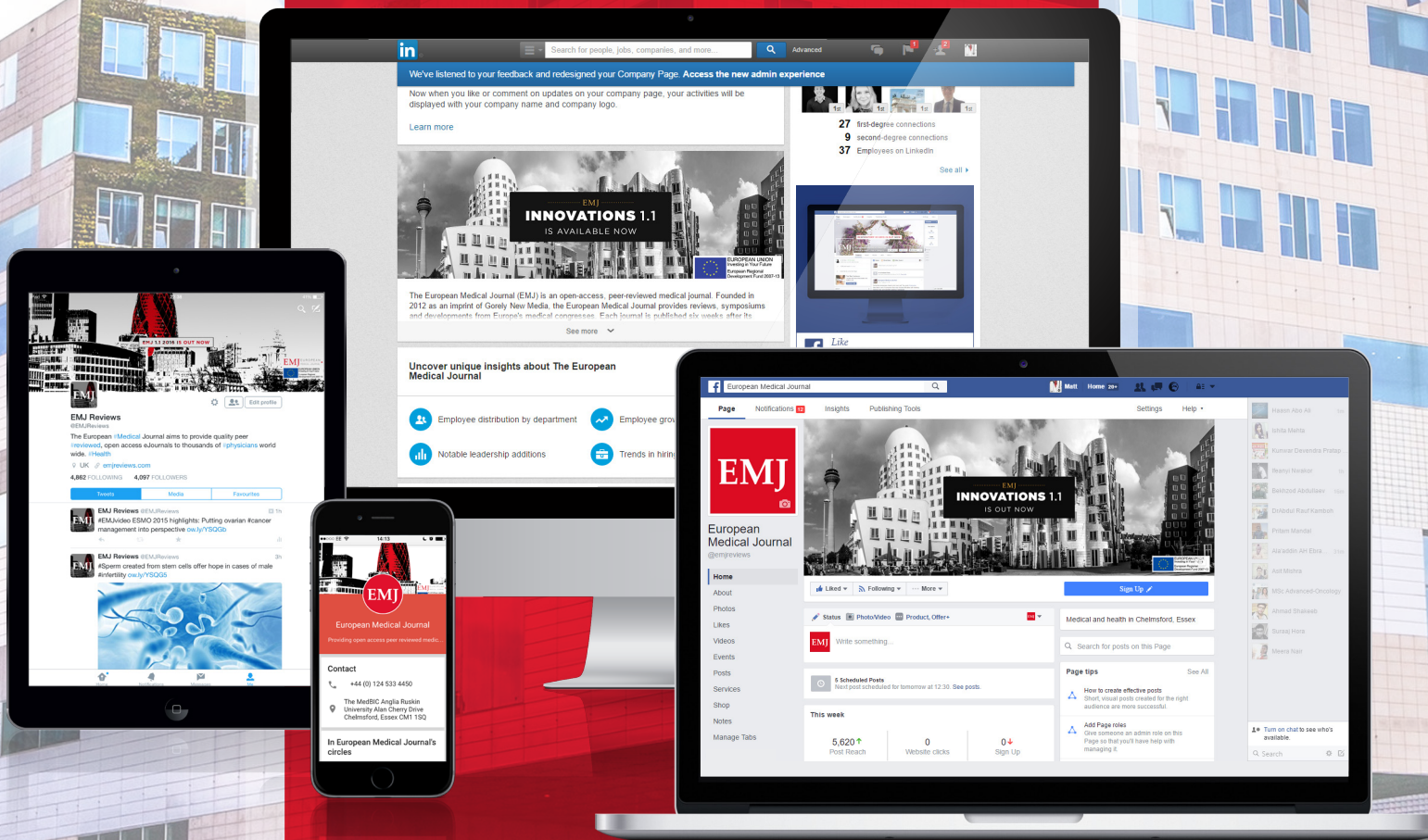


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# Foreword


## Prof Markus Peck-Radosavljevic

*Professor of Medicine and Chairman, Department of Gastroenterology and Hepatology, Endocrinology and Nephrology, Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria.*


Dear Colleagues,

I would like to welcome you to this new issue of *European Medical Journal Hepatology* 2017. After returning from the International Liver Congress™ (ILC), it was good to see that the European Association for the Study of the Liver (EASL) is still top of the list of all the large international liver meetings. Despite the fact that the focus in liver disease has shifted a bit, ILC was again able to feature a large number of very interesting and scientifically relevant presentations.

Liver cancer was one of the hop topics in this year's congress. Particular interest was fostered by the presentation of the first large Phase III trial featuring selective internal radiotherapy (SIRT) with $^{90}$Yttrium and comparing it to sorafenib. Despite high hopes, the trial was negative, missing the primary endpoint of showing superiority of SIRT compared to sorafenib. The broad introduction of such treatments without proper scientific proof through positive Phase III trials should be strongly discouraged and more trials testing SIRT are yet to come.

Drug treatment of liver cancer (hepatocellular carcinoma [HCC]) is still a large focus in hepatology, in particular after the recent success of regorafenib in second-line treatment of HCC. At the ILC, further data from the CheckMate 040 trial (the first evaluation of the immunotherapeutic PD-1 inhibitor nivolumab in HCC) were presented. At the ILC, the presentation was focussed on the comparison of virus-induced HCC patients versus other HCC patients, all of whom had been pretreated with sorafenib.

Last but not least, an absolute highlight of the conference was the ANSWER trial, a study of long-term regular outpatient albumin-infusions in patients with cirrhosis and uncomplicated ascites that was able to show improvement in all parameters studied, including most importantly, a highly significant improvement in overall patient survival. This trial has the potential to be truly practice-changing for the management of patients with moderate-to-advanced stage cirrhosis.

For those unable to attend, the main highlights of the event are documented inside this edition. There are also a number of reviews of presentations from the congress, contributed by the presenters themselves, with particular highlights being magnetic resonance imaging (MRI) of diffuse liver diseases and the role of civil society in eliminating hepatitis C.

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 2018 hosted in Paris, France.


## Markus Peck-Radosavljevic

Professor of Medicine and Chairman, Department of Gastroenterology and Hepatology, Endocrinology and Nephrology, Klinikum Klagenfurt am Wörthersee, Klagenfurt, Austria; Fellow, Austrian College of Physicians; Member, 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, and the Austrian Society for Internal Medicine (ÖGIM).

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### Light Sheet Fluorescence Microscopy Combined with Optical Clearing Methods as a Novel Imaging Tool in Biomedical Research

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### What do we Mean by Innovation in Healthcare?

- Leighann Kimble, M. Rashad Massoud

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# ILC ANNUAL CONGRESS 2017

RAI AMSTERDAM,
AMSTERDAM, NETHERLANDS
19TH–23RD APRIL 2017

## Welcome to the European Medical Journal review of the 52nd Annual Meeting of the International Liver Congress™.

The diverse city of Amsterdam, Netherlands, hosted this year's International Liver Congress™ (ILC) of the European Association for the Study of the Liver (EASL). Known as 'the little Venice of the North' the city boasts impressive architecture and a rich history, much of which can be viewed in its historic centre. Its quirky and lively atmosphere, with cycling routes throughout the city and beautiful canals, makes it the perfect place for the flagship event of the hepatology calendar.

The EASL aims to "promote liver research and improve the treatment of liver diseases worldwide" with its overall vision being: "Many Ways, One Aim: Beating Liver Disease." As well as recognising the outstanding work of professionals in the field, the congress promoted public awareness about liver diseases, encouraged scientific research into the study of the liver, and endorsed state-of-the-art education for physicians and scientists. By hosting this annual event, the EASL hopes "to reduce the burden of liver disease in Europe by advocating for more research funding and effective prevention policies."

This April's 5-day spectacle certainly embodied the goals of the EASL as it brought industry experts and healthcare professionals from around the globe to showcase their expertise in the field of hepatology. This year hosted a plethora of fields surrounding hepatology including gastroenterology, internal medicine, cell biology, transplant surgery, infectious diseases, microbiology and virology, pharmacology, pathology and radiology, and imaging. The event attracted >10,000 delegates and 250 media representatives from 127 countries, making this a momentous occasion with opportunities to learn more about the latest surrounding liver research. The USA provided the greatest number of delegates, closely followed by the UK, Germany, Italy, France, Spain, China, Netherlands, Belgium, and Switzerland. Among the 2,576 abstracts submitted this year, a total of 1,682 abstracts were accepted. There were 1,510 poster sessions, whilst the remaining 172 were oral sessions.

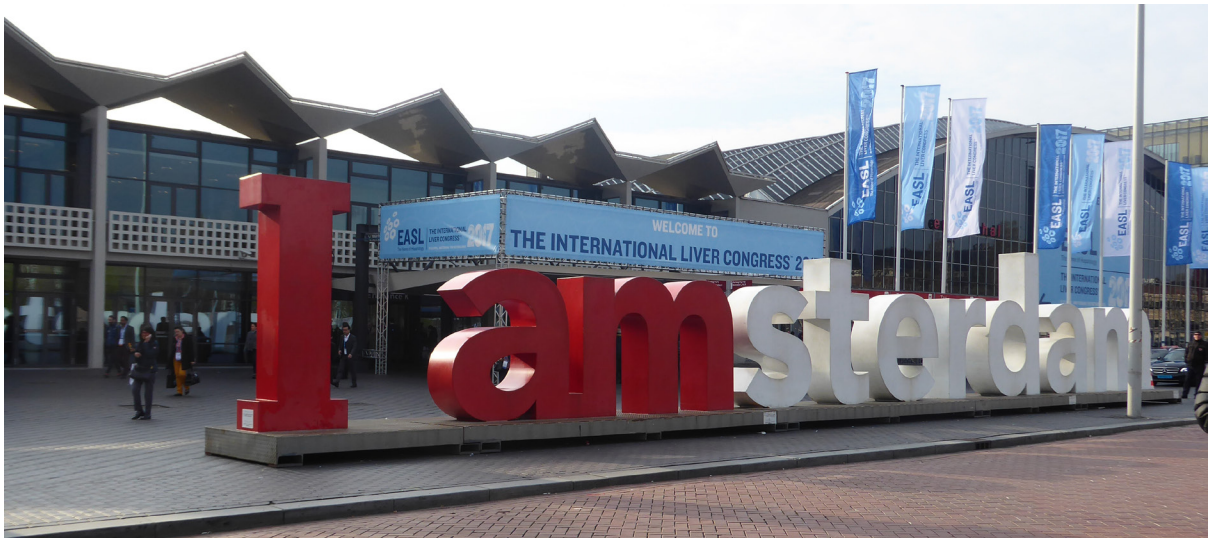
To honour some of the work in this field, the EASL presented three recognition awards to acknowledge those individuals who are making their mark in the field of hepatology and to thank them for their incredible efforts in advancing the

study of the liver. Among these recognition awards was British paediatrician Giorgina Mielli-Vergani (UK), Gustav Paumgartner (Austria), who provided seminal work in polycystic liver disease, and Kenjiro Wake (Japan) for discovery of the Sternzellen in the liver. Now in its third year, the EASL Young Investigators Award category, which is dedicated to young investigators based on their commitment to international liver research and their achievements to date, went to Evaggelia Liaskou (UK) and Jean-Charles Nault (France) who also received a cash prize.

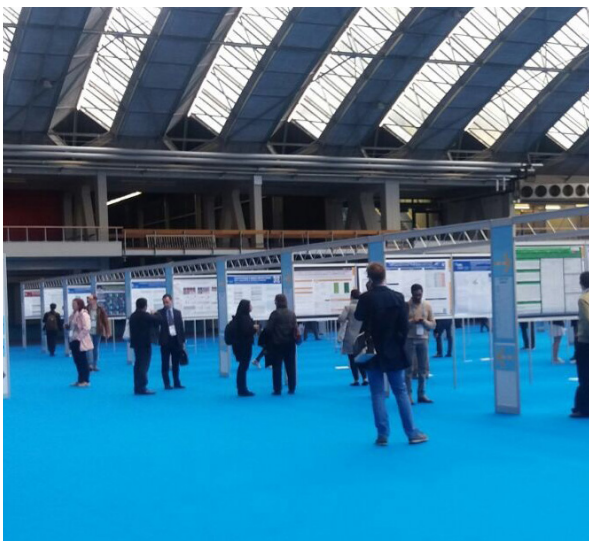
In the following pages, you will find a review of some of the fascinating updates this year's congress had to offer, alongside some groundbreaking new research into the areas such as hepatitis, cirrhosis, and liver disease. We do hope this year's congress review section will remind those who attended of some of the fascinating developments in the field that you were lucky enough to have witnessed. For those who did not attend, we hope this brings to light what you missed and truly excites you as you delve further into our journal.

Next year's congress, which is sure to be even more fascinating and packed with the latest hepatology updates, will be hosted in April from the dates of 11th–15th April in the 'City of Love', Paris, France.

To honour some of the work in this field, the EASL presented three recognition awards to acknowledge those individuals who are making their mark in the field of hepatology...








## Potential Advance in Treatment for Paediatric Cholestatic Liver Disease

RESULTS of a study of a novel ileal bile acid transport inhibitor, known as A4250, were presented at this year's ILC meeting and reported in an ILC press release dated 22nd April 2017. The inhibitor was shown to reduce the levels of blood (serum) bile acids in children who have cholestatic liver disease. Children can be vulnerable to diseases that either destroy or impair the development of the biliary tree, leading to other problems such as progressive liver injury, cirrhosis, and acid bile retention, which is related to pruritus. Patients and clinicians urgently need novel therapies to help manage pruritus and the increased levels of bile acid serum. A4250 has been demonstrated to reduce the elevated levels of bile serum acids in previous studies without any severe side effects.

> "The study results are crucial as they address pruritus, a significant issue in chronic cholestatic diseases. Currently, there are few therapeutic options, with limited efficacy, so new treatment strategies for pruritus are of great importance for clinical practice."

Four patients received five doses of A4250 (0.01–0.2 mg/kg), and single doses were initially administered to individuals with intractable itching and cholestatic disease. The drug was administered in tablet form for 4 weeks. Therapy with rifampicin or ursodeoxycholic acid (UDCA) was also available throughout the study if needed. Nineteen individuals, aged between 1 and 17 years, were enrolled reporting itching by a visual itch score using patient data.

Results showed that pruritus improved in 14 of 19 cases and the mean level of serum bile acid was reduced. Seven out of nine individuals displayed considerable reductions in serum bile acid. There were no serious side effects of the drug, and few mild side effects could be related directly to the drug. A4250 blocks the ileal bile acid transporter in the last part of the small intestine, which consequently reduces the levels of bile acids in serum. Reabsorption of intestinal bile acids was inhibited;[1] thus, they cannot be recirculated and secreted, making A4250 a highly effective inhibitor of the ileal bile acid transporter.

Prof Marco Marzioni, Clinic of Gastroenterology, Università Politecnica delle Marche "Ospedali Riuniti", University Hospital of Ancona, Ancona, Italy, commented: "The study results are crucial as they address pruritus, a significant

issue in chronic cholestatic diseases. Currently, there are few therapeutic options with limited efficacy, so new treatment strategies for pruritus are of great importance for clinical practice."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## Norfloxacin Improves Prognosis of Patients with Child–Pugh C Cirrhosis

AN IMPROVEMENT in prognosis for patients with advanced liver disease has been demonstrated as a result of long-term orally administered antibiotic therapy in a multicentre, randomised controlled clinical trial. Norfloxacin administered for 6 months resulted in a reduced risk of infection and death at 6 months in patients diagnosed with Child–Pugh class C cirrhosis. The results of this study were reported in an ILC press release dated 20th April 2017.

> "This study shows that long-term oral antibiotic therapy may improve the prognosis of patients with life-threatening liver disease. However, overuse of broad spectrum antibiotics is a subject that has been thoroughly debated over the years."

"This study shows that long-term oral antibiotic therapy may improve the prognosis of patients with life-threatening liver disease. However, overuse of broad spectrum antibiotics is a subject that has been thoroughly debated over the years," explained Dr Richard Moreau, Liver Unit, Beaujon Hospital, Clichy, France, the lead study author. He added: "The results from this study provide evidence that 6 months of norfloxacin therapy reduces the risk of infections and death in the short-term, but not in the long-term."

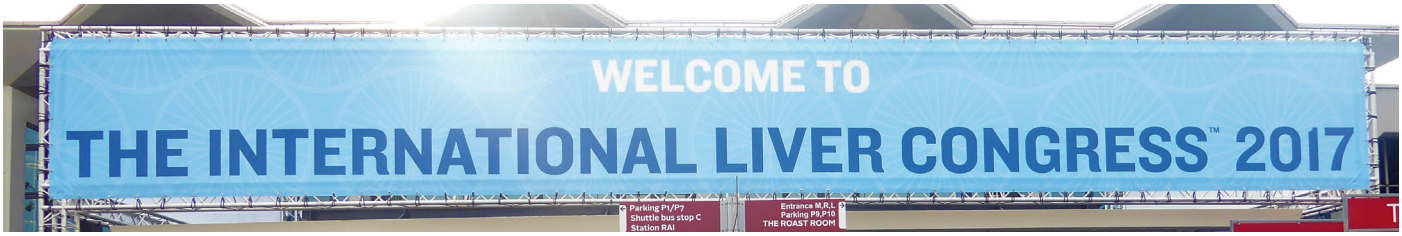
The Child–Pugh classification scoring is used to evaluate and estimate the prognosis of patients with cirrhosis. Scoring ranges from A–C, with the most advanced cases falling within the C category. Decompensation of the liver, whereby the functioning of the liver is hindered due to extensive scarring, is seen in patients with Child–Pugh class C cirrhosis.

Patients diagnosed with Child–Pugh class C (N=291) were enrolled in to the Phase III study and received either a daily dose of 400 mg norfloxacin or placebo for a 6-month period. On completion of the 6-month treatment phase, it was noted that fewer patients had died in the norfloxacin subgroup compared to placebo (22 [15.3%] versus 36 [24.5%], respectively). Further comparison confirmed the number of patients who had received a liver transplant (norfloxacin [n=17] versus placebo [n=15]).

Patient follow-up was performed for an additional 6 months, subsequent to the 6-month treatment phase, to monitor infection, death, and additional liver-related complications. Cumulative mortality incidence was lower in the active norfloxacin subgroup than the placebo group (15.5% versus 24.8%, respectively). However, at 12-month follow-up the cumulative incidence of death was comparable across the two groups. Analysis also confirmed that at 6-month follow-up, patients who had been administered norfloxacin had developed fewer infections than those receiving placebo (30 [20.8%] versus 46 [31.3%], respectively).

## Cognitive Impairment Improved by Faecal Microbiota

FAECAL transplantation of bacteria given from healthy individuals to those suffering from hepatic encephalopathy has been shown to improve cognitive function. The study, presented in an ILC press release dated 21st April 2017, explained that the number of hospitalisations that followed antibiotics and faecal transplantation was 2, compared to 11 in the standard of care arm (lactulose and rifaximin). A significant decline in hospitalisation for recurrent encephalopathy was reported. The treatment was well-tolerated with no serious side effects.




> “The results from this study demonstrate that in patients with hepatic encephalopathy, a faecal transplant improves brain function more than standard of care as well as reducing the number of hospital admissions, including those for recurrent hepatic encephalopathy.”

Dr Jasmohan Bajaj, Virginia Commonwealth University, Richmond, Virginia, USA, commented: “The results from this study demonstrate that in patients with hepatic encephalopathy, a faecal transplant improves brain function more than standard of care as well as reducing the number of hospital admissions, including those for recurrent hepatic encephalopathy. Faecal transplantation is an innovative and promising approach to treat this condition, and we look forward to more studies being conducted to confirm our results.”

Twenty men with cirrhosis and who had experienced recurrent episodes of hepatic encephalopathy were randomised to either broad spectrum antibiotics with a single faecal transplantation with a healthy donor and antibiotics for 5 days as well as standard of care treatment, or only rifaximin and lactulose. They were followed-up 150 days after they received treatment. In the faecal transplant group significant cognitive improvements were found on the Psychometric Hepatic Encephalopathy Score and the Stroop App. The results also showed that The Model for End Stage Liver Disease (MELD) score returned to baseline following the faecal transplant (delta -0.2; p=0.5), yet it increased after antibiotic treatment (delta 1.7; p<0.001). In addition, only 1 patient from the faecal transplant group demonstrated a decline in cognitive function, yet following the faecal transplantation returned to baseline.

## Researchers Investigate New Ways to Accurately Predict Severe Liver Disease

OBESITY, high alcohol consumption, diabetes, lipid abnormalities, and insulin resistance were described as major contributors to the development of severe liver disease in an ILC press release dated 22nd April 2017. Diabetes was also stated to be the most significant predictor of severe liver disease for individuals who consume substantial amounts of alcohol daily (>140 g/week in women and >210 g/week in men).

Researchers examined which metabolic factors best predicted severe liver diseases whilst classifying the results based on the amount of alcohol consumed reported in the Finnish Health Study 2000, a nationally representative cohort. Waist circumference, total cholesterol, age, and ‘homeostatic model assessment’ (HOMA)-index were used to predict the development of liver disease in those who have no or mild alcohol consumption.

Lead author, Dr Fredrik Aberg, Transplantation and Liver Surgery Clinic, Helsinki University, Helsinki, Finland, commented: “The results of this study can help us identify which people are at risk of developing severe liver disease, so that we can work with them to reduce those risks.” He continued: “It is important that the risk factors identified in our study are considered for use in future risk models so that doctors can identify and counsel those patients at risk for developing liver disease.”

The study used individuals who were representative of the Finnish population and who had participated in the Health 2000 study, conducted between 2000 and 2001. A total of 6,732 individuals were included, all without known liver disease. The research collected follow-up data over the next decade of liver-related cancer, hospital admissions, and deaths.

> The results of this study can help us identify which people are at risk of developing severe liver disease, so that we can work with them to reduce those risks.

Prof Philip Newsome, Centre for Liver Research, University of Birmingham, Birmingham, UK, added: "These data emphasise the important role of diabetes and metabolic syndrome in the development of liver disease, reinforcing the need to consider liver disease in such patient groups."

## High Animal-Protein Diet Increases Non-alcoholic Fatty Liver Disease Risk

AN EPIDEMIOLOGICAL study, reported in an ILC press release dated 21st April 2017, has demonstrated that an increased risk of non-alcoholic fatty liver disease (NAFLD) is associated with a diet high in animal protein, which is predominantly observed in overweight, elderly individuals.

NAFLD is an accumulation of fat within the liver, which can ultimately result in cirrhosis, an increased risk of cancer, cardiovascular disease, and kidney malfunction.[1] NAFLD has a worldwide incidence of approximately 1 billion people.[2] Early stages of NAFLD can be treated with changes to diet and lifestyle to aid weight-loss, however there is speculation, now more than ever, as new evidence emerges proposing that diet composition rather than increased calorie intake is fundamental factor in the development of NAFLD.

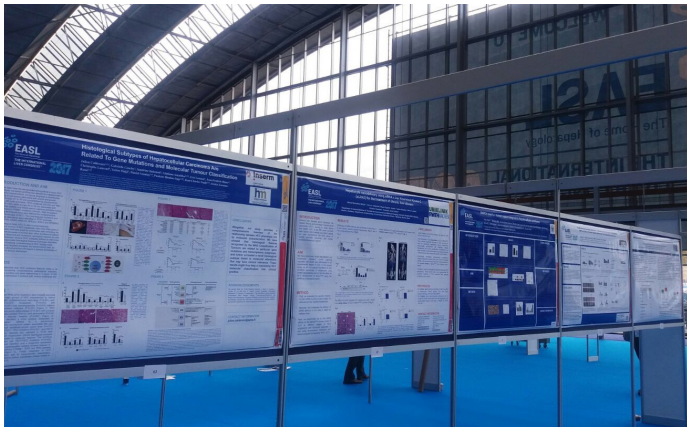
"A healthy lifestyle is the cornerstone of treatment in patients with NAFLD, but specific dietary recommendations are lacking," explained lead study author, Dr Louise Alferink, Department of Gastroenterology and Hepatology, Erasmus Medical Centre, University Medical Centre, Rotterdam, Netherlands.

The study included 3,440 individuals; 30% were lean (BMI <25 kg/m$^2$) and 70% were overweight (BMI of ≥25 kg/m$^2$). The average age was 71 years and NAFLD was present in 35% of patients. The intake of macronutrients was monitored using a validated food-frequency questionnaire and data were analysed in quartiles using the nutrition density methodology. When considering the association between macronutrient intake and NAFLD, noteworthy findings were seen predominantly amongst overweight patients.

Initial results indicated that total protein was associated with increased likelihood of NAFLD (odds ratio [OR]: Q4 versus Q1 1.37; 95% confidence interval [CI]: 1.08–1.73, p=0.005). Furthermore, this association was primarily driven by animal protein (OR: Q4 versus Q1 1.50; 95% CI: 1.17–1.92; p=0.003).

Further consideration of metabolic factors resulted in the acceptance that it was animal protein, rather than total protein, that was mainly responsible for the development of NAFLD.

> This large population-based study indicates that increased dietary protein, in particular of animal origin, increases the likelihood of developing NAFLD and should be taken into account when counselling patients at risk of developing NAFLD.



EASL Governing Board Member, Prof Philip Newsome, Centre for Liver Research, University of Birmingham, Birmingham, UK, commented: "This large population-based study indicates that increased dietary protein, in particular of animal origin, increases the likelihood of developing NAFLD and should be taken into account when counselling patients at risk of developing NAFLD."

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## SIRT Versus Sorafenib: A Dispute Between Overall Survival and Tolerance

PATIENTS with inoperable and locally advanced hepatocellular carcinoma (HCC) have been demonstrated to have a median overall survival score of 8.0 months when treated with selective internal radiation therapy (SIRT), compared to a median of 9.9 months when treated with sorafenib. Results from the SARAH trial, a randomised, open-label, controlled, multicentre investigator-initiated Phase III trial, were reported in an ILC press release dated 22nd April 2017.

The study included 459 individuals, 237 of whom received SIRT, from 25 French clinical centres. In the sorafenib and SIRT groups the median progression-free survival (PFS) was 3.7 months and 4.1 months, respectively (p=0.765). Furthermore, it was found that the cumulative incidence did not differ in either of the studies. In the SIRT group there were 1,297 treatment-related adverse events (AEs) with 230 of these Grade ≥3. In contrast, in the sorafenib group there were 2,837 treatment-related AEs and 411 of these were Grade ≥3. The Global Health Status scale of the EORTC QLQ-C30 questionnaire assessed quality of life, reporting that it was significantly better in patients who received SIRT over sorafenib (p=0.005).

Prof Valérie Vilgrain, Hôpital Beaujon Service de Radiologie, Paris, France, stated: "While SIRT demonstrated significantly reduced side effects, better quality of life, higher response rates, and more effectively controlled tumour progression in the liver, the overall survival of patients was not higher than in the sorafenib group. Nonetheless, this study provides evidence that SIRT may be a better-tolerated alternative for managing this complex and difficult-to-treat disease, deserving further evaluation."


"While SIRT demonstrated significantly reduced side effects, better quality of life, higher response rates, and more effectively controlled tumour progression in the liver, the overall survival of patients was not higher than in the sorafenib group. Nonetheless, this study provides evidence that SIRT may be a better-tolerated alternative for managing this complex and difficult-to-treat disease, deserving further evaluation."


Prof Alejandro Forner, BCLC group, Liver Unit, Hospital Clinic, Barcelona, Spain and EASL Governing Board member, highlighted the importance of this study: "The SARAH trial is the first reported randomised controlled trial evaluating the survival benefit of SIRT in locally advanced HCC compared to sorafenib." Prof Alejandro also explained that although SIRT was recognised as safe, it was not shown to have superior survival against sorafenib. Unfortunately, the study did not meet the primary endpoint, indicating that further trials are needed to ensure it can be a treatment option for patients.

## Nivolumab: Acceptable Safety and Promising Efficacy Profiles for Sorafenib-Experienced Patients with Advanced Liver Cancer

NIVOLUMUB, an immune-oncology drug, has been shown to produce robust responses in addition to long-term survival rates for sorafenib-experienced patients with advanced liver cancer. Findings were consistent, irrespective of whether or not patients were infected with the hepatitis B or hepatitis C virus. The results were announced in an ILC press release dated 21st April 2017. Interim results from the dose expansion phase of the CheckMate 040 study demonstrated that the overall objective response rate (ORR) by blinded independent central review (BICR) was 14.5% and investigator assessment ORR was 19.3% for sorafenib-experienced patients. The safety profile of nivolumab was comparable to that of other tumour types.

> "The durable responses and survival rates that were achieved with nivolumab are very welcome, especially as the side effects were manageable."

Sorafenib is currently the only approved systemic treatment for hepatocellular carcinoma (HCC); if a patient is unable to tolerate this medication there is currently no effective treatment available.[1] Nivolumab has already been shown to be an effective treatment option for other cancer types; however, it has not yet gained regulatory approval for HCC in the European Union (EU).

"The durable responses and survival rates that were achieved with nivolumab are very welcome, especially as the side effects were manageable," said study author, Dr Bruno Sangro Gómez-Acebo, Hepatology Unit, Clínica Universidad de Navarra, Pamplona, Spain.

The Phase I/II study enrolled patients (N=145) with advanced HCC who were not suitable candidates for surgery. The trial was a multi-cohort, open label study investigating the use of intravenously administered nivolumab (3 mg/kg every 2 weeks). Treatment was maintained until either the cancer progressed, or adverse events became intolerable. The primary endpoint was determined by BICR ORR.

Cancer progression was observed in 91% of patients (n=132), with 8.3% (n=12) intolerant of nivolumab. Interim analysis data of the dose expansion study phase indicated a median follow-up time of 12.9 months. The median duration of response (DOR) has not so far been achieved, 8 out of 21 responders had a DOR of >12 months. The median overall survival was 16.7 months, and it was noted that this was not achieved in patients with chronic viral hepatitis B and C. Responses to nivolumab occurred regardless of tumour programmed death-1 (PD-1) ligand expression. Treatment-related adverse events (Grade 3/4) were seen in 16.6% of patients.

Nivolumab works by acting as a programmed-death-1 (PD-1) immune checkpoint inhibitor, which can restore T-cell-mediated anti-tumour activity, enabling the body to fight against the cancer cells.

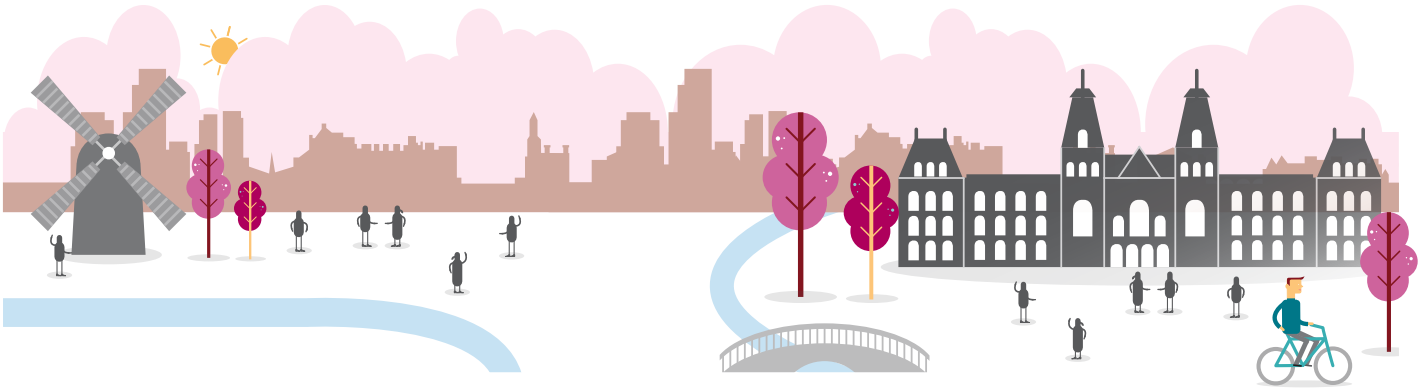
### REFERENCES

1. European Association for the Study of the Liver (EASL), European Organisation for Research and Treatment of Cancer (EORTC). Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908-43.

## Human Albumin Improves Survival of Patients with Decompensated Cirrhosis

SURVIVAL rates increased in patients with decompensated cirrhosis who had long-term administration of human albumin, as presented from the ANSWER study in an ILC press release dated 22nd April 2017. Management of ascites and quality of life were improved by human albumin treatment; additionally, the number of severe complications and hospitalisations were reduced.

Prof Mauro Bernardi, University of Bologna, Bologna, Italy, stated: "There has been a lack of scientific evidence proving that long-term human albumin can treat cirrhosis with ascites." Prof Mauro continued: "The ANSWER study has now clarified this issue, showing that human albumin extends survival and helps better manage ascites, as well as reducing the incidence of severe complications of this very serious disease."



The ANSWER study used a control trial of 440 randomised participants with cirrhosis and uncomplicated ascites, comparing standard diuretic therapy with human albumin (requiring 40 g intravenously twice a week for the first 2 weeks, followed by once a week) to standard diuretic therapy. All the participants were followed up 18 months after the study.

> “The ANSWER study has now clarified this issue, showing that human albumin extends survival and helps better manage ascites, as well as reducing the incidence of severe complications of this very serious disease.”

The results showed that patients who received standard therapy and human albumin had a significantly higher survival rate than those who received standard therapy alone. The patients who received standard therapy and human albumin had a 38% reduction in their risk of death. Quality of life, management of ascites, hospital admission, and complications of cirrhosis were demonstrated to have significant benefits for patients administered human albumin. Overall survival was the primary endpoint.

Prof Annalisa Berzigotti, University Clinic for Visceral Surgery and Medicine, University of Bern, Bern, Switzerland, and EASL Governing Board Member said: “The reduction in mortality observed in the albumin-treated arm of this randomised controlled study is a novel and important piece of information. Based on these data, weekly administration of albumin should be considered in patients with cirrhosis and ascites to prevent life-threatening complications.”

## Association Between Climate and Cirrhosis

A RECENT study has revealed an intriguing association between climatic and geographical factors and rates of alcoholic cirrhosis according to the results of a study reported in an ILC press release dated 22nd April 2017. The study’s lead author, Dr Neil Shah, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA, explained: “Our research reveals that a country’s climate and geographical location have a startling influence on the burden of liver cirrhosis.”

It was hypothesised that geographical location would influence alcohol consumption, thus influencing rates of alcoholic cirrhosis. For instance, it was suggested that individuals might increase alcohol intake in order to deal with cold and dark conditions. As the first stage in investigating this hypothesis, researchers collated and analysed a range of data across 193 countries taken from World Health Organization (WHO) and World Meteorological Organization (WMO) databases.

The results of the univariate analysis revealed an inverse association between mean average temperature and mean annual sunshine hours with the alcohol attributable factor (AAF) of cirrhosis; additionally, there was a positive association with absolute latitude ($p<0.05$) and the AAF of cirrhosis. After adjusting for the percentage of binge drinkers among active drinkers and alcohol consumption, it was found that average temperature and sunshine hours were still independently associated with the burden of alcohol-attributable liver cirrhosis. According to the data, a 1°C temperature increase was linked with a 0.3% decrease in the AAF of cirrhosis.

> Our research reveals that a country's climate and geographical location have a startling influence on the burden of liver cirrhosis.

A significant amount of further research needs to be carried out based on this initial finding. Firstly, the correlation of these factors does not necessarily imply causality so further testing of this hypothesis should be carried out. Additionally, there is a vast range of other factors, such as culture and religion, that may influence this association. The design of geographically specific healthcare policies that consider climatic variables will doubtlessly prove to be highly debated and require a lot of supporting evidence.

## Severe Periodontitis Associated with Mortality in Cirrhosis

SEVERE periodontitis was found to be a strong predictor of mortality in patients with cirrhosis in a recent study. The prediction held true after adjustments were made for a number of risk factors. The results of this prospective study were reported in an ILC press release dated 20th April 2017.

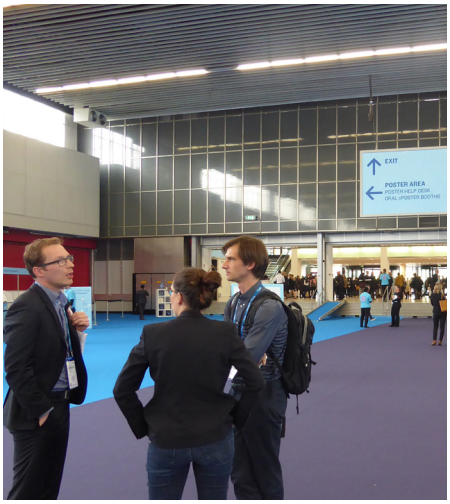
Currently, periodontitis is prevalent amongst adults: 10–15% have a severe form of the disease and >35% have periodontitis. Furthermore, periodontitis is found frequently in patients with cirrhosis and previous research has implied that periodontitis is linked to the progression of liver diseases.

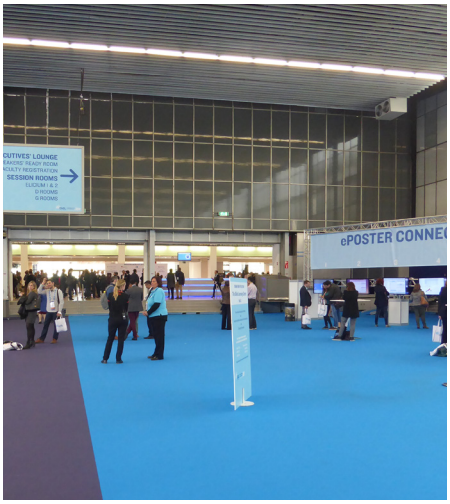
> Periodontitis may act as a persistent source of oral bacterial translocation, causing inflammation and increasing cirrhosis complications.

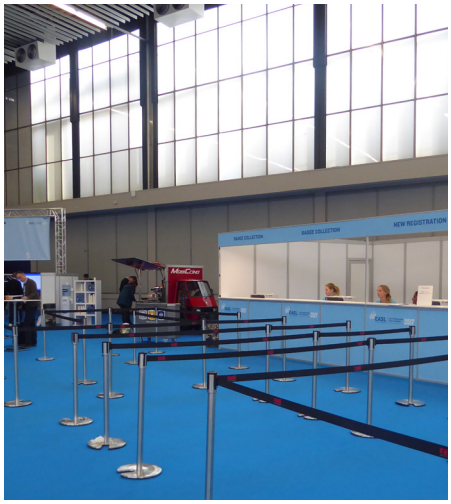
Researchers followed-up 184 consecutively enrolled patients with cirrhosis for an average of 1 year and assessed their oral health. At the beginning of the study, 44% of the patients were found to have severe periodontitis, as assessed by standard periodontology criteria. Over the follow-up period, almost half of the patients in the study died; the primary cause of death was complications of cirrhosis. Analyses were carried out on the association of periodontitis with mortality after adjustments were made for present alcohol use, smoking status, sex, age, comorbidity, Child–Pugh score, Model of End-Stage Liver Disease (MELD) score, cirrhosis aetiology, and nutritional risk score. The analyses revealed an association between severe periodontitis and greater all-cause mortality.

Lead study author Dr Lea Ladegaard Grønkjaer, Aarhus University Hospital, Aarhus, Denmark, spoke about the possible mechanism behind this finding, and explained that: "Periodontitis may act as a persistent source of oral bacterial translocation, causing inflammation and increasing cirrhosis complications. As it can be treated successfully, however, we hope that our findings motivate more trials on this subject."

With an association between gum disease and increased mortality having been demonstrated, the next research direction is to investigate whether improvements in gum care will result in improved outcomes in patients with liver cirrhosis.



## Successful Results of Trial of Fibrate Therapy in Primary Biliary Cholangitis Patients

PRIMARY biliary cholangitis (PBC) patients who have an inadequate response to treatment with ursodeoxycholic acid (UDCA) can demonstrate significant improvements to their condition through fibrate therapy, according to the results of a study presented at this year's ILC meeting and reported in an ILC press release dated 22nd April 2017.

> "The study provides evidence supporting the use of a combination of fibrates and UDCA in this population, with normalisation of liver function tests, improved symptoms, and prevention of liver disease progression."

Many PBC patients respond well to the administration of UDCA, with disease progression slowed and improved liver function tests often taking place. However, >30% of patients do not respond adequately to UDCA and remain at a high risk of disease progression, which could lead to lower survival rates and the need for a liver transplant. A study was therefore undertaken to test a new treatment method for such patients.

The BEZURSO study was a randomised, double-blind, placebo-controlled trial of bezafibrate in combination with UDCA in 100 patients who had an inadequate biochemical response to UDCA, as defined by the Paris-2 criteria. The patients were randomised to either 400 mg/day of bezafibrate or placebo in addition to UDCA for 2 years. The results showed that the primary endpoint of normalisation of liver function tests was met in 15 (30%) of the patients, compared with no patients in the placebo group.

Additionally, alkaline phosphatase normalisation was achieved in 67% of patients in the bezafibrate group as opposed to no patients in the placebo group. There were also significant reductions in surrogate markers of liver disease (liver stiffness and ELF score, which are predictors of liver failure and mortality) and fatigue and itching in the bezafibrate group compared to the placebo group. Rates of serious adverse events and end-stage liver complications were the same across both groups.

Dr Christophe Corpechot, Head, Reference Center for Inflammatory Biliary Diseases, Paris, France, and lead author of the study commented: "The study provides evidence supporting the use of a combination of fibrates and UDCA in this population, with normalisation of liver function tests, improved symptoms, and prevention of liver disease progression."

## New Research Avenue for Fatigue in Primary Biliary Cholangitis

STUDY of fatigue in the rare disease primary biliary cholangitis (PBC) has begun, with results of the first randomised, controlled, double-blind trial being reported at this year's ILC Congress, according to an ILC press release dated 20th April 2017.


PBC is a rare, chronic, autoimmune disease that can damage bile ducts, often leading to cirrhosis, liver failure, or cancer. Symptoms such as pruritus and fatigue can cause a very low quality of life for sufferers; roughly 25% of patients' work and social lives are detrimentally affected by fatigue, a symptom which does not correlate to disease severity. The RITPBC study examined whether rituximab could reduce the severity of fatigue symptoms in PBC patients, as well as assessing the safety and tolerability of rituximab, and the sustainability of the positive effects of the drug. The improvement of fatigue domain score of the PBC-40, a disease-specific quality of life questionnaire, at 12 weeks was the primary objective of the trial.

> "The current trial shows that although rituximab was not effective in reducing fatigue, there is nevertheless still a connection between the symptom and the immunopathological process."

The team randomised 57 PBC patients with moderate-to-severe fatigue to receive two doses of rituximab or placebo on Days 1 and 15, with follow-up of ≤12 months. No statistically significant difference was noted in fatigue score between the two arms of the study, but an improvement in fatigue for patients in both groups from the beginning of the study was reported. The rituximab arm also showed improved anaerobic threshold compared with placebo. Four serious adverse events were reported in the trial: one death before the drug was started and three events in the placebo arm.

Prof Marco Marzioni, Clinic of Gastroenterology, Università Politecnica delle Marche "Ospedali Riuniti", University Hospital of Ancona, Ancona, Italy, commented: "The current trial shows that although rituximab was not effective in reducing fatigue, there is nevertheless still a connection between the symptom and the immunopathological process. Therefore, further characterisation of the type of fatigue experienced by PBC patients may be crucial in helping identify optimal treatment."

## Risk Factors for Paediatric Liver Disease Confirmed

CHILDHOOD infection of hepatitis C virus (HCV) has been linked to serious long-term liver disease in a recent study reported in an ILC press release dated 20th April 2017. The study found that >33% of young people age <18 who had HCV went on to develop liver problems, including 5% developing liver cancer and >4% eventually receiving a liver transplant.

The study, from a group of researchers at Birmingham Children's Hospital, Birmingham, UK, considered data from patients who were estimated to have contracted HCV infection when they were <18 years of age taken from the HCV Research UK database. The data were collected between July 2012 and October 2016 from 51 adult and 7 paediatric centres, with a total of 1,014 patients, of whom 72% were male. The team found that intravenous drug abuse was the predominant cause of infection (n=535), followed by blood products (n=224), and acquisition at birth (n=116). Genotype 1 was the most common type of infection (57% of patients) but 35% had genotype 3, the most difficult strain to cure.

In the cohort, liver disease was present in 354 patients (33%) and 269 patients had cirrhosis (27%); 55 had hepatocellular carcinoma (5%), with 47 (5%) having received a liver

transplant. Patients who contracted HCV at birth were shown to have developed cirrhosis at a younger age than those who contracted it from intravenous drug use (median 36 years versus 48 years, respectively).

> “Our study showed that more than one-third of young people infected with HCV in childhood have serious long-term liver disease.”

It is hoped that by defining those most at risk of liver disease, more could be done to detect and treat the diseases amongst the most vulnerable groups. First author Dr Lin Modin, Birmingham Children’s Hospital, commented: “Our study showed that more than one-third of young people infected with HCV in childhood have serious long-term liver disease. Detection of HCV should be aimed at relevant risk groups, particularly young intravenous drug abusers.”

## Improved Patient Reported Outcomes for Direct-Acting Antiviral Treatment Combination Therapy

HEPATITIS C virus (HCV) and cirrhosis patients experience greater improvement of patient-reported outcome (PRO) scores in comparison to patients without cirrhosis when treated with the direct-acting antiviral (DAA) combination sofosbuvir (SOF) and velpatasvir (VEL), with or without voxilaprevir (VOX), according to an analysis presented at ILC 2017.

This treatment combination, already shown to be safe and effective against all HCV genotypes in different populations, showed significant improvements in PROs, particularly regarding cirrhosis patients, following achievement of sustained virologic response at 12 weeks (SVR12).

The study analysed information from 1,908 chronic HCV patients enrolled in the POLARIS 1, 2, 3, and 4 studies, which assessed the efficacy and safety of SOF/VEL/VOX in treating HCV infected patients. Questionnaires ascertained outcomes from 26 PRO domain scores, relating to quality of life, fatigue, work productivity, and activity impairment in these patients. The results showed that in patients with and without cirrhosis in both the SOF/VEL/VOX and SOF/VEL treatment groups, the overall cure rate (SVR12) was 94%. Following successful treatment, cirrhosis patients gained major improvements in their PRO scores compared to when treatment began. These were similar to or greater than patients without cirrhosis. No PRO improvements were seen in those cirrhosis patients treated with placebo.

“This analysis showed that although patients with HCV and cirrhosis have significantly impaired PROs, they experience the greatest improvement during treatment with SOF/VEL with or without VOX, when compared to those without cirrhosis,” explained Dr Zobair Younossi, Center for Liver Diseases, Washington DC, Washington, USA, lead author of the study, in an ILC press release dated 20th April 2017. “We also found that achieving a SVR with the drugs was associated with substantial gains in outcomes.”

> “This analysis showed that although patients with HCV and cirrhosis have significantly impaired PROs, they experience the greatest improvement during treatment with SOF/VEL with or without VOX, when compared to those without cirrhosis.”

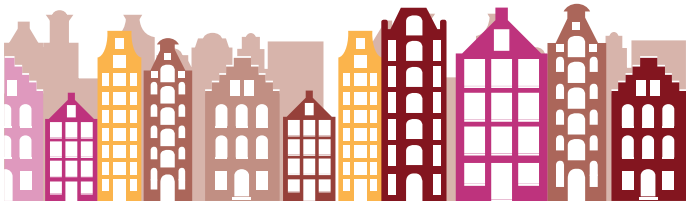
The improvements seen in PROs of HCV-related cirrhosis patients from this particular DAA therapy are likely to impact the significant indirect, as well as direct costs linked to HCV-related cirrhosis in the future.

## Optimism for Treatment of Challenging Hepatitis C Virus Subgroup

ENDURANCE-3, a Phase III, open-label, active-controlled study, has demonstrated a 95% sustained virologic response rate at 12 weeks post-treatment (SVR12) for patients being treated for hepatitis C virus (HCV). The study assessed the oral, once-daily treatment regimen of glecaprevir/pibrentasvir (G/P) in comparison to the more common treatment of sofosbuvir and daclatasvir against the most challenging subgroup of HCV patients: genotype 3.

> "While there has been great progress made in the treatment of patients with hepatitis C, there remain limited options for those with genotype 3 disease. As such, we are pleased to see that the investigational combination of G/P achieved high SVR12 rates in treatment-naïve, non-cirrhotic patients."

For the study, 348 treatment-naïve, non-cirrhotic HCV genotype 3 patients were randomised to receive a daily dose of G/P for 12 weeks or sofosbuvir and daclatasvir. Thereafter, 157 patients were given G/P for 8 weeks. The percentage of patients who achieved SVR12 was the primary endpoint, and this was achieved by 95% of patients who received G/P for 12 weeks (222/233; 95% confidence interval [CI]: 93–98) and 97% of patients who received sofosbuvir and daclatasvir for 12 weeks (111/115; 95% CI: 91–99). In both groups, 1% of patients relapsed. Of the cohort who received G/P for 8 weeks, 95% achieved SVR12 (149/157; 95% CI: 92–98) and 3% relapsed. Adverse events were reported to be mostly mild (71%), and no treatment-related adverse events were serious.



Lead author of the study Dr Graham Foster, Queen Mary University of London, London, UK, explained: "While there has been great progress made in the treatment of patients with hepatitis C, there remain limited options for those with genotype 3 disease. As such, we are pleased to see that the investigational combination of G/P achieved high SVR12 rates in treatment-naïve, non-cirrhotic patients." He added: "Treatment with this once-daily regimen for 8 weeks could provide a highly efficacious and well-tolerated option for treatment-naïve, non-cirrhotic patients with hepatitis C, genotype 3, if approved by the regulatory authorities."

## Treatment Regimen May Offer Cure for Hepatitis C-Infected Children

CHILDREN infected with hepatitis C virus (HCV) can be cured of the condition through the use of direct-acting antiviral (DAA) treatment, according to the results of a study presented in an ILC press release dated 21st April 2017. The study analysed an investigational dosage of once-daily ledipasvir 45 mg/sofosbuvir 200 mg (LDV/SOF) in a cohort of 6–11 year olds.

Unlike adults with HCV, who are regularly treated with DAAs, children with the condition mainly receive 24–48 weeks of pegylated interferon plus ribavirin (RBV), a treatment which can cause severe side effects.

> "These data establish the use of the oral DAAs as an important treatment option in HCV-infected children aged 6–11 years old."

For this ongoing, open-label study, 90 children aged between 6 and 11 years with chronic HCV were enrolled. HCV genotype 1 infected children who received either 12 weeks (n=85) or 24 weeks of LDV/SOF treatment (n=1) if they had cirrhosis and failed previous pegylated interferon plus RBV treatment. Genotype 3 patients received 24 weeks of LDV/SOF plus RBV (n=2) and genotype 4 patients were given LDV/SOF for 12 weeks (n=2). Most of the children in the group were white, male, treatment-naïve, and vertically infected.

Following completion of 12 weeks of treatment, 99% (n=89/90) of the group had undetectable levels of HCV/RNA. There was only one case of relapse: a genotype 1, treatment-naïve patient with cirrhosis. There were no instances of severe or life-threatening adverse events, and no children discontinued the study due to side effects. The most common side effects reported in 10% of patients were headache, fever, and abdominal pain.

"DAAs have transformed the treatment of adults with chronic HCV, however, studies of these new therapies in children are required," stated Dr Karen Murray, University of Washington School of Medicine and Seattle Children's Hospital, Seattle, Washington, USA. "These data establish the use of the oral DAAs as an important treatment option in HCV-infected children aged 6–11 years old."

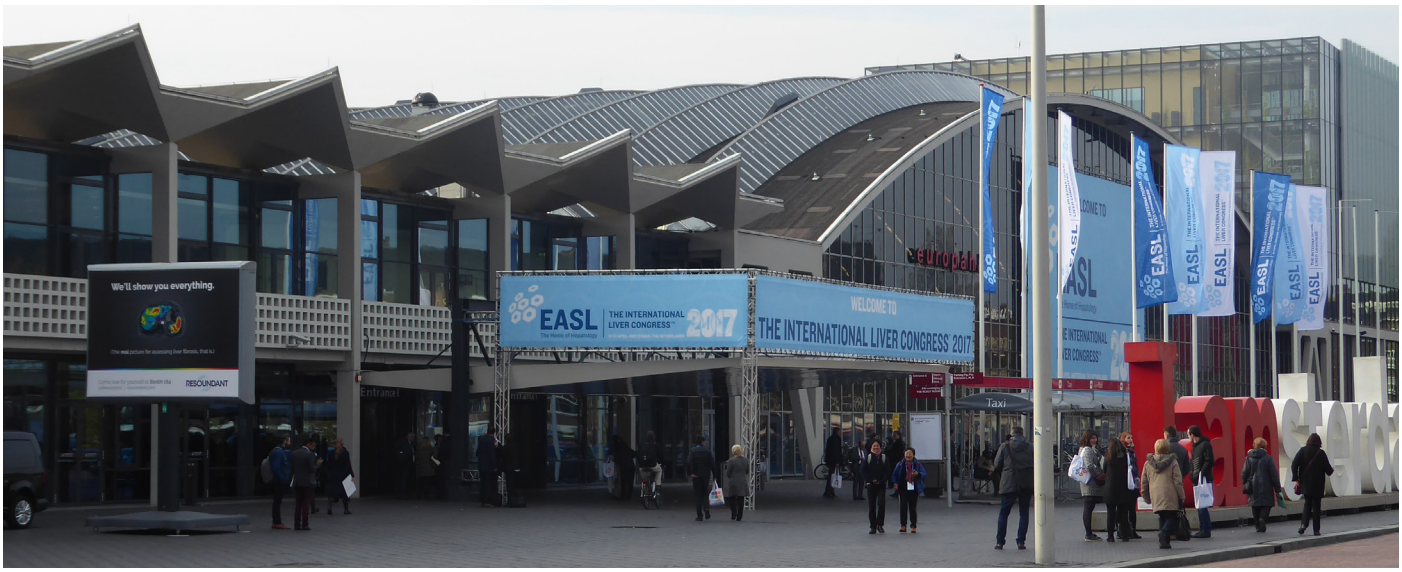
## Higher Levels of Hepatitis E in Germany Than Previously Recorded

THE FREQUENCY of hepatitis E virus (HEV) RNA was found to be higher in Germany than had been reported in the past, according to the results of a study reported on in an ILC press release dated 21st April 2017. These findings add support to calls for screening for HEV RNA at blood donation centres, which is currently non-compulsory.

> "Since HEV infection can have serious consequences among the immunocompromised and a single positive donor can give rise to HEV infection in several other patients, there is a need for longer term studies to analyse the effectiveness of routine HEV blood donor screening, and to determine whether this process should be implemented at blood clinics everywhere."

The study authors used a HEV polymerase chain reaction (PCR) assay to screen pools of 24 donations; reactive pools were then individually examined to detect HEV RNA positive donations. Overall, 13,441 blood donations at the University Hospital Hamburg-Eppendorf, Hamburg, Germany, were tested and 15 (0.11%) were found to be HEV RNA positive. One of these 15 positive donors presented with acute self-limiting hepatitis, and the remaining 14 were healthy and asymptomatic. One of the asymptomatic patients had donated blood products that had been transfused into nine immunocompromised patients prior to this screening. One of these nine patients had detectable serum HEV RNA before their death, which was caused by acute-on-chronic liver failure complicated by pseudomonas sepsis.

These findings are pertinent as they lend weight to the argument that HEV RNA should be screened for at blood donation centres.




HEV infection is often asymptomatic, so blood donors may not realise they are infected; thus, HEV RNA positive blood may be transfused into patients, as indeed is what occurred in this study. Lead study author Dr Dirk Westhölter, University Hospital Hamburg-Eppendorf, Hamburg, Germany, spoke about this concern and the need for further research, and stated: "Since HEV infection can have serious consequences among the immunocompromised and a single positive donor can give rise to HEV infection in several other patients, there is a need for longer term studies to analyse the effectiveness of routine HEV blood donor screening, and to determine whether this process should be implemented at blood clinics everywhere."

## Sustained Removal from Liver Transplant List After Hepatitis C Treatment

PATIENTS who had been taken off the liver transplant list after undergoing successful direct-acting antiviral (DAA) therapy for chronic hepatitis C virus (HCV) and severe liver damage were found to have a favourable outcome >1 year later. The results from this study were reported in an ILC press release dated 21st April 2017. Today, chronic HCV is the most common reason for liver transplantation in adults. With >8,500 patients in Europe on the waiting list for a liver transplant, this number only expected to increase. Studies such as this one, tracking the outcomes of those removed from the transplant list are of great importance. Lead study author, Dr Luca Belli, Gastroenterology and Hepatology Liver Unit, Niguarda Hospital, Milan, Italy, announced that: "The results of this study are very encouraging with clinical improvement due to DAA therapy lasting over a year in nearly all patients."

In this retrospective study, the authors tracked 38 patients who were treated with DAA therapy for decompensated cirrhosis without liver cancer and, following clinical improvement, were taken off the liver transplant list. After a median follow-up time of 15 months from being taken off the list, 37 patients were still alive and 1 of these patients had been relisted for clinical re-decompensation. The patient who died did so due to rapidly progressing hepatocellular carcinoma. Results showed that median Model of End-Stage Liver Disease (MELD) scores were 9, 78 weeks since the start of DAA therapy, which was an improvement from 14 at the start of therapy; furthermore, median Child–Pugh scores improved from 9 at the beginning of therapy to 6 after 78 weeks.

Dr Belli spoke about the need for further study, stating: "We still need to follow these patients for much longer to confirm the results and assess the long-term risks of deterioration, but so far the risk of dying after delisting is much lower than that of dying after receiving a liver transplant."

> "The results of this study are very encouraging with clinical improvement due to DAA therapy lasting over a year in nearly all patients."

## New Clinical Practice Guidelines Unveiled for Liver Disease Management

FOUR Clinical Practice Guidelines (CPGs) were unveiled at this year's ILC Congress, designed to support healthcare professionals' clinical decision-making across a number of specific liver diseases. The announcement was initially made in an official ILC press release issued on 19th April 2017. Each individual guideline was also presented in detail during the event.

Broadly, CPGs are used to assist the decision-making processes related to a specific clinical circumstance and can be useful to a varied audience, including; physicians, health service providers, patients, and caregivers. In this instance, these four CPGs have been developed to outline best practice advice for the management of patients with certain liver diseases.

The recently announced CPGs included the revision of one existing guideline on the 'Management of Hepatitis B virus infection' and the publication of an additional three original guidelines, including:

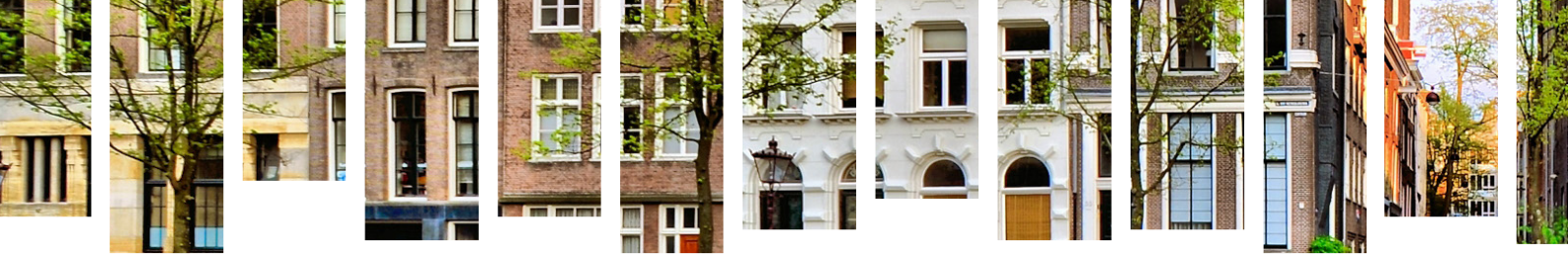
- Management of Acute (fulminant) Liver Failure
- Role of endoscopy in Primary Sclerosing Cholangitis (PSC)
- The treatment and management of patients with Primary Biliary Cholangitis

Specific focus was placed on the European Society for Gynaecological Endoscopy/ European Association for the Study of the Liver (ESGE/EASL) guideline on the role of endoscopy in PSC.

> "The EASL Primary Biliary Cholangitis guidelines are meant to support clinicians in establishing a long-term commitment with patients and their disease, providing indications on how to stratify the risk from diagnosis to disease progression. The guidelines will also help identify which patients require second-line treatment, in which advances are fortunately being made."


"The EASL Primary Biliary Cholangitis guidelines are meant to support clinicians in establishing a long-term commitment with patients and their disease, providing indications on how to stratify the risk from diagnosis to disease progression. The guidelines will also help identify which patients require second-line treatment, in which advances are fortunately being made," explained Prof Marco Marzioni, Clinic of Gastroenterology, Università Politecnica delle Marche "Ospedali Riuniti", University Hospital of Ancona, Ancona, Italy.

Prof Marzioni, who was involved in writing the guideline, added: "The EASL-ESGE guidelines have been developed by the two societies to identify the optimal approach for the management of PSC. As extra-hepatic bile ducts are involved in the disease, a multidisciplinary approach is a key factor for the success of management. The guidelines provide a tool to fine tune the endoscopic and medical treatments of patients with PSC."




## Markus Peck-Radosavljevic

Professor of Medicine and Chairman, Department of Gastroenterology and Hepatology, Endocrinology and Nephrology, Klinikum Klagenfurt am Wörthersee, Klagenfurt, 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, and the Austrian Society for Internal Medicine (ÖGIM).


**Q:** Who or what was the greatest inspiration for you to pursue a career in medicine and/or the field of hepatology?

**A:** My greatest inspiration to pursue a career in hepatology was both my first European Association for the Study of the Liver (EASL) Annual Meeting (now termed the International Liver Congress™ [ILC]) and my first Falk-meeting, which was centred around portal hypertension and advanced stage liver disease.

**Q:** Could you tell us about the main duties and responsibilities you have as Chairman of the Department of Gastroenterology/Hepatology, Endocrinology, and Nephrology, Klinikum Klagenfurt am Wörthersee? Are there any particular challenges that you face in this position currently?

**A:** My duties are centred around organising the clinical service of the department, which also includes the centralised endoscopy service and the Emergency Department of the Klinikum. At the same time, education and training of our fellows is an equally important task as well as organising the clinical trials facilities within our department. The particular challenges are the same as for most other institutions in Austria: they are centred around underfunding of manpower and the severe lack of understanding to straightforward neglect from parts of the administration for long-term planning of regional healthcare services.

> “In liver cancer, we went from no treatment for advanced stage patients to effective first and even second-line drug treatment...”

> “It is hard to judge what really led to the development of thrombopoietic substances...”

**Q:** You have been an investigator in a number of clinical trials throughout your career; which one of these completed trials has had the biggest impact in terms of the overall outcome of the study results?

**A:** This is very difficult if not to impossible to say; some of my earlier work in pathophysiologic aspects has really helped to improve the understanding of mechanisms that have led to therapeutic advances in the longer run, while the clinical observations and trials have had a much more direct impact on patient care. Whether you look at elucidating the role of thrombopoietin in liver disease, studies on the impact on beta-blockers in advanced cirrhotics, or the work on patient selection for transarterial chemoembolisation, I consider several of them quite relevant for my colleagues.

**Q:** Thrombocytopenia is a significant issue in liver disease and liver transplantation patients; what initiatives have emerged in recent years to try and combat this?

**A:** In recent years, several attempts have been made to develop thrombopoietic substances for the treatment of thrombocytopenia associated with advanced stage liver disease and portal hypertension. All of them seem to accomplish the goal of raising thrombocyte counts in cirrhotic patients but so far none of them has shown improvements in harder endpoints like a reduction in platelet transfusions before interventions or fewer bleeding complications.

**Q:** Have there been any changes in the prevalence of patients requiring a liver transplant due to infection with the hepatitis C virus (HCV) in recent years? If so, what are the main reasons for such a trend?

**A:** Yes, most clearly there has been a very significant drop in patients on the liver transplant waiting list with liver failure due to chronic hepatitis C. The main reason for this is the advent of very effective and at the same time highly tolerable therapies for chronic hepatitis C. It is really amazing how quickly this has led to such a significant reduction of transplant candidates, at least in the West.

**Q:** Could you tell us about the reports you have published on the haematological side effects of antiviral therapy in chronic hepatitis C? Have these effects had a significant impact on the development of treatments for this condition?

**A:** We published reports on the occurrence and pathophysiology of thrombocytopenia both in patients with advanced stage cirrhosis as well as in patients undergoing interferon (IFN)-based antiviral therapies. It is hard to judge what really led to the development of thrombopoietic substances to support patients undergoing IFN-based antiviral therapies, but it could well be that the publications of my group have had a relevant impact. In the end, the trials showed an improvement of thrombocytopenia with these therapies, but the overall impact on the virological cure for HCV was rather modest due to the low efficacy of IFN-based antiviral therapies in patients with advanced stage cirrhosis.

**Q:** To what extent have treatments improved for portal hypertension, viral hepatitis, and hepatocellular carcinoma since you first began your career?

**A:** Therapies for all of these conditions have improved significantly: the most significantly for viral hepatitis, where we can now control chronic hepatitis B virus infection and cure chronic HCV infection in almost all patients. In liver cancer, we went from no treatment for advanced stage patients to effective first and even second-line drug treatment and have now also much improved means to deliver optimal local ablative therapy. In portal hypertension, we are so effective in preventing variceal bleeding today that this has become almost a rare complication these days.

**Q:** How would you describe the state of healthcare in Austria? How does it compare with the rest of Europe and other parts of the world?

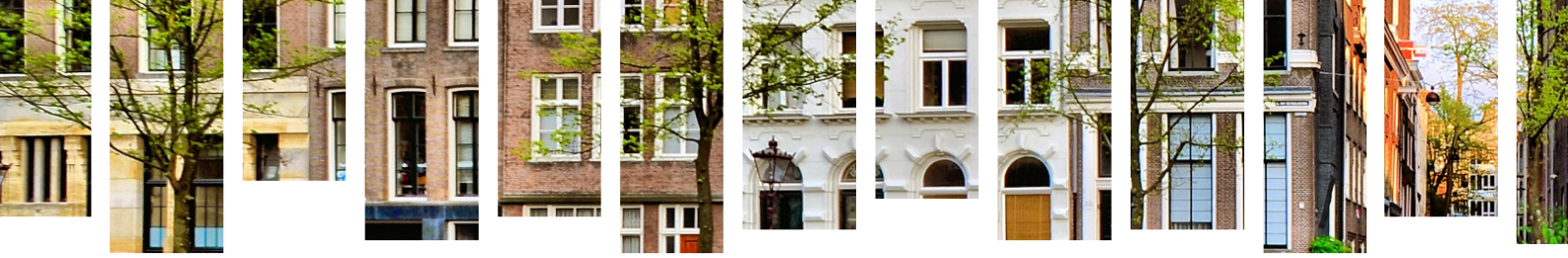
**A:** I think the state of healthcare in Austria is quite good: we have good universal coverage and access to all kinds of therapies through our national insurance system. In this sense, we are very comparable to Germany and many other Western European countries. Traditionally, we have a lower level of centralised planning and too high a frequency of hospital admissions since we are lacking effective mandatory gatekeepers, who would keep patients in ambulatory care for longer.

**Q:** As a prominent member of a number of major international hepatological societies, what is the most important role these bodies play in your opinion? In what ways do you think they could build on their current activities?

**A:** I think the major international liver societies have several important tasks: they serve as an important platform for scientific exchange and collaboration, and they provide a high level of medical education to both fellows as well as experienced colleagues. They also serve as a meeting point for interactions with the medical industry. Where they could build on their ongoing activities would be in getting involved with global health issues and policy making and in providing advice to local and regional health authorities. They could also expand their reach and provide (online) education for regions of the world underserved with medical education.

**Q:** What aspects of the field should medical students who are thinking of pursuing a career in hepatology be most aware of in your opinion?

**A:** I think the areas of portal hypertension/liver failure, liver cancer, and metabolic and cholestatic liver disease are all areas to watch out for and offer a number of opportunities for people keen on becoming hepatologists.



## Omar Sued

Clinical Research Director, Fundación Huésped, Buenos Aires, Argentina.

**Q:** As a Clinical Research Director for the Fundación Huésped, Buenos Aires, Argentina, can you give a brief insight into your roles and responsibilities?

**A:** Fundación Huésped is a non-profit organisation that works to raise awareness, provide direct services, and perform clinical research for HIV, hepatitis C, and other infectious diseases, which has had a public health impact in Argentina since 1989. As a research centre, Fundación Huésped is known as the most prestigious centre for conducting clinical trials in the area of HIV in the country and has enrolled >3,000 participants in >150 studies since 1994. Here, I co-ordinate the research team, which involves defining research strategies, mentoring young researchers, and the supervision of activities.

**Q:** What made you want to specialise in infectious diseases, in particular HIV, after completing your MD?

**A:** In the last years of my degree, I started to hear about a rare disease called AIDS. I immediately felt shocked about how this disease was changing the way doctors reacted, due to fear and stigma, and was also very intrigued about how to make a change. Two years later, at the internal medicine residence programme, my first AIDS patient, a 26-year-old male, married with a 2-year-old child, died after struggling to fight Kaposi's sarcoma of the lung. I spent many nights with this patient during my night shifts, realising how unfair life can be to some people. Fortunately, a couple of years later, an effective treatment was identified; although these situations still occur, they are less frequent.

**Q:** Can you give examples of some of the most pressing issues to be addressed in 2017 within the field of infectious diseases?

**A:** Without any doubt, we need to continue focussing on HIV. Now, we have the opportunity to make big changes after knowing that by coupling 'treatment as prevention' and pre-exposure prophylaxis (PrEP) it is possible to dramatically reduce new cases. In this sense, hepatitis C presents a really big opportunity. There is no other chronic disease that has a status of global epidemic scale that can be so easily cured with newly discovered drugs. Just 2–3 months of oral treatment can avoid cirrhosis, cancer, and death of affected individuals as well as cut transmission rates. But we need to be innovative to ensure low prices and high access. Tuberculosis is a major issue, particularly in Eastern Europe, where its incidence is increasing. Global warming and its effects in mosquito-rich areas is posing a big challenge with the increase of viral conditions such as dengue fever, Zika, and chikungunya, but surely other viral diseases will also spread.

**Q:** When working as a clinical investigator in Argentina, you organised The National Acute HIV cohort and TB-HIV clinic at the Juan A. Fernández Hospital, Buenos Aires, Argentina. How great of an impact do movements such as these have in the fight against HIV?

**A:** The production of local knowledge is critical to identify local priorities, inform guidelines, and develop and establish treatment standards. These cohorts resulted in changes to our national guidelines, specifically topics such as which antiretroviral to use in patients receiving rifampicin, facilitating the adoption of rifabutin, and the decision to start the treatment of all acute HIV patients in 2009.

**Q:** During your time as Regional Advisor for HIV treatment for Latin America and the Caribbean, part of the Pan American Health Organization (PAHO), you were involved in the development of 18 national guidelines. Why do you believe guidelines are important? Do you think they can ever hinder the adaptability of some professionals faced with atypical cases?

**A:** The guidelines are very important for countries. Standard treatment facilitates purchasing, logistics, training, and treatment expansion in most countries, increasing the public health benefit of public investment. But guidelines do not replace medical judgement and therefore are not written in stone. A guideline is designed to be a guide, but in medicine many patients do not follow the standard path and other interventions might be needed or at least explored for the individual benefit of that patient. All countries have mechanisms to use or request authorisation to use different products outside of labelled indications, not present in the guidelines, and doctors should take care not to deny the best intervention for their patients based only on the lack of it in a guideline.

**Q:** The topic of infectious disease within the media has recently been overwhelmed with epidemics caused by both the Zika and Ebola viruses, with little mentioned regarding HIV. Have the rates of HIV been improving or do they remain an ever-growing issue?

**A:** Emerging pathogens create a great level of concern globally, and it is normal for them to take up a significant space in media and newspapers. It is difficult to sustain public awareness of a chronic disease if no bad or good news changes the course of the epidemic. Fortunately, the HIV epidemic changed during the course, and a significant reduction of new cases is starting to be seen, particularly in countries with high treatment coverage.

**Q:** On a positive note, people with HIV are living longer than ever before because of continuous medical advances in the field. Patients are, however, still at risk of global discrimination. Should governments and media outlets be making more of an effort to educate the public? What is currently being done to accomplish this?

**A:** Yes, this is a result of the efficacy of treatment. Today, a young individual (e.g. 25 years old) that may have just acquired HIV and who has started treatment can expect to have an almost 'normal' life expectancy. Studies show that the possibility of sexually transmitting the virus to partners is extremely low if the individual is under stable treatment and with undetectable viral load. This is the principal message: HIV is not a death sentence. Free HIV treatment is available in almost every place, but we continue to have some gaps due to people not being tested frequently enough. Therefore, you must know your status and if you have minimal risk, you should continue to be tested periodically, and start treatment as soon as possible.

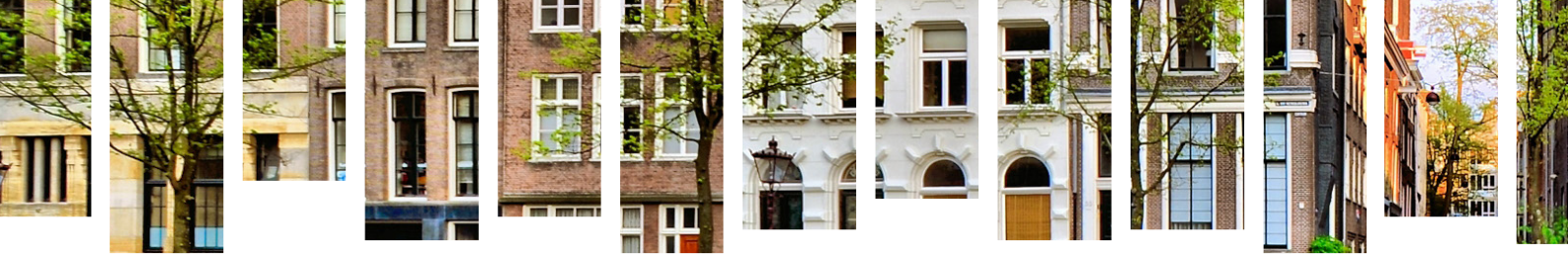
**Q:** This April's European Association for the Study of the Liver (EASL) International Liver Congress™ (ILC) was held in Amsterdam, Netherlands. How important are these events as a platform for networking among professionals? Which aspects of this congress particularly piqued your interest?

**A:** One of the most important aspects of the medical congress is networking. Today we have a lot of new technologies that allow access to medical information, and most topics being discussed at congress can be followed on the internet. But participation allows attendees direct exposure to the new topics and allows them to network with people with the same interests as their own, which allows collaborative work.

**Q:** What is your most prized achievement from across your extensive career in improving and developing strategies for the treatment and prevention of HIV?

**A:** I see patients on a regular basis, and having the opportunity to see one HIV patient become undetectable after starting treatment, or knowing that one HCV patient was cured are what I continue to consider as my most important prizes. I have had other good opportunities also, such as being awarded 2.5 million US dollars for a HIV prevention project in Central America, and have also recently been accepted in the HIV Prevention Network Trials for carrying out projects based on HIV prevention in Argentina.

> "Free HIV treatment is available in almost every place, but we continue to have some gaps due to people not being tested frequently enough."



## Heidar Sharafi

Molecular Hepatologist; Research Director, Iran Hepatitis Network, Tehran, Iran;
Member of the European Association for the Study of the Liver (EASL).

**Q:** What particularly inspired you to pursue a career as a molecular hepatologist?

**A:** When studying physiology and biochemistry as a student of the medical laboratory sciences, I realised that as the largest endocrine organ, the liver has numerous vital activities and its hepatocytes harbour many complicated molecular procedures to maintain metabolic and detoxification activities. Malfunction of the liver can be investigated from both molecular and cellular viewpoints. The molecular aspects of liver diseases can result in elucidation of the clinical features of disease. Our knowledge regarding the molecular aspects in this field of medicine can help us to innovate the diagnosis and treatment of liver diseases. The most prominent instance for the latter is the development of new hepatitis C virus (HCV) direct-acting antiviral agents (DAAs) based on the elucidation of a molecular mechanism of HCV replication and functional proteins involved in the life cycle of this virus. All of the mentioned facts inspired me as a student of biomedical and laboratory sciences to pursue a career as a molecular hepatologist.

**Q:** The first ever Global Viral Hepatitis Strategy 'NOhep' was adopted last year, with a goal to eliminate viral hepatitis as a major public health threat by 2030. Do you think this goal is achievable? What steps are being taken to achieve this?

**A:** With recent advances in the diagnosis and treatment of viral hepatitis B and C, we can clear these viral infections in the next 13 years; however, it needs global action as we are all responsible for the elimination of hepatitis B and C globally. Unfortunately, the prevalence of viral hepatitis is high in countries where there is no wide access to new HCV antiviral therapies or even the hepatitis B vaccine. 'NOhep' global action can help the elimination of viral hepatitis by raising the awareness of the general population regarding the hepatitis B and C transmission routes and risk factors. Public awareness regarding availability of an efficient vaccine for hepatitis B and treatments for hepatitis B and C is important as well. Moreover, the availability and affordability of new DAAs of hepatitis C and finding patients with HCV infection to study are the steps that should be followed by governments to achieve hepatitis C elimination by 2030.

**Q:** Could you give us a brief overview of what your role as Research Director, Iran Hepatitis Network, Tehran, Iran, entails? What are your responsibilities?

**A:** First of all, I would like to introduce the Iran Hepatitis Network (IHN) as an active, non-governmental organisation connecting Iranian and Middle Eastern research centres and researchers in the field of liver diseases and viral hepatitis. We have major research and educational activities in Iran and even neighbouring countries. One of our most prominent activities is the Tehran Hepatitis Conference (THC). It is a biannual, international conference of liver diseases with an attendance of specialists from all over Iran and other countries of region. The previous THC was a great success with the collaboration of the European Association for Study of Liver (EASL) in 2015 and participation of many scholars from Europe and USA. Tehran, as the capital for the treatment and research of liver diseases, will host the 7$^{th}$ conference (THC7) on 6$^{th}$–8$^{th}$ September 2017 and we will have the kind collaboration of the EASL for the 'Best of EASL' day. As scientific manager of the THC7, I would like to invite everyone to participate in the meeting and share their experiences with other attendees. Another great activity of the IHN is a Hepatitis Monthly journal publishing scientific articles from all around the world in the field of hepatology. Beside these, we conduct multicentric studies with

the help of our network of research centres, utilising IHN as the hub for connecting these centres. Other than research and educational activities, IHN has activities aimed at public awareness with the help of the Hope Health Club and Young Investigators Club of IHN. For example, during and after the recent World Hepatitis Day of 2016 with introduction of the 'NOhep' movement, we had a few public awareness campaigns with the help of (bio)medical students in cities within Iran and these activities attracted global attention. Overall, with these activities the IHN has the main goal to decrease the burden of liver diseases in Iran and the Middle East.

As the research director of IHN, I get involved in designing and managing research projects in Iran to collect evidence in order to develop our regional model for the elimination of hepatitis C. Moreover, for reliable evidence to be obtained we need multicentric studies in Iran, which I always try to facilitate the procedure of in the setting of IHN. Do not forget, as the scientific manager of THC7 I will be very busy as well!

**Q:** Serology is a well-established method for the diagnosis and screening of viral hepatitis, with rapid diagnostic tests based on this technique, particularly that of the ELISA test. Are there any alternative molecular biological techniques that could reasonably replace this as the main means for diagnosis?

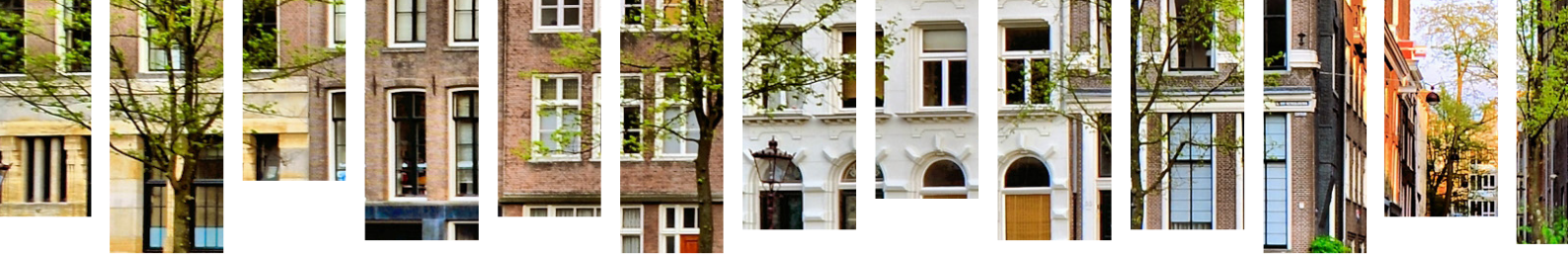
**A:** As you know, the treatment of hepatitis C has greatly changed through the previous few years. Beside good treatment strategies we need good screening and diagnosis methods for the elimination programmes of hepatitis C. Fortunately, the technical development of point of care testing will help the feasibility of screening and diagnosis; however, the greatest pitfall in this part is the diagnosis of viraemia in patients with a positive result for the HCV antibody. For the diagnosis of viraemia, we need molecular methods such as RT-PCR and TMA, which are both expensive and need highly technical instruments and specialised personnel, making the procedure infeasible for community-based programmes of HCV elimination in prisons and especially in people who inject drugs (PWID). This being said, there are alternatives for the detection of HCV RNA such as detection methods for the HCV core antigen which are in development or validation. I would like to remind readers that screening and diagnosis in the elimination programs of HCV should have certain characteristics: feasibility, a rapid turnaround time from sampling to result, a reasonable price, and an acceptable clinical sensitivity and specificity.

**Q:** Some of your more recent publications have focussed on the molecular tracing of hepatitis C virus genotype isolates and the distribution of hepatitis C genotypes. How important is it to determine the prevalence of specific genotypes across different geographical locations?

**A:** As a researcher in the field of molecular studies, I have a great interest in molecular epidemiology. With new HCV pan-genotypic DAA regimens, the clinical need for genotyping of HCV is fading. However, looking for the genome of HCV to see where it comes from is of great interest and I have a few ongoing studies investigating the source of HCV in special groups of patients. While the latter is true, the genotyping of HCV will be forgotten very soon which will make the diagnosis feasible and straightforward.

**Q:** How has the area of hepatology evolved since the start of your career, and what impact has this had on your role within the field?

**A:** I entered the field of hepatology in 2009 exactly when three Genome Wide Association Studies (GWAS) found the impact of host genetics and polymorphisms near *IFNL3* on the natural history, treatment response, and outcome of HCV infection. Following this great finding, the roles of a few other genes such as *ITPA*, *PNPLA3*, and *IFNL4* were found to govern the outcome or treatment of liver diseases. Beside these achievements, approval of the first direct-acting antivirals for the treatment of HCV in 2011 and first all-oral interferon-free HCV antiviral regimen in 2014 changed the future of viral hepatitis C. Regarding the treatment of HBV infection, tenofovir became the best oral regimen and the recent introduction of tenofovir alfenamide optimised hepatitis B treatment. As a result, the field



of hepatology has evolved rapidly during the past decade and it is a great opportunity for me as a researcher of liver diseases to get involved.

**Q:** In your opinion, what do you think the biggest challenges and/or obstacles will be for hepatologists in the next 5 years?

**A:** I believe that patient findings and community-based treatment of patients with HCV infection, especially PWID, are the greatest challenges faced against the elimination of HCV infection.

Another challenge in the field of hepatology is the need for effective hepatitis B and D treatments for the elimination of these infections by 2030. With the availability of effective treatments for hepatitis C, there is a great demand from clinicians and patients with hepatitis B to have short-course elimination treatments. Moreover, the prevalence and burden of non-viral liver diseases such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis have increased through the previous two decades and will be the main reasons for advanced liver diseases in the near future.

**Q:** How important is the annual International Liver Congress™ 2017 (ILC 2017), organised by EASL, and similar congresses to hepatologists?

**A:** The annual ILC event held in European countries and the Best of EASL day held outside of Europe, both organised by EASL, can play a great role in decreasing the global burden of liver diseases. In addition, other international meetings such as the THC, Paris Hepatitis Conference, and the annual meetings of the American Association for the Study of Liver Diseases (AASLD) and Asian Pacific Association for the Study of the Liver (APASL) are great forums for clinicians and researchers in the field of liver diseases to share their knowledge and experiences and to get updated regarding the latest findings of hepatology.

**Q:** What would you say your proudest career achievement has been so far?

**A:** In fact, I am very young to have such achievement. However, I can tell you that I am very happy to have been involved in the laboratory diagnosis of viral hepatitis for >7 years. Helping people with viral hepatitis is always pleasant.

**Q:** What guidance would you give to clinicians and researchers who are at the beginning of their career, considering hepatology as their specialism?

**A:** Congratulations for their decision! I guarantee that if they try to find something new in hepatology, they will never be disappointed in their decision.

## Ashwani Singal

Associate Professor, Department of Medicine, Division of Gastroenterology and Hepatology;
Co-Director, Porphyria Center, University of Alabama at Birmingham, Birmingham, Alabama, USA;
Fellow, American College of Gastroenterology (ACG).

**Q:** Could you describe some of your main roles and responsibilities as an Associate Professor of Medicine and Co-Director at the Porphyria Center?

**A:** As an associate professor, I have three core responsibilities: i) Clinical: seeing and managing patients with various liver diseases in both outpatient and inpatient settings, working out the appropriate patients for liver transplantation, and triaging porphyria patients to be seen in clinic or enrolling in studies of the porphyria consortium; ii) Education: our division regularly schedules rotation of residents, students, and fellows on service as well as clinics from within and outside of The University of Alabama at Birmingham (UAB), international medical students, and graduates. This provides me with a great opportunity to teach them. I also take great interest in teaching junior faculty and nurses. Furthermore, I encourage the residents and students who work on various

research projects. iii) Research: about half of my time is dedicated to research activities, which includes enrolling suitable patients on various ongoing clinical trials and studies. I supervise my mentees in their research activities and support them at various levels.

**Q:** You are known for your expertise in alcoholic liver disease; what was it that inspired your research in this direction?

**A:** I have always been interested in liver diseases from my residency training days in India, where I went to medical school and undertook further studies. In the beginning, I was interested in hepatitis B infection; however, after moving to the USA in 2005, working with great mentors like Dr Scott Friedman from The Mount Sinai Hospital, New York City, New York, USA, I became interested in alcoholic liver disease, especially while reading literature on this topic for developing a review article. Recognising the potential for research in this field, given the lack of effective and safe medications for treatment of alcoholic liver disease with huge population burden, along with working with my mentors throughout the journey, including Dr Steven Weinman, Liver Center, University of Kansas Medical Center, Kansas City, Kansas, and Dr Don Powell, University of Texas Medical Branch, Galveston, Texas, USA, Dr Vijay Shah and Dr Patrick Kamath at Mayo Clinic, Rochester, Minnesota, USA, and basic science collaborators with the same interest, namely Dr Victor Darley-Usmar and Dr Shannon Bailey, The University of Alabama, Birmingham, Alabama, USA, expanded my enthusiasm and interest in this field.

**Q:** Are there any areas across the field of hepatology that you believe are currently ripe for further research? What developments would you like to see made over the next 10 years?

**A:** Besides alcoholic liver disease and non-alcoholic fatty liver disease, other areas need attention and need exploring for newer therapies. These include liver cancer, hepatitis B virus infection, and genetic liver diseases. For alcoholic and non-alcoholic fatty liver diseases, there is a clear unmet need for developing guidelines on managing these diseases, newer, more effective, safer drugs, and prevention of these common clinical problems.

**Q:** With the UAB Porphyria Center being part of the Porphyria Consortium, what are some of the main opportunities offered by collaboration? Can we expect to see an expansion of collaborative research in an increasingly networked society?

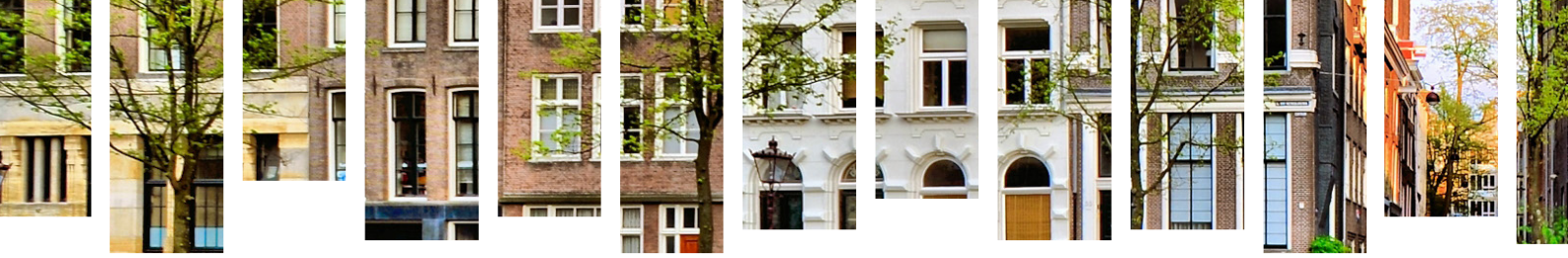
**A:** The Porphyria Consortium provides a number of great opportunities. These include networking with leaders in this field, learning more about the disease by attending national and international conferences on porphyria, conducting studies within the consortium, ancillary studies related to the disease, as well as taking leadership roles with increasing experience in the field. The consortium is already working on including more sites as satellites centres with focussed approaches in managing and researching porphyria.

**Q:** On the other hand, are there any challenges that need to be overcome in developing collaborative research efforts?

**A:** The challenges experienced include funding for the ancillary studies, especially when the budget for the main funded study is limited. It would be better to have a formally developed plan on encouraging emerging scholars and co-principal investigators, and nurturing and boosting them into the forefront as a basis for a continuing legacy in this field.

**Q:** We understand that you are involved in mentoring work. Which aspects of this do you find most rewarding? Additionally, could you give us any tips on what makes an effective mentor?

**A:** The most rewarding aspect of mentoring is an opportunity to nurture the younger generation and to help them advance in their careers, enabling them to emerge as scholars and rising stars in the field. Qualities of an effective mentor, from my own experience, are finding time for the mentee, being available with an open-door policy, providing constructive criticism with a non-intimidating approach, being open to ideas and encouraging them, but most importantly being selfless with advice in advancing their careers, and giving them wings to fly to their chosen field/destination.



**Q:** This April's European Association for the Study of the Liver (EASL) International Liver Congress™ (ILC) was held in Amsterdam, Netherlands. How important, in your opinion, are congresses such as this one for medical students as well as experienced clinicians? Is there any advice you would give to attendees looking to make the most of a congress?

**A:** Large liver meetings such as American Association for the Study of Liver Diseases (AASLD) and ILC, in my mind, serve a couple of purposes. It is a very good source of updating yourself with latest advances in the field on an annual basis, especially those participating in postgraduate courses and courses for students and residents, as well as fellows, physicians, and practitioners. Secondly, investigators, learners, and researchers focussed in a specific field can participate in their respective field attending educational talks, networking with leaders and collaborators in the field, and going over the presentations (podium and poster) throughout the meeting. However, compensation and budget at academic centres is a main limitation in participating in these activities, especially in international courses and symposia.

**Q:** Could you give us an insight into the projects you are currently undertaking?

**A:** We are interested in and currently working on i) studying mitochondrial function and bioenergetics of peripheral blood cells in patients with alcoholic liver disease and porphyria as a basis for developing a non-invasive biomarker to predict responsiveness to corticosteroids, ii) longitudinal and other studies of the porphyria consortium to study the natural history of this disease, iii) metabolomics of urine and serum among patients with kidney injury in cirrhosis patients as a basis for developing a biomarker predictive of renal function recovery after liver transplantation.

**Q:** What advice would you like to have been able to give yourself when you were starting out as a medical student?

**A:** I started out this journey as a medical student in India. I expected the fruit and the rewarding result. However, this field requires a focussed approach, dedication, plenty of hard work, and sometimes uncertainty. I think as a medical student, it is extremely important to be aware of the available infrastructure and curriculum focussed on mentoring students, so that each medical student can have one-to-one mentoring with experts providing them guidance to help them excel in whatever they are good at, adding more and more stars to the field.

**Q:** Finally, is there an important issue or piece of information we have not yet asked you about that you would like to draw attention to?

**A:** I think we have covered many important issues, but one aspect that needs attention is funding for research, which has always remained limited and is likely to worsen in the foreseeable future.

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# A DISCUSSION ON THE MANAGEMENT OF WILSON DISEASE

## This satellite symposium took place on 22nd April 2017 as a part of The International Liver Congress™ (ILC) in Amsterdam, Netherlands


**Chairperson**

**Peter Ferenci[1]**

**Speakers**

**Gideon Hirschfield,[2] Anil Dhawan,[3] Karl Heinz Weiss[4]**

*1. Medical University of Vienna, Vienna, Austria*
*2. University of Birmingham, Birmingham, UK*
*3. King's College Hospital, London, UK*
*4. University Hospital, Heidelberg, Germany*



**Disclosure:** Dr Peter Ferenci reports membership of the respective advisory boards for Univar, Wilson Therapeutics, and Vivet Pharmaceuticals. Dr Hirschfield reports personal fees for preparing and delivering an educational seminar from Univar, during the conduct of the study. Outside the submitted work, Dr Hirschfield is on the advisory board (PBC) for Novartis, GSK, Intercept Pharmaceuticals, and on the advisory board (Wilson Disease) for Univar. Dr Dhawan reports a speaker fee from Gilead and personal fees from Audentes. He was advisor to the clinical study and lecture for Univar outside the submitted work. Dr Weiss reports grants and personal fees from Univar, personal fees from Vivet, grants and personal fees from Wilson Therapeutics, grants and personal fees from GMPO, personal fees from Orphan Europe, during the conduct of the study; grants and personal fees from Bayer, grants from Novartis, grants and personal fees from Alexion, personal fees from BMS, and grants from MSD, outside the submitted work.
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## MEETING SUMMARY

Prof Peter Ferenci opened the meeting by providing a background to Wilson disease (WD), an enigmatic condition where no two cases are the same. He explored the aetiology, peak age of presentation, and long-term outlook.

Dr Gideon Hirschfield considered the wide variation in WD symptom presentation, the lack of a diagnostic gold standard, and the difficulties around choosing WD endpoints for clinical trials. He went on to consider how study endpoints have evolved over time, and how, in real-life clinical practice, therapies need to be tolerable for patients with negative copper balances.

Prof Anil Dhawan focussed on diagnostic challenges in paediatric WD, reviewing the size of liver biopsies needed for measurement of liver copper dry weight, the penicillamine challenge test, and Leipzig scores. Regarding treatment, he stressed that improvements in liver scores take time on chelation therapy, making it important not to rush patients to transplant. Prof Dhawan explored the development of disease severity scores for transplantation, including the revised cut-off points for the Nazer score. He provided reassuring data around the success of living related liver transplantation from parents heterozygous for WD and raised the possibility of auxiliary liver transplants.

Prof Karl Heinz Weiss considered three WD cases reflecting different aspects of the condition. The neurological case showed deterioration of neurological symptoms after starting D-penicillamine. This, Prof Weiss speculated, may relate to treatment causing shifts in the copper pool from bound copper to unbound copper. The second case involved a young woman with WD who was planning a pregnancy; Prof Weiss

showed the importance of patients remaining with therapies they are used to. Finally, he considered a patient with decompensated cirrhosis referred for liver transplantation but for whom, when reassessed with the modified Nazer score, the level did not indicate the need for transplantation. The patient showed side effects with one treatment but subsequently did well on a second treatment and was delisted for transplant.

## Introduction

### Professor Peter Ferenci

Prof Ferenci explained that many misconceptions exist around WD, including that the condition only occurs in children and young adults, is a neurologic disease, can be excluded if levels of caeruloplasmin are normal, and is a rare condition. Prof Ferenci estimated that in the USA alone, there are at least 9,000 WD patients.

The WD genetic defect affects the copper-transporting adenosine triphosphatase (ATPase) gene (*ATP7B*) responsible for incorporating copper into copper-binding proteins (including caeruloplasmin) and excreting excess copper into bile. Presentation of WD is highly variable, with no two cases resembling each other. WD patients present with liver disease (including acute hepatitis, fulminant hepatic failure, chronic hepatitis, or cirrhosis), Coomb's negative haemolytic anaemia, or neurological symptoms (including tremor, rigor, dyskinesia, inability to write, slurred speech, inability to walk, and depression).

The primary defect is hepatic copper accumulation, resulting in liver fibrosis, inflammation, and cell necrosis, which may lead to hepatitis and cirrhosis. Symptoms such as Kayser–Fleischer (KF) rings and central nervous system complications occur only when copper accumulates outside the liver.

An Austrian database involving 1,300 patients, presented by Prof Ferenci, showed the peak age of WD presentation to be between 16 and 20 years.[1] However, the oldest patient Prof Ferenci is currently treating was diagnosed when aged 74 years.

Unpublished liver histology data by Prof Ferenci revealed that rates of cirrhosis are low in children but increase slowly towards adulthood (˜60% of WD patients have cirrhosis by the age of 20 years). The huge variability, he added, is underlined by findings that some WD patients aged from 60–70 years have not developed cirrhosis.

Although a range of diagnostic tests exist for WD (including caeruloplasmin, urinary copper excretion, and hepatic copper content), no test, except genetic methods, can be considered diagnostic on its own,[2] see Figure 1.

Prof Ferenci highlighted the case of a woman diagnosed with WD at the age of 14 years in 1965, who, despite stopping treatment in 1968 (and who has not continued any other subsequent treatment), went on to have two children and showed no clinical evidence of liver damage when examined in 2016.[3]

A study exploring long-term outcomes in 229 Austrian WD patients diagnosed between 1961 and 2013 showed, at a mean observation period of 14.8 years after diagnosis, that 26% were symptom free, 25% had stabilised, 24% had improved, 17% had deteriorated, and 7% had died.[4] Such wide-ranging outcomes, said Prof Ferenci, demonstrate how little is known about the natural history of WD, including disease penetrance.

| | Abnormal | Caveat |
|---|---|---|
| Caeruloplasmin | <20 mg/dL | May be normal in hepatic WD; low in malabsorption |
| Urinary copper excretion | >100 µg/24-hr | Not useful when anuric |
| Hepatic copper content | >250 µg/g dry weight | May be <250 µg/g in 20% |
| Genetics | Two mutations | Present only in 75% |

**Figure 1: Diagnostic tests for Wilson disease.[2]**
WD: Wilson disease.

A problem for assessing treatment efficacy in WD, explained Prof Ferenci, is that the natural history of WD is unknown, and the only parameters are clinical improvement, specific tests focussed towards patients with neurological WD, and copper levels. The ultimate endpoint was survival.

## What is the Mark of Success in Treating Wilson Disease Patients?

### Doctor Gideon Hirschfield

One of the challenges of WD, said Dr Gideon Hirschfield, is the wide spectrum of symptoms that affect the liver, brain, eyes, blood, kidneys, joints, pancreas, heart, and endocrine system.[5] See Figure 2.

Such wide variation in WD presentation results in difficulties choosing endpoints for clinical trials, often making it necessary to judge treatment success according to how patients first present with WD. Current treatments for WD, including D-penicillamine, zinc salts, and trientine have been available since the late 1960s.

An additional issue is the lack of a gold standard for WD diagnosis. Diagnosis relies on a 'composite' of clinical features and laboratory findings (such as increased urinary copper excretion, reduced levels of serum caeruloplasmin, and high concentrations of liver tissue copper) and KF rings.[6,7]

Key findings for WD diagnosis include urinary copper levels >100 µg/24-hour, hepatic copper levels >250 µg/g dry weight, caeruloplasmin levels <200 mg/L, and the presence of KF rings. While mutation analysis may provide definitive diagnosis, some variants are not responsible for the disease.

Assessing treatment response therefore is a challenge and WD study endpoints have evolved over time. In 1956, Walshe[8] considered removal of copper from the body as a marker of D-penicillamine treatment success but by 1982 was using symptom improvement as a marker of success for trientine in symptomatic neurological WD patients (reviewing factors such as dysarthria and tremor).[9] The challenge for the 21st century, said Dr Hirschfield, is to define treatment safety and efficacy more precisely, using clinical scores to determine whether WD symptoms have improved. Current endpoints in clinical trials for WD include quantitative neurologic and speech tests, complete blood cell counts, liver function tests, blood levels (amylase, lipase, creatinine, urea nitrogen, uric acid, and iron variables), and urine protein levels.[10]

In a recent retrospective analysis comparing outcomes for patients who received D-penicillamine and trientine, Prof Weiss used treatment outcomes including effects on neurologic and hepatic symptoms, and adverse events leading to discontinuation of therapy, but did not include negative copper balances.[11] A second paper by Prof Weiss comparing copper chelators and zinc salts used hepatic deterioration (defined as an increase in activity of liver enzymes aspartate aminotransferase [AST], alanine aminotransferase [ALT], and gamma glutamyltransferase) as the study endpoint.[12]

| | |
|---|---|
| **Liver** | Fatty liver; resembling autoimmune hepatitis; cirrhosis, etc.; bilirubinate gallstones |
| **Brain** | Movement disorders resembling chorea; rigid dystonia; dysarthria; dysautonomia |
| **Eyes** | Kayser-Fleischer rings; sunflower cataract |
| **Blood** | Self-limited intravascular haemolysis |
| **Kidney** | Renal tubular dysfunction; Fanconi syndrome; kidney stones; hypokalaemic muscle weakness |
| **Joints** | Arthralgia or arthritis; osteoporosis, osteopaenia |
| **Pancreas** | Pancreatitis |
| **Heart** | Arrhythmias; cardiomyopathy |
| **Endocrine** | Hypoparathyroidism<br>Testicular dysfunction<br>Amenorrhoea; spontaneous abortion; infertility |

**Figure 2: Different presentations of Wilson disease.[5]**

Dr Hirschfield explained that, when considering real-life clinical practice, therapies need to be tolerable and patients need to achieve negative copper balances, as this effectively stops disease progression.

There was also a need for neurologic stability and improvement. Hepatic stability and improvement can be gauged through clinical means, bloods and imaging, and clinicians should aim where possible to achieve an absence of side effects and demonstrate measurable impacts on copper metabolism. However, Dr Hirschfield explained the field still did not know the exact treatment thresholds clinicians should aim for.

Considering practical issues, Dr Hirschfield said that non-adherence represents a major problem, with studies showing 25–45% of WD patients are non-adherent.[13,14] Although direct measurement of free copper (as opposed to protein bound copper) is possible, it is not routinely available and most measurements have yet to be standardised.[15-17]

While 24-hour urinary copper excretion reflects the fraction of free copper in serum, wide variations have been observed for WD patients. Some recommend stopping chelator treatment for 48 hours, which has low patient acceptance.[2]

Recommendations suggested by Dr Hirschfield include monitoring efficacy with 24-hour urinary copper excretion. For D-penicillamine and trientine, he suggested complete blood counts to check for cytopenia and urinalysis to check for proteinuria; while for zinc he suggests liver tests for efficacy and creatinine for toxicity.[5]

During chelation therapies, 24-hour urinary copper excretion outputs of 3–8 µmol per day (200–500 µg) denotes adequate treatment; while for zinc therapy the 24-hour copper excretion target of <2 µmol per day (125 µg) are adequate.[18]

Important considerations, said Dr Hirschfield, include adherence (once daily treatment may prove more practical), ease of use and monitoring, travel (difficulties occur around treatment refrigeration), availability (different countries have different reimbursements), and follow-up. Treatment follow-up, concluded Dr Hirschfield, needs to be both over the short and long-term and should be appropriate to the severity of WD neurological and hepatic symptoms.

## Management of Wilson Disease in the Paediatric Population, Including the Role of Transplantation

### Professor Anil Dhawan

WD, said Prof Anil Dhawan, is uncommon in children <3 years of age; it involves mainly hepatic presentation <10 years and neurological symptoms do not usually start until children reach 12–13 years. Disease response changes with age. Unusual WD presentations include haemolytic anaemia, incidental abnormalities of liver enzymes, rickets, gall stones, and neuropsychiatric symptoms. Prof Dhawan described three paediatric WD cases:

- Case 1 involved a 12-year-old girl with a 2-week history of jaundice and haemolytic anaemia, (test scores: caeruloplasmin: 0.1; urine copper: 7 mmol/L: post-D-penicillamine: 26; international normalised ratio (INR): 4; white cell count (WCC): 14; albumin (ALB): 21; AST: 163). The patient had a Wilson index score of 12, which resulted in her undergoing transplantation, after which she has remained well for 10 years. Mutation analysis showed that she was homozygous for common mutations. At birth, her sister was found to be homozygous for the same mutation but to have normal liver function. At 2 years, the sister was started on zinc acetate but at 5 years showed abnormal AST/ALT with liver biopsy revealing steatosis, mild fibrosis, and elevated liver copper. The family opted to change treatment to trientine. Prof Dhawan said that since serum zinc was higher than normal, this raised questions around whether the patient experienced zinc failure or was non-compliant.
- Case 2 involved a 14-year-old girl with decompensated liver disease, ascites, and a WD index of 8. Other centres might have sent her for a transplant. Both ascites and liver function improved over the next 2 years but 3 years later she presented with neurological features resulting in psychiatric hospitalisation. The sad outcome (the girl showed progressive neurological illness and died) raised questions about whether she might have benefited from liver transplant.
- Case 3 involved two siblings. The older boy was diagnosed aged 6 years, after presenting with abdominal pain, and was found to have incidental liver steatosis, to be homozygous for WD, and to have normal liver and neurology. Subsequently, his 3-year-old brother had homozygous mutations identified and showed abnormal liver

enzymes (AST: 67; ALT: 71) and on ultrasound was found to have mild splenomegaly.

The diagnosis of WD in children, said Prof Dhawan, includes urine copper, serum copper, serum caeruloplasmin, KF rings, liver copper, Coomb's negative haemolytic anaemia, biochemical indices, serum zinc, alkaline phosphatase, and molecular diagnostics. Liver copper >250 µg/g of dry weight has become the gold standard for WD diagnosis, with recommendations to use the entire core of the liver biopsy specimen >1 mg dry weight for valid copper content.

A study by Yang et al.[19] concluded that liver samples >1 mg dry weight are needed to reflect hepatic copper content. However, Prof Dhawan showed that liver copper could be reliably measured in children when the biopsy sample was <1 mg. This sample size could reliably distinguish WD from autoimmune disease and non-alcoholic liver disease ($p<0.001$).[20] So the message here, said Prof Dhawan, is that one or two decent-sized cores are sufficient.

The penicillamine challenge test, measuring urinary copper excretion for 24-hours after the administration of two doses of D-penicillamine (500 mg), was shown in 1992 to have a sensitivity of 88.2% and specificity of 98.2% for diagnosing WD, using cut-off levels of urinary copper >25 µmol/24-hour.[21]

When Prof Dhawan re-evaluated the penicillamine challenge test in 38 children diagnosed with WD and 60 controls with other liver disorders, he showed a sensitivity of 76% and a specificity of 93%.[22] Furthermore, he found sensitivity was better in symptomatic patients (92%) than asymptomatic patients (46%).

In 2001, a diagnostic score was proposed at the 8th International Meeting on Wilson disease, Leipzig, Germany, taking into account liver copper, rhodanine stain, urinary copper, serum caeruloplasmin, KF rings, neurological features, Coomb's negative haemolytic anaemia, and genetic *ATP7B* analysis.[23] See Figure 3.

Consensus was reached that scores of ≥4 established WD diagnosis. When Prof Dhawan retrospectively validated the Leipzig score in children he found that it showed high specificity (96.6%) and sensitivity (98.1%).[24]

With WD treatments for children, clinicians need to appreciate the length of time that may be needed for symptoms to improve. A review of outcomes of 58 children with WD treated at King's College Hospital, London UK, over 37 years showed that in the 20 children who achieved normal liver function with chelation time to normalisation was 12.2 years for INR, 9 years for AST, and 2.3 years for bilirubin.[25]

| **Scoring system by Ferenci et al.[23]** | **-1** | **0** | **1** | **2** | **4** |
|---|---|---|---|---|---|
| **Liver copper, µg/g of dry weight** | <50 | | 50–250 | >250 | |
| **Rhodanine stain*** | | Absent | Present | | |
| **Urinary copper, µmol/d** | | | | | |
| Basal 24-hour | | <1.6 | 1.6–3.2 | >3.2 | |
| 24 hours after PCT | | | | >8 | |
| **Serum caeruloplasmin, g/L** | | >0.2 | 0.1–0.2 | <0.1 | |
| **Kayser–Fleischer rings** | | Absent | | Present | |
| **Neurological feature** | | Absent | | Present | |
| **Coomb's negative haemolytic anaemia** | | Absent | Present | | |
| **Genetic *ATP7B* analysis** | | No mutation detected | Mutations in one chromosome | | Mutations in two chromosomes |

*only if quantitative copper measurement is not available.

**Figure 3: Scoring system for Wilson disease diagnosis.[23]**
The diagnostic score was retrospectively validated in children and shown to have a high specificity (96.6%) and sensitivity (98.1%).
PCT: penicillamine challenge test.

Such delays, stressed Prof Dhawan, underline the importance of not rushing to transplant. Liver transplantation, he said, has its own consequences with children swapping WD treatments for antirejection treatments. The exception was fulminant WD, which without transplantation has nearly 100% mortality.

There is undoubtedly a need to recognise WD features predicting a need for transplant. In 1986, Nazer,[26] from King's College, London, UK, developed a scoring system for the severity of hepatic dysfunction on admission. This was evidenced by prolongation in prothrombin time, AST activity, and serum bilirubin. The study showed patients with scores of ≥7 had higher likelihood of dying or needing a transplant. When Prof Dhawan and colleagues reviewed the data years later (adding WCC and ALB levels) the cut-off was extended to 11.[25]

In WD paediatric liver transplantation, parents (usually heterozygous for WD) often volunteer as donors leading to concerns around heterozygous donors increasing risk of recurrence. However, results from the Kyoto Experience (involving 11 parent donors donating parts of their liver to children aged 6–16 years) showed marked reduction in urinary copper excretion in all recipients and KF ring improvement.[27] The study concluded living related liver transplantation (LRLT) was an excellent choice for effective treatment. The data is further supported by a Chinese study showing survival in WD patients with complicated neurologic manifestations undergoing LRLT.[28] Such data, said Prof Dhawan, suggests LRLT ameliorates the neurologic consequences of WD, although the study suggests that outcomes can be related to severity of neurological symptoms at time of transplant. Another possibility is auxiliary liver transplant with Korean doctors reporting a patient who, 26 months after auxiliary liver transplant, had normal serum caeruloplasmin without neurologic problems.[29]

WD transplant outcomes are reassuring; a study following-up medical records for 121 French WD patients (transplanted between 1985 and 2009) found that actuarial patient survival rates were 87% at 5, 10, and 15 years.[30]

At King's College Hospital, increased recognition of psychological issues affecting compliance and quality of life have, in the past 5 years, led to joint WD clinics with hepatologists, neurologists, and psychologists. While expensive, said Prof Dhawan, treatment adherence and patient quality of life have greatly benefited.

## How to Manage Difficult Situations when Dealing with Wilson Disease Patients

### Professor Karl Heinz Weiss

Prof Karl Heinz Weiss considered the lessons that could be learnt from 3 WD cases: a symptomatic neurologic patient, a patient who wished to become pregnant, and a decompensated cirrhosis patient. The cases allowed him to explore a range of considerations around WD treatment.

The symptomatic neurologic case was a 21-year-old male who after referral for tremor was found to have elevated transaminases, caeruloplasmin 0.03 g/L (normal range 0.2–0.6 g/L), urinary copper excretion >2 times the upper limit of normal (ULN), and was found to be KF positive. After WD diagnosis, the patient was started on D-penicillamine (20 mg/kg bw). He had barely any evidence of hepatic disease but the tremor was severe. Prof Weiss said it was important to use a neurologic rating scale in the clinic for neurologic symptomatic patients, as it helps quantify the neurologic symptom burden and follow the clinical course of the disease. Following 1 week of therapy, this patient was unable to walk or swallow and his urinary copper excretion increased dramatically (>35 x ULN).

One of the risk factors for neuro deterioration reported in the literature has been starting on too high a dose of D-penicillamine or trientine.[31-33] A possible explanation, suggested Prof Weiss, is that chelating agents may be responsible for shifting the copper pool, i.e. moving copper from the CP-bound copper (non-toxic pool) to the non CP-bound toxic pool (toxic pool) and shifting too much copper into the circulation leading to neurological worsening.[34] When Litwin[35] used the Unified Wilson Disease Score scale on 143 symptomatic WD patients, diagnosed between 2005 and 2009, he found neurological worsening following treatment initiation in 11.1% of patients. But the role of free copper remains unclear as it was not assessed in that paper.

The current patient underwent D-penicillamine dose reduction and achieved slow stabilisation over the next 8 weeks but showed no improvement making it necessary to consider other treatment options. In

the Heidelberg Vienna cohort study, a retrospective analysis of 288 WD cases for hepatic treatment failure, Prof Weiss and Prof Ferenci showed that hepatic treatment failure occurred least often with chelating agents ($p<0.001$) and actuarial survival was greater for chelating agents ($p<0.001$).[12] On this basis, the patient was prescribed a combination treatment with trientine and zinc. The advantage of a combination is that drugs have different modes of action, but the disadvantage is that the regimen is complicated as both drugs need to be taken at widely spaced intervals to avoid interference between zinc and chelator ions.[36,37] After 6 months, the patient showed neurologic recompensation. In treating symptomatic neurological disease, said Prof Weiss, the rule should always be to start low and go slow.

The second patient, a 25-year-old woman with no neurologic symptoms diagnosed with WD at the age of 14 years after mild transaminase elevation, said that she hoped to become pregnant. Ultrasound showed no cirrhosis, no ascites, and mild splenomegaly. Transaminases, ALB, INR, bilirubin, and haemoglobin were within normal range.

As long as a woman's liver is healthy enough to become pregnant, hepatic worsening does not appear to be a frequent finding. A study by Pfeiffenberger et al.[38] reviewing pregnant women showed that hepatic worsening is a rare event for all treatments.[38]

Considering the possibility of switching treatments, Prof Weiss said it might take 6–12 months for the transaminases to increase in cases who were non-responders to one treatment.[12] By far the easiest approach, said Prof Weiss, would be to advise the patient to stay with the same treatment but to decrease the dose.

Decompensated cirrhosis. The third patient was a 22-year-old male diagnosed with WD after referral for ascites. He had elevated transaminases, caeruloplasmin 0.05 g/L, urinary copper excretion >2 x ULN, KF positive, INR 1.9, ALB 26 g/L and was listed for transplant. However, on the Modified Nazer score he had a score of 6, which is well below the transplant 'cut-off' of 11.[25] Over 6 months of treatment the patient showed slow recompensation and was de-listed for transplant.

Medical therapy, said Prof Weiss, should always be indicated even for very sick patients with subacute liver failure. The overall prognosis for WD patients if they are adherent and on drugs is excellent. A study by Bruha et al.[39] that followed 117 WD patients showed that their long-term survival did not differ from the general Czech population ($p=0.95$).[39]

## Discussion

The wide-ranging audience questions considered the value of liver biopsies, doses, genetic testing, and issues around pregnancy. Regarding liver biopsies, Prof Ferenci said he had reviewed data on 100 patients with established WD and found 18% did not have cut-off levels for copper. Other studies have found that copper is unevenly distributed in the liver and a biopsy may not accurately assess its level. Urinary copper analysis was complementary to biopsy, he said.

The challenge of genetics, said Dr Hirschfield, is that people with the same genotype do not necessarily have the same phenotypes. Someone with significant illness can have a sibling with the same mutation who is completely asymptomatic. Prof Ferenci stated that when patients are diagnosed with WD it should be mandatory to offer genetic testing to first degree relatives.

With regard to chelator dose, Prof Weiss said that, if adult patients are doing well with normal liver function tests and there is no residual neurologic disease, clinicians should aim to lower the dose. Dr Hirschfield agreed that there was likely to be a point where less copper chelation was needed to keep the patient in clinical equipoise, adding that dose reduction had benefits for side effects and cost. Prof Dhawan explained that because paediatric patients are growing, unchanged doses are equivalent to a reduction.

With regard to female WD patients contemplating pregnancy, Prof Ferenci said that the likelihood of their partners carrying the gene was low. Partners should not be considered for genetic testing but once born the child should be tested to confirm that it is heterozygote.

In pregnancy, the risk of an untreated mother dying far outweighs any potential teratogenic effects of therapy. Also, added Prof Ferenci, women with WD who do not take therapy have high risks of miscarriage. Prof Weiss said that in pregnancy the best choice for most women is to remain on existing treatments they have been previously stable on.

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# THE IMPORTANCE OF CIVIL SOCIETY INVOLVEMENT IN ELIMINATING HEPATITIS C

***Eberhard Schatz**

*Correlation Network, Amsterdam, Netherlands*
**Correspondence to eschatz@correlation-net.org*

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This is an historic moment for hepatitis C treatment. With the advent of direct-acting antivirals (DAAs), we can now massively reduce the harm from an infectious disease that affects millions of people and causes hundreds of thousands of unnecessary deaths every year. Interferon-free DAA regimes are short, highly tolerable, and simple to deliver, with cure rates of >90%. One year ago, the first World Health Organization (WHO) Global Health Sector Strategy on Viral Hepatitis[1] was approved with the ambitious goal of hepatitis C elimination as a public health threat by achieving a 90% reduction in new infections and a 65% reduction in deaths from the hepatitis C virus (HCV) by 2030.

The use of unsterile drug-injecting equipment is a primary contributor to the HCV epidemic in Europe. Over 90% of new infections are among people who inject drugs (PWID).[2] Other populations at high risk for HCV include migrants from high prevalence countries/regions, prisoners, people who are homeless, sex workers, people living with HIV, and men who have sex with men.

Major European and international agencies such as the WHO, United Nations Office on Drugs and Crime (UNODC), Joint United Nations Programme on HIV/AIDS (UNAIDS), European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), and European Centre for Disease Prevention and Control (ECDC) consider viral hepatitis, especially among PWID, to be a serious public health problem. However, at present, policies responding to HCV are inconsistent, or non-existent across Europe. The broad range of issues pertaining to HCV have not been exhaustively included in European and/or national policies or comprehensively dealt with among designated stakeholders.

HCV prevention, screening, early diagnosis, and treatment among PWID have been proven to be both effective and cost-effective.[3] Research exploring the values and preferences of PWID with regard to HCV treatment has found that concerns exist about side effects, limited HCV knowledge, rationed treatment expectations, experiences of treatment refusal due to drug use, stigma and discrimination within healthcare settings, and difficulties associated with hospital systems pose significant hurdles for HCV treatment access and uptake.[4]

Consequently, hepatitis C elimination will require an enormous scale-up in testing and treatment along with comprehensive harm reduction services, including in prisons. The other essential requirement for achieving the elimination of hepatitis C is a sustained collaborative effort to combat the stigma, discrimination, and criminalisation[5] faced by PWID and other priority communities, such as migrants and men who have sex with men. Again, community and civil society actors hold a vital key to succeeding in this effort.

On 18th–19th of April, just before the International Liver Congress™ (ILC) organised by the European Association of the Study of the Liver (EASL) in Amsterdam, Netherlands, a Community Summit on hepatitis C came together with an urgent call to policy makers, healthcare providers, health insurance providers, and the pharmaceutical industry to work in collaboration with the affected communities and their organisation, as well as low-threshold services, to achieve the scale-up. Communities and community representatives must participate in formulating and implementing hepatitis C prevention, testing, and affordable treatment strategies because these stakeholders have unique knowledge about what will be accessible, acceptable, and effective. Without their close ongoing involvement, the effort to eliminate hepatitis C is likely to fail.

In a 'Civil Society Declaration on the Importance of Civil Society Involvement', it is stated:

"Together, we can improve access to care for marginalized populations and hold governments accountable to their commitment to the Global Health Sector Strategy on Viral Hepatitis."

The declaration is signed by numerous European networks and organisations and is accessible at www.hepatitiscommunitysummit.eu.

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# STATINS IN CIRRHOSIS AND PORTAL HYPERTENSION

***Juan G. Abraldes, Lourdes Cabrera**

*Division of Gastroenterology, University of Alberta, Edmonton, Alberta, Canada*
**Correspondence to juan.g.abraldes@ualberta.ca*

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Keywords: Simvastatin, oesophageal varices, acute-on-chronic liver failure, portal pressure, nitric oxide.

Statins have been used for almost three decades as cholesterol lowering drugs, yet additional effects beyond lipid lowering, known as pleiotropic effects, have been found. These include an improvement in endothelial dysfunction, antifibrotic, and anti-inflammatory properties. These are relevant pathogenic events in cirrhosis, leading to portal hypertension and providing the rationale for repurposing statins for this indication. The first study assessing this was published in 2004 and analysed the acute haemodynamic effects of simvastatin in patients with cirrhosis. This study showed that a single oral dose of 40 mg of simvastatin decreased hepatic resistance. It was followed by several studies in animal models of cirrhosis that further refined this concept and led to a Phase II study assessing the effects of 1-month oral simvastatin (40 mg once daily) or placebo on portal pressure. Simvastatin induced a mild decrease in portal pressure (~8%) that was associated with an improvement in hepatic clearance of indocyanine green, suggesting that simvastatin could also improve liver function by improving hepatocyte perfusion. In a second small, randomised trial including less severe patients, 3-month simvastatin decreased portal pressure by 15%. In both studies, simvastatin did not have an effect on arterial pressure.

To date, only one randomised trial has evaluated the effects of statins on relevant clinical endpoints in patients with cirrhosis (the BLEPS study). This study assessed whether adding simvastatin to standard therapy (variceal ligation+non-selective beta-blockers) could improve clinical outcomes after variceal bleeding. The study included 158 patients with cirrhosis from different aetiologies (70% alcohol cirrhosis), treated with simvastatin 40 mg orally once daily or placebo for up to 24 months. The primary endpoint was a composite of rebleeding and death. The addition of simvastatin did not further decrease the risk of rebleeding; however, it was associated with a significant survival benefit, which was mainly associated with a decrease in mortality related to bleeding and infection. The rate of adverse events was not different between the groups. However, two patients

in the simvastatin group developed rhabdomyolysis (both patients with severe liver dysfunction), suggesting that in these patients statin treatment requires close monitoring. Since the benefit was shown on a secondary endpoint, further confirmation would be desirable in new trials designed with death as the primary endpoint. In addition, these results point to new clinical scenarios in which statins could be assessed, such as in patients with ascites with or without variceal bleeding, and in patients with, or at high risk of, infections.

# MAGNETIC RESONANCE IMAGING OF DIFFUSE LIVER DISEASES

***Luis Martí-Bonmatí,[1] Manuela França,[2] Angel Alberich-Bayarri[3]**

*1. Radiology Department, La Fe University-Polytechnic Hospital and Imaging Biomarkers Research Group, Valencia, Spain*
*2. Radiology Department, Centro Hospitalar do Porto, Porto, Portugal*
*3.Imaging Biomarkers Research Group, Instituto de Investigación Sanitaria La Fe and QUIBIM, Valencia, Spain*
**Correspondence to marti_lui@gva.es*

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Keywords: Steatosis, iron overload, steatohepatitis, magnetic resonance, liver inflammation, liver fibrosis, quantitative radiology, imaging biomarkers.

Virtual liver biopsy (VLB) can be considered a multicomponent imaging biomarker able to provide fundamental information on the presence, distribution, and amount of different pathological changes in diffuse liver diseases by controlling the effect of fat, iron, inflammation, and fibrosis on the magnetic resonance imaging (MRI) signal. VLB assesses quantitative changes. The provided imaging biomarkers, extracted by MRI signal computational processing techniques, are resolved in space (parametric images) and time (follow-up studies) (Figure 1). VLB addresses the main biopsy limitations, such as a small sample size, heterogeneous tissue distribution of the pathological hallmarks, large inter and intra-observer scoring variability, and procedure-related morbidity.

MRI is sensitive and efficient in measuring liver fat and iron on a voxel-by-voxel basis as the signal is proportional to the fat (chemical shift effect) and iron (T2* decay) content. For an accurate determination, several biases must be controlled. The main confounding factors to be corrected are the T1 bias, as initial signal intensities of water and fat are different; the T2* decay bias to correct for the effect of iron in the signal; and the spectral complexity of the fat spectrum frequencies. The T1-independent T2*-corrected multi-echo chemical shift encoded (MECSE) sequence, with magnitude and phase information, allows determination of both the proton density fat fraction (PDFF; %) and the iron-related R2* water signal component ($s^{-1}$). Simultaneous modelling of the signal improves the robustness and accuracy of these imaging biomarkers of liver steatosis and iron.[1] MRI quantification of fat and iron provides numerical and continuous measurements, rather than categorical or semi-quantitative grades as liver biopsy does (Figure 1).

PDFF and R2* are highly related to liver biopsy results, both ordinal scores and absolute values. In our recent publication on 109 patients with histology as gold standard, a high correlation was observed.[2] We found that the calculated PDFF measurements showed significant differences ($p<0.001$) among steatosis grades, being unaffected by iron, inflammation, or fibrosis. A strong positive correlation was also observed between R2* measurements and iron grades ($R_s=0.71$, $p<0.001$). Therefore, MECSE-gradient echo breath-hold (12 seconds) calculations were accurate for the simultaneous identification and quantitation of liver steatosis and siderosis, as they had excellent

linearity, negligible bias, and high precision across different field strengths, vendors, and reconstruction algorithms (Quantitative Imaging Biomarkers Alliance [QIBA]). MECSE MRI is considered more precise than liver biopsy for therapy, monitoring steatosis and iron overload.

Regarding inflammation, the short tau inversion recovery (STIR) sequence allows a semi-quantitative evaluation because a healthy liver signal is similar to fat (TI ≈ 150 ms at 1.5T; 180 ms at 3.0T), while liver brightness increases with the water content related to the necro-inflammatory development.[3] Unfortunately, signal also relates to the iron content, which is a confounding variable.

To improve the MRI accuracy, the diffusion-weighted images allow the evaluation of the signal decay due to the diffusion restriction associated to the intracellular oedema and macrophage infiltration. The signal decay observed by using multiple b-values and a bi-exponential fitting allow the application of the intra-voxel incoherent motion model. The apparent diffusion coefficient (ADC), perfusion related (D*), vascular fraction (f), and pure diffusion (D) metrics can be calculated.[4] ADC and f values were significantly lower with higher fibrosis stages (p=0.009, p=0.006, respectively), and with higher necro-inflammatory activity grades (p=0.02, p=0.017, respectively). However, only fibrosis seemed to present a significant effect on ADC and f measurements (p<0.05). It was found that the D values were influenced by steatosis and iron overload. The intra-voxel incoherent motion measurements were not accurate enough to clinically stage liver fibrosis or necro-inflammatory activity in diffuse liver diseases.

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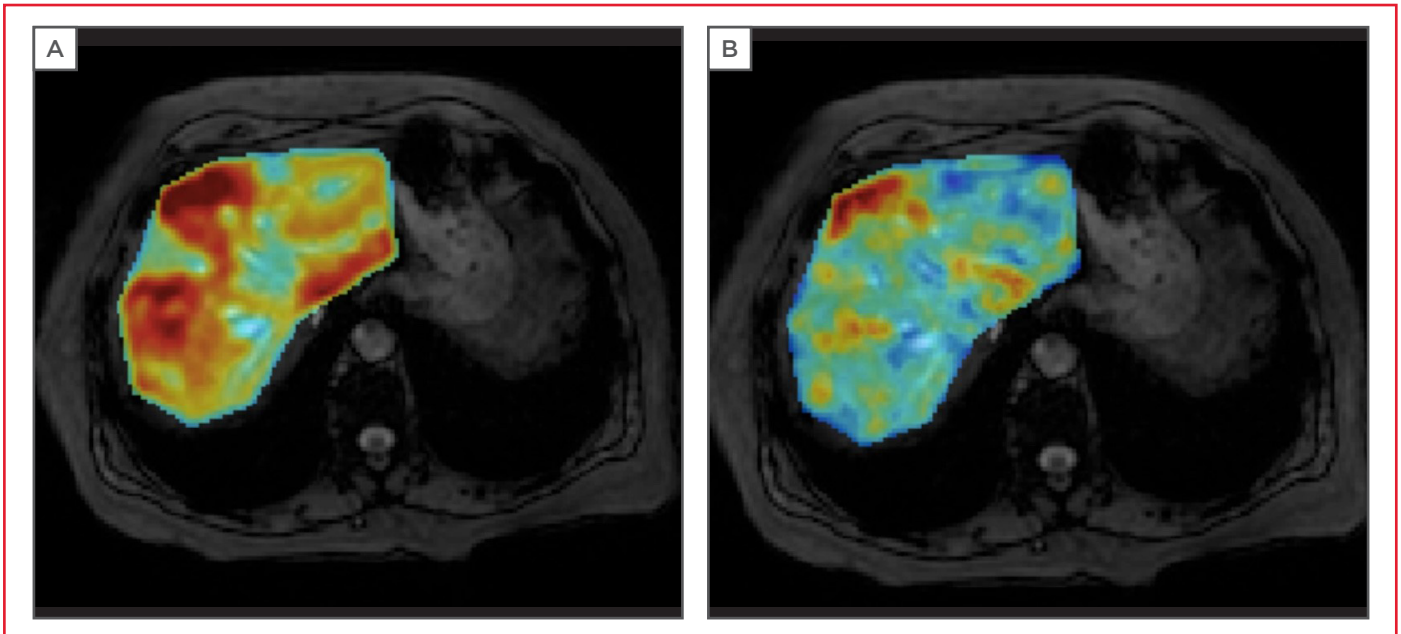


**Figure 1: Parametric liver MRI showing the quantitative analysis of a female patient, 69 years old, with increased serum ferritin (394 ng/mL). The PDFF (A) and R2* (B) parametric maps demonstrate liver steatosis and an increased amount of hepatic iron deposits, both with a heterogeneous distribution throughout the parenchyma.**
MRI: magnetic resonance imaging; PDFF: proton density fat fraction.

# ADENOSINE 2A RECEPTOR STIMULATION PREVENTS THE MULTIPLE PROCESSES THAT LEAD TO HEPATIC 'IMMUNO-LIPOTOXICITY' IN MICE FED A METHIONINE CHOLINE-DEFICIENT DIET AND BLOCKS NON-ALCOHOLIC STEATOHEPATITIS DEVELOPMENT

***Elisa Alchera,[1] Simona Rolla,[2,3] Chiara Imarisio,[1] Valentina Bardina,[2,3] Guido Valente,[4] Francesco Novelli,[2,5,6] Rita Carini[1]**

*1. Department of Health Science, University of Piemonte Orientale, Novara, Italy*
*2. Center for Experimental Research and Medical Studies (CERMS), Azienda Ospedaliera Città della Salute e della Scienza di Torino, Torino, Italy*
*3. Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy*
*4. Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy*
*5. Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy*
*6. Immunogenetics and Transplantation Biology Unit, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Turin, Italy*
**Correspondence to alchera@med.uniupo.it*

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Keywords: Steatosis, immune-reactions, adenosine, hepatocyte, cytoprotective signalling.

Non-alcoholic fatty liver disease (NAFLD) is a benign condition, but in ~20% of patients it can evolve into non-alcoholic steatohepatitis (NASH) with chronic hepatic inflammation, parenchymal injury, and possible evolution into fibrosis, cirrhosis, and cancer. No therapies are yet available for NAFLD and NASH patients. Their development, however, is urgent since NAFLD is the most frequent hepatic pathology in western countries and its incidence is continuously increasing.

In recent studies, we found that NASH development in mice is critically associated to a biphasic increase of T helper (Th)17 and that interleukin 17 (IL-17) was able to exacerbate the lipid-induced damage of mice hepatocytes (lipotoxicity). We have also previously shown that the pharmacological stimulation of the adenosine 2A receptor (A2aR) was able to reduce hepatocytes lipotoxicity and NASH severity in rats fed on a methionine choline-deficient (MCD) diet by phosphoinositide 3-kinase-mediated inhibition of c-Jun $NH_2$-terminal kinase (JNK).

At the International Liver Congress™ (ILC) 2017, hosted in Amsterdam, Netherlands, I presented our latest findings about the effects of A2aR stimulation on NASH development in mice. Adenosine is a powerful cytoprotective agent but is also known as a critical modulator of immuno-inflammatory reactions. We thus investigated its capacity to interfere with NASH development by modulating Th cell responses in order to avoid an immune-mediated potentiation of lipotoxicity. Indeed, we found that the treatment of steatotic mice with A2aR agonist CGS-21680 blocked NAFLD evolution into NASH and that such capacity was associated with multiple immuno-inhibitory and cytoprotective effects. These effects acted at three main levels: i) prevention of Th cell infiltration through the reduction of immuno-inflammatory signals involved in their recruitment/polarisation, ii) reduced activation of inflammatory Th subsets (Th17, Th22, Th1 cells) and increased immunosuppressive activity of regulatory T (Treg) cells, and iii) reduction of lipotoxicity and of its exacerbation induced by IL-17, through PI3-kinase stimulation and inhibition of the negative regulator of PI3-kinase, phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*), which was upregulated by IL-17.

These data offer novel insights on NASH pathogenesis and indicate a possible therapeutic approach to contrast its development and, consequently, its further evolution toward the more severe forms of liver disease (cirrhosis and cancer/cirrhosis). The finding that immuno-reactions and lipotoxicity co-operate to induce NAFLD development into NASH suggests, in fact, that this process, which we named 'immuno-lipotoxicity' might represent the critical factor responsible for NASH appearance. Moreover, the capacity of A2aR agonists to act as, contextually, cytoprotective and immunomodulatory agents strongly supports their therapeutic potential to inhibit NASH progression in early NASH patients.

# A NEW SCREENING STRATEGY FOR VARICES BY LIVER AND SPLEEN STIFFNESS MEASUREMENTS IN CIRRHOTIC PATIENTS: A RANDOMISED CONTROLLED TRIAL

***Grace Lai-Hung Wong**

*Institute of Digestive Disease, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong*
**Correspondence to wonglaihung@cuhk.edu.hk*

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Keywords: Chronic viral hepatitis, liver stiffness measurement, spleen stiffness measurement, splenomegaly, transient elastography, varices.

Variceal screening with upper endoscopy is recommended in cirrhotic patients but unfortunately is costly, uncomfortable to patients, and has low uptake. Non-invasive assessment with transient elastography for liver stiffness measurement (LSM) and spleen stiffness measurement (SSM) is accurate in detecting varices. A new screening strategy for varices guided by LSM and SSM results (LSSM-guided) with transient elastography may prove as good as universal endoscopic screening in detecting clinically significant varices in patients with cirrhosis.

Our research group aimed to test the hypothesis that a new LSSM-guided screening strategy for varices was non-inferior to universal endoscopic screening in detecting clinically significant oesophageal and/or gastric varices in patients with liver cirrhosis in a pragmatic clinical trial.

This was a non-inferiority, open-label, randomised controlled trial. The detection rates of clinically significant oesophageal and/or gastric varices in patients with liver cirrhosis were compared between the LSSM-guided screening strategy and the conventional approach. Individuals were randomised in a 1:1 ratio to either a LSSM-guided or conventional arm. Patients randomised to the LSSM-guided arm first underwent a transient elastography examination; those with high LSM (≥12.5 kPa) or SSM (≥41.3 kPa) results then underwent an upper endoscopy examination to screen for varices. Patients randomised into the control arm directly underwent an upper endoscopy examination.

Between October 2013 and June 2016, 548 patients were randomised to the LSSM arm (n=274) and the conventional arm (n=274); these patients formed the intention-to-treat (ITT) population. Patients in both study arms were predominantly middle-aged men with viral hepatitis-related cirrhosis in 85% of cases. Around 30% of patients had splenomegaly. Among the 264 patients who attended transient elastography examinations, the LSM and SSM values were 14.0±9.6 kPa and 37.5±20.7 kPa, respectively. In the ITT analysis, 11 out of 274 participants in the LSSM arm (4.0%) and 16 out of 274 in the conventional arm (5.8%) were found to have clinically significant varices. The difference between the two groups was -1.8% (90% confidence interval: -4.9–1.2%, p<0.001). The number of patients with

clinically significant varices detected was 5 out of 16 (31.3%) fewer in the LSSM arm.

In conclusion, the non-inferiority of the LSSM-guided screening strategy to the convention approach cannot be excluded by this randomised trial. This approach should be further evaluated in a cohort with a larger sample size with more clinically significant varices.

# PREDICTING SEVERE LIVER DISEASE IN THE GENERAL POPULATION: ABDOMINAL OBESITY, INSULIN RESISTANCE, DIABETES, CHOLESTEROL, AND ALCOHOL

***Fredrik Åberg**

*Transplantation and Liver Surgery Clinic, Helsinki University Hospital, Helsinki University, Helsinki, Finland*
**Correspondence to fredrik.aberg@helsinki.fi*

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Keywords: Liver cirrhosis, prediction, risk factor, alcohol, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome.

Liver disease is the 13th leading cause of death worldwide, and hepatocellular carcinoma is the 2nd leading cause of cancer death. Finland has one of the highest liver-mortality rates in Europe.

Alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) are the two most common types of chronic liver disease in the population. However, only 15–20% of heavy alcohol drinkers will ever develop liver cirrhosis, and <5% of NAFLD patients die from a liver-related cause. Individual susceptibility to progressive liver disease remains incompletely understood.

ALD and NAFLD are distinguished from each other by an arbitrary threshold of average alcohol intake of 30 g per day for men and 20 g per day for women. However, even lower amounts of alcohol intake have been linked to increased liver mortality. Weekly or monthly binge drinking may affect progression of NAFLD even when average alcohol intake is within the aforementioned limits. On the other hand, metabolic factors may affect progression of alcoholic liver disease. Both diseases also share some of the genetic risk factors (for instance, patatin-like phospholipases [PNPLAs]). This calls for a more holistic approach, where alcohol use and metabolic factors are taken into account at the same time in order to identify the individuals with a high risk of severe liver complications.

We investigated which metabolic factors best predict severe liver disease in the population with stratification by average alcohol intake. The study included 6,732 individuals without known liver disease who participated in the Health 2000 Study, which was conducted from 2000–2001. The cohort was representative of the general Finnish population. Follow-up data on liver-related hospital admissions, deaths, and liver cancer were collected until 2013.

We found that, at baseline, 46% of the adult population had metabolic syndrome and 22% were obese. Of these subjects, 12–13% were also alcohol risk drinkers (>30 g/day for men, >20 g/day for women). Of alcohol risk drinkers, 49% had metabolic syndrome, and 8% did not have at least one component of metabolic syndrome. This clearly shows that increased alcohol intake (alcohol risk use) and metabolic factors often coexist in the population.

In multivariate modelling, average alcohol use, total cholesterol, very low-density lipoprotein cholesterol, diabetes, homeostasis model assessment of insulin resistance, waist circumference, and low BMI emerged as significant predictors of severe liver disease. In the subgroup of alcohol risk users, the only factor that predicted liver disease was diabetes.

A high waist circumference to BMI ratio emerged as a novel predictor of liver disease. This ratio was significant both among subjects with BMI 19–25 kg/m$^2$, 25–30 kg/m$^2$, and >30 kg/m$^2$. It can be speculated that a high ratio reflects a metabolically unhealthy state, even when a person is considered lean merely by their BMI value.

Metabolic factors should not be overlooked in alcohol risk users. The dysmetabolic state that confers risk for complicated liver disease, on the other hand, cannot be evaluated by single variables such as BMI. For a comprehensive liver-risk assessment, lipid abnormalities, abdominal obesity, insulin resistance, diabetes, and alcohol use should all be addressed at the same time. These are also factors to be considered in future scores for quantifying individual risk for liver disease in the general population.

# JAPANESE GUIDELINES AND REAL-WORLD DATA ON THE MANAGEMENT OF HEPATITIS C

***Masayuki Kurosaki, Jun Itakura, Namiki Izumi**

*Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan*
**Correspondence to kurosaki@musashino.jrc.or.jp*

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Keywords: Hepatitis C virus (HCV), direct-acting antiviral (DAA), resistance-associated substitution (RAS).

The guideline committee of the Japan Society of Hepatology (JSH) have promptly updated the treatment guideline for the hepatitis C virus (HCV) after approval of the new direct-acting antiviral (DAA) regimen. Treatment options and recommendations are based on the results of the clinical trials and therefore should be validated by real-world data.

The Japanese Red Cross (JRC) Liver Study Group cohort is a nationwide, multicentre study group involving 92 Red Cross Hospitals. Hepatitis C patients treated with interferon-free DAAs are registered and prospectively observed. To date, 1,136 patients treated by daclatasvir+asunaprevir (DCV+ASV), 1,756 patients treated by ledipasvir/sofosbuvir (LDV/SOF), 344 patients treated by ombitasvir/paritaprevir/ritonavir (OMV/PTV/r), and 1,127 genotype 2 patients treated by SOF+ribavirin (RBV) are registered.

The first-line treatment for genotype 2 in the JSH guideline is SOF+RBV. Treatment duration is fixed to 12 weeks. The real-life JRC cohort revealed that the rate of sustained virologic response (SVR) was 97% overall and was 90% in interferon (IFN)-experienced cirrhosis.

For DAA naïve genotype 1, SOF/LDV for 12 weeks, OBV/PTV/r for 12 weeks, elbasvir/grazoprevir (EBR+GZR) for 12 weeks, beclabuvir (BEC)/DCV/ASV for 12 weeks, and DCV+ASV for 24 weeks could all be a treatment choice. NS5A resistance-associated substitution (RAS) testing is recommended for DCV+ASV and OBV/PTV/r because NS5A RAS attenuated the rate of SVR in Phase III trials. In real-life JRC cohorts, RASs in NS5A at baseline were analysed by population sequencing to facilitate the selection of an optimal DAA regimen. The prevalence of NS5A Y93H RAS in patients treated by DCV+ASV was low compared to the general population. The overall rate of SVR in the real-life cohort was significantly higher than the Phase III trial, which was possibly due to optimal patient selection based on NS5A RASs. The prevalence of NS5A Y93H RAS in patients treated by LDV/SOF was significantly high compared to the prevalence in the general DAA naïve population. The rate of SVR was >95% irrespective of baseline NS5A RASs. These results may reflect the preference of physicians in real-life to select LDV/SOF for treating patients with baseline NS5A RASs in accordance with the recommendations by the JSH guideline.

By the nationwide analysis of NS5A RAS after DCV+ASV failure, the prevalence of signature RASs at position Y93, L31 increased significantly compared to baseline. Among patients with signature L31/Y93 RASs, the incidence of triple RASs was 40% and the incidence of quadruple RASs was 10%. The interim analysis of re-treatment by LDV/SOF showed that SVR12 was around 60%. The rate of SVR correlated with the number of RASs. Therefore, the JSH guideline recommends determining NS5A RAS before re-treatment in patients who failed DAA treatment and to defer re-treatment in those having multiple RASs.

# WHAT IS THE ROLE OF URSODEOXYCHOLIC ACID FOR THE FUTURE MANAGEMENT OF PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS IN THE CLINIC AND IN TRIALS?

***Roger W. Chapman[1,2]**

*1. Nuffield Department of Medicine, Oxford University, Oxford, UK*
*2. Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford, UK*
**Correspondence to roger.chapman@ndm.ox.ac.uk*

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Keywords: Primary sclerosing cholangitis (PSC), cholangiocarcinoma, cholestasis, inflammatory bowel disease, microbiome.

Primary sclerosing cholangitis (PSC) is a rare, progressive, cholestatic, immune-mediated hepatobiliary disease. It is characterised by inflammation, stricturing, and concentric obliterative fibrosis of the biliary system, ultimately leading to biliary cirrhosis, portal hypertension, and eventually, in the majority of patients, hepatic failure.

It is predominantly found in males (male–female ratio 2:1) and is closely associated with inflammatory bowel disease, usually in the form of ulcerative colitis (UC). PSC is a premalignant disease with a high prevalence of hepatobiliary and colonic malignancy. The overall median survival is 21 years; however, mortality rates are ≤30% at 6 years, with the majority of deaths (40–50%) due to cancer and around 30–40% due to liver failure. Phenotypes of patients who have a bad prognosis include male patients with associated UC and both intra and extrahepatic involvement of the biliary tree.

There is no proven medical therapy for PSC. Trials of immunosuppressants and antifibrotic agents have been unsuccessful. A naturally occurring bile acid, ursodeoxycholic acid (UDCA), has been established as first-line therapy in primary biliary cholangitis, improving survival. UDCA has a number of actions that should be of benefit in cholestatic liver disease, including producing a choleresis (increase in bile flow), beneficial anti-apoptotic effects, and an increase in biliary bicarbonate secretion. In addition, it decreases bile toxicity by reducing the level of toxic hydrophobic bile acids, cholic acid, and chenodeoxycholic acid in the total bile acid pool.

The efficacy of oral UDCA in the treatment of PSC remains controversial. All studies since the 1980s have shown a significant drop in serum alkaline phosphatase. However, trials of moderate dose UDCA (17–22 mg/kg/day) have not shown a clear benefit to survival, and indeed higher doses (25–30 mg/kg/day) may be harmful. Moderate doses of UDCA may have a positive chemoprotective effect in the reduction of colonic dysplasia and colonic cancer in patients with PSC and inflammatory bowel disease.

However, it seems that a subset of approximately 30% of patients may respond, as assessed by normalisation of serum alkaline phosphatase after 1 year of treatment to <1.5-times the upper limit of the normal range. This has been shown to correlate

with increased survival in PSC. The most recent guidelines suggest a trial of 6 months of moderate-dose UDCA treatment in PSC patients with elevated alkaline phosphatase and continuing long-term therapy if normalisation of alkaline phosphatase is achieved.

PSC patients have been shown to have specific gut microbiota that differ from normal subjects and UC patients without PSC. New therapeutic strategies in PSC include clinical trials of alteration of the biome with antibiotic therapy and faecal transplantation. In addition, clinical trials of agents that may alter T cell function, preventing inappropriate homing of T cells to the liver and biliary system, are being evaluated. Also, trials of drugs that cause a reduction in biliary fibrosis and toxic bile are in progress. It is likely that bile acid therapy, either with UDCA or nor-UDCA, a C23 homologue of UDCA, may be a part of combination therapy for PSC in the future. A Phase III trial of nor-UDCA is in progress.

# HEPATITIS E VIRUS INFECTION AND ACUTE NON-TRAUMATIC NEUROLOGICAL INJURY

***Harry R. Dalton,[1] Glynn W. Webb,[2] Katy Jones[3]**

*1. European Centre for Environment and Human Health, University of Exeter, Truro, UK*
*2. Peninsula College of Medicine, Truro, UK*
*3. Royal Cornwall Hospital, Truro, UK*
**Correspondence to hardalton@gmail.com*

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Keywords: Hepatitis E virus (HEV), zoonosis, epidemiology, neurology, neuralgic amytrophy, stroke, epilepsy, pathogenesis.

Hepatitis E virus (HEV) is endemic in many developed countries, with seroprevalence rates ranging from 4.7% in Scotland to 31.1% in France. Cases are caused by genotypes 3 and 4, which are porcine zoonoses (Figure 1). Infection is associated with the consumption of undercooked pork meat and is often asymptomatic.[1] In England there are an estimated 100,000 new infections each year, but <1% of cases are laboratory-confirmed.[2] A significant cause of this disparity is the currently incompletely understood clinical phenotype of HEV infection.

**Figure 1: Hepatitis E virus is a porcine zoonosis in developed countries, and pigs worldwide are asymptomatically infected.**

HEV infection has been associated with a variety of extra-hepatic manifestations, the most common of which are neurological.[3,4] To date, ~100 cases of hepatitis E-associated neurological injury have been described in case reports/series.[5] HEV infection has been linked to a number of neurological conditions including neuralgic amyotrophy (NA), Guillain-Barré syndrome (GBS), and meningoencephalitis. Recent studies have found that 5–11% of GBS patients[6] and 10% of NA patients[7] had evidence of HEV infection at the onset of their neurological illness. Importantly, in these cases neurological signs and symptoms dominated the clinical picture; patients were mostly anicteric and had normal or only mildly elevated liver enzymes. The diagnosis of HEV is easily, and probably commonly, overlooked.

To further elucidate the range and frequency of HEV-associated neurological injury we conducted a multicentre study across four centres in the UK, France, and the Netherlands. Four hundred and sixty-four consecutive patients who presented with acute non-traumatic neurological illnesses were prospectively tested for HEV by serology and polymerase chain reaction (PCR). Of these, 11 patients (2.4%) had evidence of current or recent HEV infection, 7 (1.5%) of whom were HEV PCR positive. Neurological presentations were wide ranging and included NA (n=3, all PCR positive), cerebrovascular event (n=4), seizure (n=2), encephalitis (n=1), and an acute combined facial and vestibular neuropathy (n=1). In all cases, the hepatitis was mild and no patients were jaundiced. All strains were classified as genotype 3. The cases of NA were all middle-aged males with bilateral involvement of the brachial plexus.

The most important question regarding the above observations is: 'Is the relationship between HEV infection and neurological injury just a chance association or is the relationship causal?' In NA, our view is that this relationship is causal. Our reasoning for this assertion is based on very recent findings from another multicentre European study of 118 patients with NA, ~50% of whom were associated with HEV infection.[8] This study showed that bilateral involvement of the brachial plexus is the clinical hallmark of HEV-associated NA. The cases of NA we observed in the current study all had bilateral involvement of the brachial plexus. This observation, together with a temporal relationship between HEV infection and NA in differing geographical locations in Europe, together with laboratory data which show that HEV is potentially neurotropic,[9] is strong evidence supporting a causal relationship between HEV infection and NA.

Our finding that 1.5% of subjects with acute non-traumatic neurological injury have HEV viraemia at presentation is striking but needs to be contextualised. The prevalence of HEV genotype 3 viraemia among asymptomatic blood donors ranges from 0.035% in the UK (2013–4),[2] 0.045% in France (2012–3), and 0.17% in the Netherlands (2013–4).[10] Although the HEV viraemia rate in neurological cases we observed is an order of magnitude higher than we would have expected from these blood donor studies, this by itself does not prove causality. The reason for this is that the incidence and prevalence of HEV infection in developed countries varies both geographically and over time. Careful case-control studies are now needed to further determine the role of HEV in patients with neurological injury, other than NA.

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# CLINICAL HEPATOCYTE CELL THERAPY: CHALLENGES AND OPPORTUNITIES

***Stephen C. Strom,[1] Carl Jorns[2]**

*1. Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden*
*2. Division of Transplant Surgery, Karolinska University Hospital, Stockholm, Sweden*
**Correspondence to stephen.strom@ki.se*

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Keywords: Hepatocyte transplant, metabolic liver disease, cell therapy, rejection.

The clinical application of hepatocyte transplants to treat patients with liver disease extends back to the early 1990s. Hepatocyte transplants are conducted for three general purposes. One is to extend the life of a patient listed for an orthotopic liver transplant (OLT) but who, because of deteriorating conditions, is expected to die while on the waiting list. Hepatocyte transplants provide temporary liver function to 'bridge' and sustain the person for up to several days, which provides time for a suitable organ to be identified and transplanted. A second group of patients are a subset of the first. Some patients with acute liver failure have received what was intended to be a bridging hepatocyte transplant, yet following the cellular therapy the acute liver failure reversed, and the patient no longer required an OLT. The third and most common current use of a hepatocyte transplant is to correct monogenetic liver diseases that would otherwise require an OLT. In these cases, the cellular therapy is intended to support the one critical liver function that is missing in the patient while the patient's native liver supports all other liver functions. Although results in preclinical studies with small animal models of monogenetic liver defects have been quite successful, hepatocyte transplants in patients have shown consistent but low-level support of the missing liver function, and the hepatocytes appear to show metabolic activity ranging from 0.5–2 years post-transplant in most published studies. The reasons for the loss of function are unknown but may be related to the senescence of the donor cells and/or the rejection of the cell graft.

In the preclinical studies, the hepatocytes are sustained (but usually for <1 year) through the use of preconditioning treatments to the recipient liver that inhibit the growth of the native liver or otherwise offer a growth stimulus to the donor cells. This allows donor cells to proliferate and form large colonies of cells in the recipient liver. It is likely that colonies of donor cells are sustained and renew themselves in the recipient, while cells that engraft and remain as single cells or small clusters fail to proliferate and either senesce or are more readily rejected. Most preclinical conditioning regimens are considered too toxic for use on patients. Two preconditioning treatments that are considered safe enough for clinical trials are surgical liver resection or low-dose radiation of a portion of the liver prior to the hepatocyte transplant. Liver resection should induce a regeneration response in the remnant liver, including donor cells, while liver radiation should limit the growth of native hepatocytes in the irradiated area, which will provide a growth advantage to the donor cells.

Two recent reports have addressed the problem of preconditioning and also investigated the immune response to the transplants. Jorns et al.[1] reported that surgical resection of segments 2 and 3 was safe and that the metabolic activity provided by the donor hepatocytes was perhaps better than expected from the transplantation of that number of cells. Two patients with Crigler–Najjar syndrome Type 1 underwent partial hepatectomy immediately before hepatocyte infusion. Both patients showed a 50% decrease in total serum bilirubin, and graft function was confirmed by detection of bilirubin diglucuronides in duodenal bile. The presence of donor hepatocytes was confirmed in a liver biopsy 4 months after transplantation. However, long-term graft loss occurred in both patients after transplantation associated with detection of *de novo* donor specific human leukocyte antigen antibodies, suggesting that the graft was rejected.

Diagnosis of rejection is particularly difficult in clinical hepatocyte transplantation, as no suitable marker of graft function nor marker for graft rejection currently exists. Repeated liver biopsies are not applicable due to sampling error and risk of complications.

Soltys et al.[2] reported that 10 Gy irradiation of ≤35% of the liver of non-human primates resulted in the repopulation of the liver with ≤15% donor hepatocytes. A similar irradiation protocol prior to hepatocyte transplant in an adult patient with phenylketonuria was found to be effective in reducing blood phenylalanine levels ≤200 days post-transplant. Two patients aged 4 and 7 months with urea cycle defects received 5 or 7.5 Gy, respectively, prior to the hepatocyte transplant. Both showed a minimal therapeutic response post-transplant, and both showed a rejection response within 75 days of the transplants.

In summary, two new preconditioning protocols, surgical liver resection and liver irradiation, have been introduced into clinical hepatocyte transplant trials. Both were deemed to be safe. In three of the five reported cases the authors reported that the correction of the enzymatic defect seemed to be greater than might be expected from the number of cells transplanted and that additional studies will be needed to fully assess efficacy. Both studies confirmed the recipient to the donor cells initiated an immunological response. Additional studies will be needed to determine if the immune response is related to the loss of cell graft function.

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# SPECIALIST NURSES IN EUROPE: WHERE DO WE STAND?

***Françoise Charnay-Sonnek**

*President of ESNO; Educational Head at FNCLCC-EFEC, Paris, France*
**Correspondence to francoise.charnaysonnek@bbox.fr*

**Citation:** EMJ Hepatol. 2017;5[1]:60-62. Abstract Review No. AR11.

Keywords: Nurse specialist, advanced practice nursing, recognition at European level, common training framework, mobility, security and quality of care.

## CONTEXT

Nurses and specialist nurses are playing a growing role in the healthcare system. Alongside the specialities already existing, such as nurse anaesthetists, operating room nurses, intensive care nurses, mental health nurses, and paediatric nurses, new specialties have also emerged, or are about to emerge, such as cancer nurses, endocrinology nurses, diabetes nurses, and cardiology nurses.

Despite the fact that formal training for certain specialties has existed for years, practice, status, training duration, and content may be drastically different from one country to another. Some other specialist roles have been established in Europe, with a professional transnational collaboration, including diabetes, dialysis, urology, and oncology. Moreover, the development of higher medical technologies and more sophisticated treatment requires specialist nurses.

According to the World Health Organization's (WHO) 'Health 2020. A European policy framework and strategy for the 21st century', health for all should be improved and health inequalities reduced.[1] We can observe a higher demand for healthcare services and physician-specialists alone cannot respond to this demand; therefore, nurse specialists have a role to play. Nevertheless, their role is still not homogeneously defined across Europe.[2-7] There is no mention of nurse specialists

in the European directive 55/2013/EU[8] on the recognition of the professional qualification and there is no harmonisation in terms of education, practice, and status.

ESNO is committed to recognising nurse specialists at the European level. We represent the interests of the member organisations and we provide expert advice and share best practice on issues shaping specialism in nursing. Our scope of interventions is large and includes advocacy, education, lobbying, and contributing to different actions run by European organisations, institutes, and agencies. FEND and ESNO are leading a survey titled 'Nurse specialists in Europe Definition of a Common Diabetes Nurse Specialist Training Framework- a Feasibility Study (DiaFram)'. We have started with the specialty of diabetes, but the study corpus can be duplicated to other specialties, in order to allow them to develop their own common training framework according to their own specificities.

Our long-term strategies aim to set the status of nurse specialists in the healthcare arena at master's, post-master's, and doctoral level with an automatic recognition of their qualification. The harmonisation of practice and scope of competences is essential to permit mobility at a pan-European level and, last but not least, to ensure the security and quality of the care.

ESNO's members consist of individual European specialist nurses, and the number of members is still growing. Today, we number 15 members representing different specialties:[9]

- Association for Common European Nursing Diagnoses, Interventions and Outcomes (ACENDIO)
- European Dialysis and Transplant Nurses Association/European Renal Care Association (EDTNA/ERCA)
- European federation of Critical Care Nursing associations (EfCCNa)
- European Nurse Directors Association (ENDA)
- European Oncology Nursing Society (EONS)
- European Operating Room Nurses Association (EORNA)
- Foundation of European Nurses in Diabetes (FEND)
- International Federation of Nurse Anesthetists (IFNA)
- European Association of Urology Nurses (EAUN)
- European Society of Gastroenterology and Endoscopy Nurses and Associate (ESGENA)
- European Respiratory Nurses Association (ERNA)
- European League Against Rheumatism/Nurses section (EULAR)
- European Society for Emergency Nursing (EuSEN)
- European Association of Neuroscience Nurses
- European Veterinary Nurses

Candidate

- European Society of Endocrinology Nurses (ESE)
- European Conference on Mental Health (ECMH)
- IBD, Inflammatory Bowel Diseases

Observer

- Skin and Dermatology Nurses Europe

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# SUPPORTING LIFESTYLE CHANGE THROUGH NURSE-LED MOTIVATION

***Kathrin Husi**

*Inselspital, University Hospital, Bern, Switzerland*
**Correspondence to kathrin.husi@insel.ch*

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Keywords: Training hepatology care, motivation and contract, nurse-led.

## TRAINING HEPATOLOGY CARE

The main focus of training hepatology care is to gain skills and enable nurses to specialise in hepatology. Skills and specialisation are gained through six core modules, each requiring 1 day of training.

The six core modules are Viral Hepatitis, Steatohepatitis, Cirrhosis, Liver Transplantation, Hepatocellular Carcinoma, and Rare Liver Diseases. The modules are focussed on improving patient wellbeing, saving money in healthcare, and bringing benefits for physicians and nurses. Achieving these goals relies on already trained nurses. This contribution is one piece of the module Steatohepatitis and its concerns with therapy. Most of the patients benefit if they lose weight or gain physical conditioning with lifestyle changes.[1]

## MOTIVATION AND CONTRACT

A common perception of lifestyle change is that modification of diet or therapy is sufficient; this is an illusion that can jeopardise long-term success.[2] With fact-based motivation, we help patients establish self-responsibility.[3] The most important part of establishing self-responsibility is to reach the patient's intrinsic motivation. Let the patient define their one soft spot of readiness for change within their different areas of life. The deeper the soft spot lies within the ego, the more likely the changes are to be full-hearted and to endure better in terms of lifestyle. With this patient-driven information, the nurse and the patient define together a contract and sign and date it. The contract is based on the collected soft facts and the patient's willingness to change (Figure 1).

The monthly adjusted lifestyle change contract contains several items:

- 30% of the contract is focussed on the nutritional change in quantity and quality, e.g. consuming 500 kcal per day fewer
- Another 30% focusses on increased moderate movements in daily life and vigorous exercise three times per week (pw) or 6 days pw for 10 minutes each
- The final part contains details such as the patient's soft spots, steps towards a better self-esteem, identification and naming of patient's important persons, the promise to progress only one step after another, the promise to take part in recreation 1 day pw, verbal conversations, written down objective measurements, and the change in nutritional quality

## NURSE-LED

To accompany the contract, which includes the patient's information and ideas, transparent workflow and data management for institutions, physicians, nurses, and patients is needed. The main

care part is completed by nurses, which is more time efficient for patients and physicians, is simpler for patients, and brings job-enrichment to nurses. After the physician's description of lifestyle change therapy, nurses take over the care of the whole procedure.[4] If the situation changes and problems emerge, physicians will be involved and give supplemental advice to nurses. It is best to work in a tandem-pattern: nurse and physician together. To release the patient from the contract, measurable values are declared such as questionnaires, lists, workflows, contracts, and evaluations. This allows quality checks and ensures safety.

## DEMISSION OF THE PATIENT

Of importance are measurable hard and soft facts before the cessation of a patient's therapy. They must be experienced in the new lifestyle, motivated to move forward, and willing to take over the full responsibility. It is best to also have a questionnaire for quality and outcome control.

## CONCLUSION

The idea of bringing such a complex situation to nurses' practical focus is timely. We need a new, transparent, organised workflow to bring back physicians' and nurses' enjoyment of professional competencies, which will lead to a bigger output and best care for patients.

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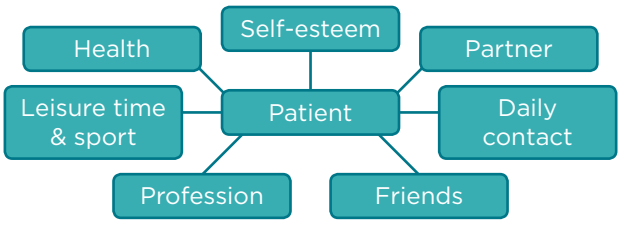
Patient's data: .....

I am ready to improve my lifestyle starting DD/MM/YYYY

**Mark your personal soft spots:**




Therefore I am ready to (one step per topic and month):

- Increase my **daily movements**
- Increase my **vigorous exercises**
- Reduce my **amount of calories by 500 per day**
- Replace my food intake with **healthier nutrition**

To boost my self-esteem I .....
I am sharing the plan to change my lifestyle with: .....
I need added support from / with / in .....

I agree to be interviewed by my caregiver and I am willing to answer the needed questions.
We are in a transparent relationship concerning progress tracking, results, and measurements.

Date and signature of care receiver

.....

Date and signature of care provider

.....

Next appointment and contract : ☐ in 2 weeks ☐ in 1 month at .....

**To fill at next appointment**
I reached my goals (answer at our next appointment): .....
☐ exceeded ☐ yes ☐ partly yes ☐ partly no ☐ no

**Define all conditions to the point**



**Figure 1: Contract for Start of Lifestyle Changes.**

# DIAGNOSIS AND STAGE-DEPENDENT TREATMENT OF HEPATOCELLULAR CARCINOMA

***Sadahisa Ogasawara,[1] Masao Omata[2,3]**

*1. Department of Gastroenterology, Graduate School of Medicine, Chiba University, Chiba, Japan*
*2. Yamanashi Prefectural Central Hospital, Kofu, Japan*
*3. Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
**Correspondence to sadahisa@me.com*

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Keywords: Hepatocellular carcinoma (HCC), Asian Pacific Association for the Study of the Liver (APASL), guideline, treatment algorithm; diagnostic algorithm.

Hepatocellular carcinoma (HCC) is currently the second most common cause of cancer-related death worldwide, with the majority of all cases of HCC found in the Asia-Pacific region. Indeed, HCC is one of the leading public health challenges in the Asia-Pacific region. The Asian Pacific Association for the Study of the Liver (APASL) HCC guideline was published in 2010, and it has been the oldest of the major guidelines to date. The 'Toward Revision of the APASL HCC Guideline' meeting was held at the 25th annual conference of the APASL, Tokyo, Japan, on 23rd February 2016. The new guideline is evidence-based and is generally accepted in the Asia-Pacific region, which has a diverse range of medical environments. This latest guideline includes two variations of diagnostic algorithms based on the use of multi-modalities, mainly gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) and contrast-enhanced ultrasound (CEUS), and only using dynamic computed tomography (CT) or MRI. This is because this guideline focusses on the universal usage in the Asia-Pacific region. The updated treatment algorithm has several unique points. Firstly, recommended treatments are separated into 'standard treatment' and 'treatment being widely performed' in the Asia-Pacific region. Secondly, the indication of resection is not defined and decided in discussions between surgeon and hepatologist in Child–Pugh classified A and B patients without extrahepatic metastasis. Although these ideas seem to go against the era of evidence-based medicine, we believe that this latest treatment algorithm gives priority to HCC patients in the Asia-Pacific region, with a wide variety of medical environments. The latest APASL HCC guideline is going to be published in several months.

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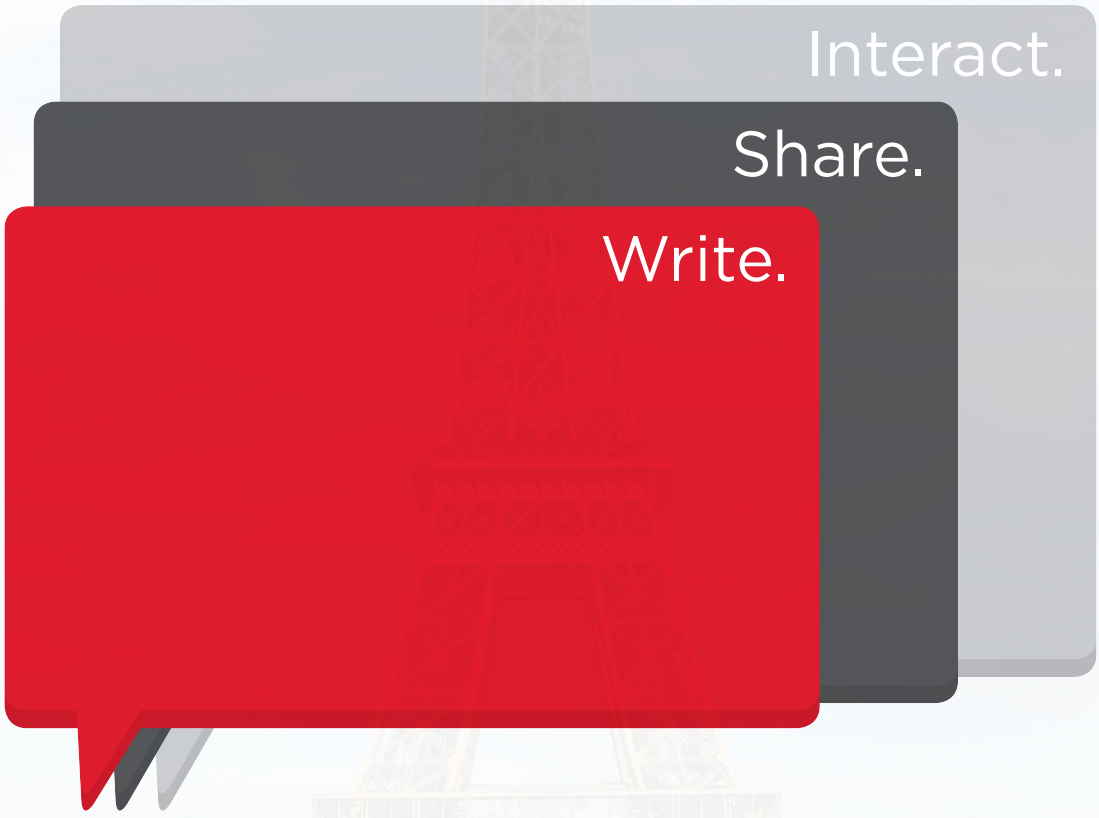


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SPECIAL POSTER FEATURE

# AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES (AASLD): THE LIVER MEETING® 2016 COVERAGE

***Chris Ontiveros**

*ApotheCom, New York City, New York, USA*
**Correspondence to Chris.Ontiveros@apothecom.com*

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## MEETING SUMMARY

The Liver Meeting®, the official meeting of the American Association for the Study of Liver Diseases (AASLD), held in Boston, Massachusetts, USA, supported the presentation of recent clinical and basic research in liver disease by renowned experts, to target the diverse needs of hepatology professionals. A summary of posters presented on viral hepatitis and orthotopic liver transplantation (LT) and oral presentations discussing chronic hepatitis infection were given.

## VIRAL HEPATITIS AND ORTHOTOPIC LIVER TRANSPLANTATION POSTER SESSION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are among the most common causes of cirrhosis and hepatocellular carcinoma (HCC), with cirrhosis secondary to chronic HCV the leading indication for LT globally.[1] The Viral Hepatitis and Orthotopic Liver Transplantation poster session at The Liver Meeting® covered important topics related to LT including whether HCV antibody (Ab) positive livers can safely be transplanted into HCV $Ab^-$ recipients and whether regional differences exist in the characteristics of patients with HCV-related LT. Outcomes for chronic HCV have improved with the introduction of interferon (IFN)-free direct-acting antiviral (DAA) regimens,[1,2] and studies presented in this poster session largely relate to the safety and efficacy of DAAs, some of which were conducted in special patient populations such as HIV-HCV co-infected individuals.

Late breaking abstracts of large clinical studies and high-impact translational studies, which featured the most current research, were presented at The Liver Meeting 2016.

### The Use of Hepatitis C Virus-Positive Donors in Hepatitis C Virus-Negative Liver Transplant Recipients

There has been an increasing emphasis on using HCV $Ab^+$ donor livers in HCV $Ab^-$ LT candidates with the introduction of DAAs; however, data are lacking to support these potential donor-expanding policies.

Dr George Cholankeril, University of Tennessee, Memphis, Tennessee, USA, presented a study that evaluated post-LT survival rates in HCV $Ab^+$ donor/HCV $Ab^+$ recipient (+/+) and $HCV^+$ donor/HCV $Ab^-$ recipient (+/-) populations, using the United Network for Organ Sharing (UNOS) Database (2003–2014).[3] Survival was also compared in the HCV $Ab^+$ donor cohorts with the HCV $Ab^-$ donor/HCV $Ab^-$ recipient populations (-/-).

Of 1,953 HCV $Ab^+$ donor livers, 1,883 (96.4%) were allocated to HCV $Ab^+$ LT recipients (+/+)

and 70 (3.6%) to HCV Ab$^-$ LT recipients (+/-). Compared with the HCV Ab (+/+) group, the HCV Ab (+/-) group contained both older donors (43.5 versus 41.5 years; p<0.01) and younger recipients (52.0 versus 54.6 years; p<0.01).

HCV Ab (+/-) LT recipients had a lower 1-year and 5-year post-LT survival rate compared with HCV Ab (-/-) and HCV Ab (+/+) recipients (p<0.001). The 1-year and 5-year survival rates post-LT were, respectively, 90.1% and 81.7% for HCV Ab (-/-), 89.9% and 77.1% for HCV Ab (+/+), and 85.7% and 65.7% for HCV Ab (+/-).

A subanalysis of findings from the years 2013–2014 (era of DAA) was performed. During this 2-year period, 32 of the 70 (45.7%) HCV Ab (+/-) LT surgeries occurred. The subanalysis found that the 1-year survival rate post-LT in the HCV (+/-) group was 90.6%, comparable to both the HCV Ab (-/-) and HCV Ab (+/+) groups (91.7% and 92.5%, respectively).

The investigators of this study demonstrated a worse survival rate post-LT in HCV Ab$^-$ recipients who received livers from HCV Ab$^+$ donors. However, during the era of DAA therapy, an improvement was noted in the 1-year survival rate post-LT, raising the possibility that the donor pool may be safely expanded in the future.

## Peritransplant Treatment with Direct Acting Antivirals of HIV-Hepatitis C Virus Co-Infected Patients

New DAAs have significantly improved sustained viral response (SVR) rates in the treatment of HCV, including special patient populations, such as HIV-HCV co-infected individuals and LT recipients. However, there are limited efficacy and safety data for DAAs in HIV-HCV co-infected patients in the peritransplant setting.

Dr Carmen Vinaixa, Hospital Universitari i Politècnic La Fe, Valencia, Spain, presented an observational case-controlled study that investigated the efficacy, safety, and drug–drug interactions with DAA treatment in HIV-HCV co-infected patients in the pre-transplant setting (wait list) and post-transplant setting.[4] The HIV-HCV co-infected patients were compared to HCV mono-infected patients by matching each co-infected patient with two mono-infected patients by HCV genotype, fibrosis stage, antiviral treatment regimen, and by Model of End-Stage Liver Disease (MELD) and Child–Pugh scores if cirrhosis was present.

In the pre-transplant setting, data were collected for nine HIV-HCV co-infected patients and 18 HCV mono-infected patients. The most common treatment regimen in co-infected patients was sofosbuvir (SOF)+daclatasvir (DCV)±ribavirin (RBV) (70%), and in mono-infected patients was SOF/ledipasvir (LDV)±RBV (43%). A rapid virological response occurred in 67% of co-infected and 73% of mono-infected patients. At Week 4, a SVR occurred in 67% of co-infected and 89% of mono-infected patients, and these rates were unchanged at 12 weeks. There were two non-responses in the HIV-HCV (genotype 4) co-infected patients treated with the suboptimal regimen SOF-RBV, and one relapse occurred in a single HCV mono-infected patient.

In the post-transplant setting, data were collected for 15 HIV-HCV co-infected patients and 30 mono-infected patients. The most common treatment regimen was SOF/LDV±RBV in co-infected patients (67%) and mono-infected patients (64%). The rapid virological response rate was 57% in co-infected and 80% in mono-infected patients; the SVR rates at Week 4 and Week 12 were 93% in co-infected and 97% in mono-infected patients. One relapse occurred in a HIV-HCV co-infected patient. Compared with mono-infected patients, co-infected patients had higher rates of anaemia (57% versus 50%), use of erythropoietin (EPO; 33% versus 17%), and transfusions (33% versus 10%).

The investigators concluded that antiviral treatment with DAAs in HIV-HCV co-infected patients in the pre and post-transplant setting is effective, safe, and easily applicable, even when co-administered with HIV antiviral drugs. In the co-infected group in the pre-transplant setting, there was a higher incidence of infections, whereas more anaemia, use of erythropoietin (EPO), and transfusions were evident in the post-transplant setting. The investigators recommend closer on-treatment monitoring in HCV-HIV co-infected patients in the peritransplant setting.

## Hepatitis E Virus Infection and Hepatic Graft Versus Host Disease In Allogenic Hematopoietic Stem Cell Transplantation Recipients

In immunocompromised patients, such as those with allogenic haematopoietic stem cell transplantation (alloHSCT), hepatitis E virus (HEV) genotype 3 infection can lead to the development of chronic HEV infection and liver cirrhosis.[5,6] Increased alanine

aminotransferase (ALT) levels occur in most patients with HEV infection and at least one episode of elevated transaminase levels is experienced by most alloHSCT patients post-transplantation.[7] Elevated ALT levels are usually ascribed to drug toxicity, graft versus host disease (GvHD), or iron deposition.[8]

Dr Donna Bezuur, Academic Medical Center, Amsterdam, Netherlands, presented a retrospective cohort analysis on the prevalence of HEV infection in patients with elevated ALT levels following alloHSCT between 2005 and 2015.[9] Of the 130 alloHSCT patients, 123 had ≥1 episodes of elevated ALT levels (defined as ALT >50 U/L for ≥4 consecutive weeks), recurrent elevated ALT levels (>50 U/L for a shorter period of time with normal ALT levels in between), or an episode of peaking ALT levels (>100 U/L during a period of <4 weeks). To confirm an active viral presence at the time of ALT elevation, HEV RNA was isolated and identified using real-time quantitative PCR.

Of the 123 patients with ALT elevations, 5 (4%) had HEV infection; their age ranged from 37–70 years, 2 were female, and the underlying diseases were chronic lymphoid leukaemia (n=2), acute lymphatic leukaemia (n=1), chronic myeloid leukaemia (n=1), or Hodgkin lymphoma (n=1). In the entire cohort of 130 alloHSCT recipients, 19 were diagnosed with GvHD. Three of the five HEV positive patients had signs of concomitant GvHD, and in two of these patients, RBV treatment led to rapid clearance of the virus and resolution of the GvHD.

The investigators concluded that HEV infection was prevalent among alloHSCT recipients and may be related to the presence of GvHD. Additionally, the investigators hypothesised that HEV could provoke or sustain hepatic GvHD. While further study in larger patient cohorts is required to confirm these findings and test this hypothesis, the results suggest that all alloHSCT patients with persistently elevated ALT levels should be considered for HEV infection, particularly in patients with signs of hepatic GvHD.

## Daclatasvir in Combination with Other Direct Acting Antivirals Achieves a High Rate of Virological Clearance with an Excellent Safety Profile in Liver Transplanted Patients for Hepatitis C Virus

Prior to the new DAA era, outcomes were poor for patients who underwent LT for HCV. Based on the availability of DAA combinations, the Italian Named Patient Programme (NPP) was started (2013–2014). The NPP was granted access to LT recipients who had advanced disease and a life expectancy <12 months due to severe HCV recurrence or cholestatic hepatitis.

Dr Rafaella Lionetti, IRCCS Lazzaro Spallanzani, Rome, Italy, presented a real-world study that evaluated the efficacy and safety of DAAs.[10] Italian patients in the NPP that underwent LT for HCV and who were treated with DCV+SOF or simeprevir (SMV) were included in the analysis. Of the 94 patients, the majority were infected by HCV genotype 1 (84.1%) and 79.2% of patients were cirrhotics. The treatment used was DCV+SOF±RBV in 88 patients and DCV+SMV in 6 patients, with all patients treated for 24 weeks. The addition of RBV to dual DAAs was by physician choice. HCV RNA was undetectable in 50% of patients at Week 4, 75% at Week 8, and 97% at Week 12. Of the 87 evaluable patients, the SVR at Week 12 was 88.2% and remained unchanged at Week 24. Virological breakthrough after achieving viral undetectability occurred in three patients in the DCV+SMV group without RBV, and two patients on DCV+SOF without RBV relapsed after end of treatment (EOT). Overall, RBV was administered in 53 patients (57%); all but 1 patient experienced SVR; therefore, RBV use was associated with SVR benefit (odds ratio [OR]: 15.1; p=0.012).

A significant improvement from baseline in bilirubin (p<0.001) and albumin (p<0.0001) levels was found at the EOT and at Week 12 and 24 follow-ups. Creatinine increased significantly from baseline to EOT (1.14 versus 1.2 mg/dL; p<0.004), which indicated a worsening in renal function; creatinine levels at the Week 12 follow-up (1.17 mg/dL) and Week 24 follow-up (1.19 mg/dL) were no longer significantly higher than baseline, suggesting a return to normal function. RBV was stopped in 13 patients due to anaemia (n=11), rash (n=1), or diarrhoea (n=1).

Findings from the study found that the combination of DAAs with DCV was efficacious and had an excellent safety profile in this real-world Italian study of LT HCV patients. The addition of RBV to DAAs appeared to improve SVR. Liver function improved during therapy and persisted during follow-up, whereas a mild and transient worsening in renal function occurred during treatment.

## Variation in Demographics and Comorbidities in Hepatitis C Virus Liver Transplant Recipients within United Network for Organ Sharing Regions

HCV is the leading indication for LT in the USA. Dr George Cholankeril presented an analysis of regional characteristics of HCV-related LT recipients within the USA.[11] The UNOS database was used to determine differences in HCV-related LT within the 11 UNOS regions of the USA from 2003–2014. A subanalysis then compared the two UNOS regions with the highest number of HCV-related LTs. Analysis included the evaluation of demographics, diabetes, ascites, hepatic encephalopathy, HCC, and post-transplant survival.

Overall, 20,778 HCV-related LTs were performed in the USA from 2003–2014. Region 3 (n=3,415; 16.4%) and Region 5 (n=3,150; 15.2%) were the two UNOS regions with the highest proportion (31.6%) of HCV-related LTs. Compared with all other HCV-related LTs in the USA, Region 3 and Region 5 had slightly improved 5-year post-transplant survival with 75.6% for other regions, 76.9% for Region 3 ($p<0.01$ versus other regions), and 78.1% for Region 5 ($p<0.01$ versus other regions).

When comparing characteristics between regions, Region 5 had a higher proportion of Asians (8.3% in Region 5, 1.5% in Region 3, and 2.6% in other regions; $p<0.01$), which constituted 38.7% of all HCV-related LTs within the Asian cohort. Another notable difference was the prevalence of HCC, which was 32.1% in Region 5, 22.7% in Region 3, and 29.9% in other regions ($p<0.01$). Regional differences in demographics and comorbidities of HCV-related LT recipients within the USA are considerable, though this likely represents a regional disparity in wait list time to LT. Understanding these differences in HCV-LT recipients may help identify geographical subpopulations at risk for decompensated liver disease due to HCV.

## 100% Virological Response With 3D Regimen and Significant Short-Term Liver Stiffness Improvement in Patients with Recurrent Hepatitis C Virus Following Liver Transplantation

The DAA regimen of ombitasvir/paritaprevir/ritonavir, dasabuvir, and RBV (3D+R) was approved by the US Food and Drug Administration (FDA) in December 2014 for LT recipients with genotype 1 recurrent HCV, although drug interactions may be a concern. Treatment with 3D+R is a cost-effective and outcome-improving regimen for this difficult-to-treat population of LT patients with recurrent HCV.[12]

Dr Speranta Iacob, Fundeni Clinical Institute, Bucharest, Romania, presented results from a cohort of 72 patients with recurrent HCV after LT who were treated with 3D+R for 24 weeks.[13] Liver stiffness can be used to assess inflammation and fibrosis in LT recipients and to follow these patients after HCV eradication. Liver stiffness was assessed by non-invasive Fibroscan® before therapy, at EOT, and at the time of SVR12 evaluation. FibroMAX was performed before therapy to provide a measure of baseline inflammation, fibrosis, and steatosis.

Of the 72 patients, 40 were male, the mean age was 55.2 years, and the median time since LT was 26.6 months. By Week 8 of treatment, HCV RNA was undetectable in 100% of patients, and this persisted up to 12 weeks after EOT. Liver stiffness assessed by FibroMAX at baseline differed significantly by activity grade ($p=0.0008$) and fibrosis stage ($p<0.0001$). Furthermore, liver stiffness assessed by Fibroscan significantly improved from baseline to EOT ($p=0.0016$) and further improved from EOT to SVR12 ($p=0.007$).

The study found that virological response with 3D+R in LT patients with recurrent HCV reached 100% at EOT and up to 12 weeks after. Liver stiffness significantly decreased after EOT and SVR12 and suggests improvement in liver damage with 3D+R.

## Treatment of Hepatitis C Virus with Ledipasvir, Sofosbuvir, with or without Ribavirin in Post-Liver Transplant Patients in an Academic Centre

Recurrence of HCV is challenging in the LT population, particularly with accelerated rates of fibrosis, lower SVR rates, and decreased tolerability to traditional therapies. Dr Nikolaos Pyrsopoulos, Rutgers, The State University of New Jersey, New Brunswick, New Jersey, USA, presented results of a study of 63 LT patients with HCV who were treated with SOF and LDV with or without low-dose RBV and immunosuppressive therapy.[14]

Patients included in the analysis were at least 3 months post-LT with documented recurrent HCV of genotype 1a, 1b, 3, 4, 5a, or 6, and were treated with SOF/LDV with concurrent immunosuppressive therapy (cyclosporine, tacrolimus, everolimus, or sirolimus). Pre-treatment dosing of

immunosuppressive therapy was maintained, and patients were evaluated for SVR12 and side effects of therapy.

Of the 63 patients, 48 (76%) were male and the median age was 61 years. Treatment with SOF/LDV+RBV was completed by 60 patients (genotype 1a, n=39; genotype 1b, n=14; genotype 3, n=1; genotype 4, n=3; genotype 5, n=1; genotype 6, n=2). RBV was commonly dosed at 200 mg twice daily and titrated as tolerated. Of the three patients treated with SOF/LDV, two (genotype 1a) completed the 24 weeks of treatment, and one completed only 8 weeks due to severe allograft dysfunction. All 63 patients were evaluable for efficacy, and the SVR12 was 100%. Adverse effects were reported in 23 patients and included fatigue/weakness (n=15), anaemia (n=4), headache (n=3), diarrhoea (n=2), nausea/vomiting (n=1), pruritus (n=1), and arthralgia (n=1). The four patients with anaemia received EPO alpha and three of these received blood transfusions. These findings suggest that SOF/LDV with or without low-dose RBV may be a viable treatment option for LT patients with HCV.

## Hepatitis B Reactivation Associated with Direct Acting Antiviral Therapy for Hepatitis C Virus: A Review of Spontaneous Post-Marketing Cases

Treatment for HCV with new DAAs allows for >90% chance of SVR, which is an improvement over older regimens. HBV co-infection with HCV is common in particular geographical areas where both infections are endemic and in populations at high risk of acquiring both infections due to common routes of transmission. HBV reactivation (HBV-R) can occur spontaneously but is usually triggered by immunosuppressive therapy, immunodeficiency due to HIV, autoimmune disease, or organ transplantation. HBV-R has also been reported in HBV-HCV co-infected patients treated with IFN-based therapy.

Dr Susan Bersoff-Matcha, FDA, Silver Spring, Maryland, USA, presented an analysis of the FDA's Adverse Event Reporting System (FAERS) database to identify reports of HBV-R associated with second-generation HCV DAAs and determine whether HBV-R is a safety concern with DAAs.[15] Reports of HBV-R cases among patients receiving all currently approved second-generation DAAs from 22nd November 2013 (the date of USA SMV approval) to 15th October 2016 were included.

There were 29 cases of HBV-R identified among patients receiving currently approved second-generation DAAs: 19 were reported in Japan, 5 in the USA, and 5 in other countries. All cases were temporally related to DAA initiation and the mean time from DAA to HBV-R was 53 days (range: 14–196 days). Of the 3 patients who developed decompensated liver failure, 2 died and 1 required LT. In the 16 patients treated for HBV, treatment was delayed in at least 7 cases (44%) and 1 of these patients died. Most patients had improvement in HBV DNA with treatment, as well as accompanying signs and symptoms such as elevated transaminases and malaise/fatigue. In 18 patients with a confirmed HCV genotype, 16 patients (89%) had genotype 1. A range of DAAs were received, suggesting that HBV-R is associated with the class of drug and not one particular DAA. The data should be interpreted with caution as FAERS is a voluntary reporting system and data may be incomplete, of variable quality, and subject to reporting bias and under-reporting.

The investigators concluded that HBV-R is a safety concern in patients previously infected with HBV who take DAAs. However, the benefit of a high HCV cure rate with DAAs continues to outweigh the risks, even in patients who may be at risk of HBV-R. Patients with a history of HBV require careful monitoring while on DAA therapy and further studies are required to determine the monitoring frequency and risk factors for HBV-R to identify patients who may benefit from HBV prophylaxis and treatment.

## Reduction in Liver Transplantation Wait List in the Era of Direct Acting Antiviral Therapy

DAA therapy in patients with HCV and decompensated cirrhosis can improve hepatic function and may allow the avoidance of LT. Dr Jennifer Flemming, Queen's University, Kingston, Ontario, Canada, presented a retrospective population-based cohort analysis of trends in LT wait lists to explore the potential impact of effective therapy on wait list registration.[16]

Adult patients listed for first LT for HCV, HBV, or non-alcoholic steatohepatitis (NASH) were identified from the Scientific Registry of Transplant Recipients (SRTR) database from 2003–2015. The indication for waitlisting was either decompensated cirrhosis if their biochemical MELD score was ≥15, or HCC. The era of wait list was defined as 'IFN' (2003–2010), 'protease inhibitor' (2011–

2013), or 'DAA' (2014–2015). Annual standardised incidence rates for LT wait list addition were analysed using modified Poisson regression.

Of 47,591 LT wait list registrants identified, 61% were listed for decompensated cirrhosis and 39% for HCC. Compared to the IFN era, the LT wait list rate for decompensated cirrhosis in HCV patients decreased by 4.8% in the protease inhibitor era (p=0.004) and by 31.9% in the DAA era (p<0.001). The LT wait list rate for decompensated cirrhosis in HBV patients also decreased in the protease inhibitor era (17%, p=0.002) and the DAA era (24.1%, p<0.001) compared with the IFN era. Conversely, LT wait list rates for decompensated cirrhosis in patients with NASH increased by 41.2% in the protease inhibitor era (p<0.001) and by 80.8% in the DAA era (p<0.001) compared with the IFN era. In 2015, the LT wait list rate for decompensated cirrhosis in NASH was equal to HCV. Wait list rates for HCC in both HCV and NASH patients increased in both the protease inhibitor and DAA eras (p<0.001), while wait list rates for HCC in HBV patients did not change significantly. This analysis found that LT wait list registrations have decreased more than 30% for decompensated HCV disease during the DAA therapy era in the USA. The ongoing efforts to increase HCV screening and linkage to care and improved access to DAAs will likely eliminate HCV as the leading cause for LT in the USA.

## CONCLUSION

The Viral Hepatitis and Orthotopic Liver Transplant poster session largely consisted of real-world studies that confirmed the efficacy and safety of DAA therapy in HCV patients. Of note, an FDA analysis concluded that HBV-R is a safety concern in patients previously infected with HBV who take DAAs; however, the benefit of DAAs for HCV outweigh the risks even for patients who may be at risk for HBV-R.[15] Furthermore, a large USA study found that LT wait list registrations for decompensated cirrhosis from HCV decreased >30% in the DAA therapy era compared with the IFN era.[16] Investigators are hopeful that increased HCV screening, linkage to care, and access to DAA therapy will eliminate HCV as the leading cause for LT in the USA.[17]

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# BETA-BLOCKERS IN PREVENTION OF DEVELOPMENT OF VARICES AND VARICEAL BLEEDING IN CIRRHOSIS: CURRENT MANAGEMENT, CONTROVERSIES, AND FUTURE DIRECTIONS

***Dhiraj Tripathi**

*Liver Unit, Queen Elizabeth Hospital Birmingham, Birmingham, UK*
**Correspondence to d.tripathi@bham.ac.uk*

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One of the major complications of cirrhosis is the development of portal hypertension and variceal bleeding. Varices develop at a rate of 5% per year with a 10-year cumulative incidence of 44%.[1] Variceal bleeding accounts for 10% of all admissions with gastrointestinal bleeding; it has an inpatient mortality of 15% and a 1-year mortality of ≥40%.[2] Therefore, reducing the risk of the development of varices (pre-primary prophylaxis) and the first variceal bleed (primary prevention) are important clinical goals.

Non-selective beta-blockers (NSBBs) have been used to reduce portal pressure and variceal bleeding for >35 years. There are several key mechanisms in the pathophysiology of portal hypertension in cirrhosis, namely increased intrahepatic resistance, splanchnic vasodilation, and augmented blood flow, that result in the hyperdynamic circulation.[3] NSBBs act to reduce portal hypertension through β1 blockade, lowering cardiac output, and β2 blockade, which results in splanchnic vasoconstriction through unopposed alpha-1 action.[4] Thus, there is a reduction in splanchnic inflow and portal pressure. NSBBs used in clinical practice are propranolol, nadolol, and carvedilol. Carvedilol has additional actions as a vasodilator due to alpha-1 receptor blockade, which reduces portocollateral resistance, and by acting on hepatic stellate cells, leading to a reduction in intrahepatic resistance.[5] Haemodynamic studies demonstrate a greater reduction in portal pressure than with the utilisation of propranolol, and carvedilol can be effective even in patients not responding to propranolol.[6,7]

Present guidelines recommend NSBBs to reduce the risk of the first variceal bleed (primary prophylaxis) in patients with medium to large oesophageal varices or small varices and advanced liver disease or red signs.[1,8] However, NSBBs are not recommended in patients without varices (pre-primary prophylaxis), those with small varices and compensated cirrhosis, or those with small varices in the absence of red signs due to a lack of evidence. This is an area that causes some controversy and requires further study.

Since clinical complications in cirrhosis are related to the severity of the portal hypertension, prevention of the escalation of portal pressure as early as possible would seem desirable, even prior to the development of varices or in patients with small varices. A large, randomised, controlled trial failed to show a beneficial effect of timolol in reducing the development of varices or variceal bleeding in patients with portal hypertension (hepatic venous pressure gradient [HVPG] ≥6 mmHg) but without varices.[9] The primary endpoint of the development of varices or variceal haemorrhage was 40% over 55 months in both arms. There were more adverse events in the timolol arm. There have been four randomised placebo-controlled trials studying the role of NSBBs in patients with small varices. Calés et al.[10] showed that propranolol in patients with small or no varices resulted in greater development of varices. However, patients without varices were included and there was a significant loss of patients at follow-up. The second trial showed that nadolol reduced variceal bleeding in patients with small varices by 45% without survival benefit but with increased adverse events.[11] Sarin et al.[12] did not show any effect of propranolol in patients with small varices, despite a significant effect on portal pressure. A recent randomised placebo-controlled trial showed that carvedilol reduced the progression of varices over a minimum of 24-months

follow-up, although there was no difference in bleeding or survival.[13] In this study, patients with advanced cirrhosis and ascites were included. The promising results of carvedilol in the prevention of the progression of varices were supported by an updated meta-analysis restricted to randomised controlled trials of patients with small varices. This showed a strong trend towards reduced progression of varices with NSBBs.[14]

While all the studies to date including patients with small or no varices have focussed on preventing variceal bleeding or the development of varices as the primary endpoint, most lack adequate stratification of patients at greatest risk of developing varices or complications of cirrhosis. There is emerging data showing that prior to the development of the hyperdynamic circulation or clinically significant portal hypertension (CSPH), defined as HVPG >10 mmHg, the effect of NSBBs on reduction of portal pressure is negligible.[15] The hypothesis is that at lower portal pressures increased intrahepatic resistance rather than splanchnic vasodilatation accounts for portal hypertension. Intrahepatic resistance is not amenable to most NSBBs apart from carvedilol, which can reduce intrahepatic resistance due to alpha-1 receptor blockade. Furthermore, there is good evidence that HVPG >10 mmHg predicts development of varices.[9] This may explain the inefficacy of using NSBBs in the study of patients without varices, where the threshold for inclusion was HVPG ≥6 mmHg with a significant number of patients not having CSPH.[9] Recent studies have shown that platelet count and liver stiffness (which correlates with liver fibrosis) are useful markers for predicting those at high risk of developing varices.[16-18] Liver stiffness is measured using a modified ultrasound based technique called transient elastography (TE) and is measured in kPa. Liver stiffness has also been shown to predict the patients most likely to develop other complications of cirrhosis, such as ascites or hepatic encephalopathy.[19] In this study of patients with compensated cirrhosis, the risk of developing decompensation related to portal hypertension over a 2-year follow-up period was 53% if liver stiffness was >20 kPa. Over 50% of patients in this study had no or small varices.

Further evidence came from the cross-sectional Anticipate study,[16] which investigated the role of non-invasive tools (TE, spleen size, platelet count, platelet/spleen ratio, and liver stiffness to spleen/platelet score [LSPS]) in predicting CSPH and varices. From a total of 542 patients selected from four centres, the best non-invasive tool to predict CSPH was a LSPS ratio >2.65, which was associated with an 80% risk of CSPH. A LSPS of <1.33 or liver stiffness >20 kPa combined with a platelet count ≤150,000 was associated with a <5% risk of developing varices needing treatment. However, the non-invasive markers could not reliably identify patients with compensated cirrhosis at risk of developing varices of any size. A haemodynamic response to nadolol (>10% reduction in portal pressure) was shown to reduce ascites development by 38% over a 3-year period in patients with compensated cirrhosis and large varices, compared with non-haemodynamic responders (<10% reduction in in portal pressure).[20]

NSBBs can also have beneficial effects independent of the effects on portal pressure, with studies showing reduced risk of infections (bacterial translocation).[21] Carvedilol has anti-inflammatory, anti-oxidant, and antifibrotic properties along with other roles in enhancing insulin sensitivity and improving mitochondrial function. Since carvedilol appears to be a more potent NSBB than propranolol, with potential effects on portal pressure even in early cirrhosis due to alpha-1 receptor blockade, it seems an ideal drug to study in this setting of prevention of complications of cirrhosis and portal hypertension.[5]

It is therefore clear that there is an urgent need for large multicentre controlled trials selecting patients with compensated cirrhosis at the highest risk for the development of varices or decompensation. Ideally, HVPG measurements should be performed and patients selected if >10 mmHg i.e. CSPH. There is some evidence from an abstract that showed that NSBBs in patients (n=201) with CSPH reduced decompensation or liver related deaths, although this did not influence decompensation-free survival.[22] This may reflect the small size of the trial and its lack of power. To see a crucial effect on clinical outcomes, trials need to include several hundred or even over a thousand patients. Clearly HVPG measurements are not available in many centres and the use of TE and platelets count or LSPS ratio would seem attractive.[16-18]

The clinical trials of patients with small varices have not conclusively shown a reduction in bleeding or mortality. However, this should not dissuade further study as there will be profound clinical and economic implications if NSBBs were found to be beneficial in further large, well-designed clinical trials. The findings by Bhardwaj et al.[13] of carvedilol reducing

the progression of varices should encourage further study. These results suggest that carvedilol is the ideal NSBB to study in future trials of patients with small varices.

The key is patient stratification and large-scale multicentre involvement. All patients selected for studies on the role of NSBBs in preventing liver decompensation must have evidence of CSPH with HVPG >10 mmHg where available and/or liver stiffness/platelet count/spleen size criteria.[16] It is also important to select patients with small varices and compensated cirrhosis most likely to develop high-risk varices for entry into clinical trials using the invasive or non-invasive methods described earlier. In view of the failure rate of ≤25% of TE, alternative non-invasive methods of quantifying portal hypertension, such as non-contrast quantitative magnetic resonance imaging (MRI), should be studied.[23]

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# EDITOR'S PICK

Our Editor's Pick for this edition of *EMJ Hepatology* is a tripartite paper by Charach et al. encompassing hepatocellular carcinoma, which is a common cause of cancer-related death in both men and women. This thorough review provides excellent detail on the development, clinical presentation, diagnosis, and treatment of the disease. It is worth a read for all those looking to learn more about the current status of research in this field.

Prof Markus Peck-Radosavljevic

# HEPATOCELLULAR CARCINOMA. PART 1: EPIDEMIOLOGY, RISK FACTORS, PATHOGENESIS, AND PATHOLOGY

**Lior Charach, Lior Zusmanovitch, *Gideon Charach**

*Department of Internal Medicine C, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
**Correspondence to drcharach@012.net.il*

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## ABSTRACT

This review will cover the epidemiology, risk factors, pathogenesis, and pathology of hepatocellular carcinoma (HCC). HCC is the fifth most commonly diagnosed cancer in males and second most frequent cancer-related cause of mortality worldwide. In females, it is the seventh most frequently diagnosed malignancy and sixth leading cause of death. The incidence of HCC is higher among males in less developed countries and reaches a peak around the age of 70 years. The rates of liver cancer are twice as high in males compared to females.[1,2] Various risk factors, including environmental, infectious, nutritional, and metabolic, are associated with HCC; among them viral infection has been linked to being the highest risk factor for developing HCC.

HCC is a highly vascular tumour and its pathogenesis consists of increasing angiogenesis by overexpression of various growth factors. Another cause of HCC development is thought to be mutations in different signalling pathways that lead to proliferation of the tumour cells.

Keywords: Hepatocellular carcinoma (HCC), epidemiology of HCC, risk factors of HCC, pathogenesis of HCC, pathology of HCC.

## EPIDEMIOLOGY

Hepatocellular carcinoma (HCC) is the most common primary liver cancer; it accounts for 70–85% of the total incidences of liver cancer and is the third leading cause of cancer mortality worldwide.[1] It accounts for 9.2% of the new cancer diagnoses worldwide with >748,000 new cases per year.[3] It usually appears after the age of 40 years and reaches a peak around 70 years of age,[4] with an overall 5-year survival of <12% in the USA.[5] HCC distributions differ by race, ethnicity, geography, and sex. For instance, Hispanics and African-Americans in the USA have higher rates of HCC than non-Hispanics but lower rates than Asians.[6] Furthermore, HCC has a high incidence in sub-Saharan Africa and Southeast Asia.[4] The incidence in Caucasians in the UK and USA is lower,[7] and a

probable cause for this is the different aetiologies of HCC. Table 1 demonstrates the incidence of HCC in various countries worldwide.[5]

## RISK FACTORS

### Non-Modifiable Causes

Non-modifiable causes also include factors related to age, sex, and ethnicity. It has been shown that black, white, and Hispanic men >50 years of age have an increased risk of developing HCC. In ages ranging between 35 and 49 years there has been a decrease in the incidence of HCC.[8] Males show a higher risk for HCC than females due to differences in exposure to risk factors such as hepatitis B virus (HBV) and hepatitis C virus (HCV).[9] Family history of liver cancer in first-degree relatives was associated with a significantly elevated risk of liver cancer.[10]

### Modifiable Causes

#### Hepatitis B virus

HBV infection correlates to >50% of HCC cases. In HBV endemic areas, HBV has the potential to become chronic in >90% of the individuals; the infection is transmitted by perinatal or vertical ways. In developed countries, there is low potential of chronic infection and >90% of cases resolve spontaneously, with the transmission usually associated with sexual and parenteral routes.[5]

#### Geographical region

HCV prevalence in patients with HCC can vary between 20% and 90%, depending on the geographical region.[11] The USA, Europe, and Japan have the highest incidence of HCV-infected patients. Compared to negative HCV patients, chronic HCV patients have a 17-times higher risk of developing HCC.[12]

#### Non-alcoholic fatty liver disease

In a population-based study, a statistically significant association was shown between metabolic syndrome and risk of HCC, making non-alcoholic steatohepatitis (NASH) the contributor. In cohort based studies including patients with non-alcoholic fatty liver disease (NAFLD), there was no increased incidence of HCC.[5] NASH, which is a subset of NAFLD, showed an increasing incidence of liver transplantation due to HCC, which reached ≤6% of the overall number of liver transplantations undergone in 2012 in the USA, while in 2002 this figure was 0%.[13]

Excessive alcohol consumption, defined as a daily intake of alcohol for 10–12 years with doses in excess of 40–80 g/day for males and 20–40 g/day for females, may lead to alcoholic liver disease, which in turn may lead to cirrhosis and finally to HCC. Out of long-term heavy alcoholic drinkers it is reported that only 10–35% will develop alcoholic hepatitis and only 8–20% will develop cirrhosis.[14]

#### Aflatoxins

Aflatoxins are metabolites derived from the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. They can be found in maize, ground nuts, and rice in tropical and subtropical countries. The mycotoxin optimally grows at temperatures between 25°C and 32°C in a moist environment. Aflatoxin B1 is the most potent experimental hepatocarcinogen known and is considered to be a cause of HCC development.[15]

#### Haemochromatosis

Haemochromatosis is a genetic disorder in which iron deposits in the tissues can lead to liver damage.

**Table 1: Age-standardised incidence rates of hepatocellular carcinoma per 100,000 populations at risk, in different regions of world.[5]**

| Women | Men | Country |
|---|---|---|
| 11.4 | 47.1 | North Korea |
| 11.4 | 47.1 | South Korea |
| 17.2 | 38.6 | Thailand |
| 14.2 | 37.9 | China |
| 7.6 | 23.1 | Japan |
| 5.8 | 23.7 | Vietnam |
| 5.1 | 15.9 | Italy |
| 2.6 | 11.3 | Indonesia |
| 2.2 | 10.5 | France |
| 4.9 | 5.0 | Mexico |
| 2.3 | 6.1 | South Africa |
| 2.0 | 5.5 | USA |
| 2.1 | 4.6 | Russia |
| 2.6 | 3.6 | Poland |
| 2.4 | 3.4 | Brazil |
| 2.0 | 3.7 | Sweden |
| 1.9 | 3.5 | Argentina |
| 1.7 | 3.3 | UK |
| 1.5 | 2.6 | Turkey |
| 1.9 | 1.4 | Iran |

Homozygosity of the C282Y mutation on the *HFE* gene is associated with low secretion of hepcidin, which is the regulator of iron metabolism in the body. When iron overloads over time in the body, the hepatocytes become damaged and dysfunctional, which leads to cirrhosis and HCC. HCC develops in 8–10% of patients with hereditary haemochromatosis. HCC is the major cause of mortality in individuals with hereditary haemochromatosis, where it reaches ≤45%.[16]

### Wilson's disease

Wilson's disease (WD) is an autosomal recessive disorder in which copper accumulates in the tissues. Its manifestation includes neurological, psychiatric, and liver disorders. Whether HCC develops as a result of WD is controversial. Animal studies show that the use of chelating agents decreases the risk of HCC in these patients, while in clinical and experimental trials it has been shown that tumourigenesis in WD patients is multifactorial and that chronic liver injury (rather than copper accumulation) leads to HCC.[17]

### Diabetes mellitus

Diabetes mellitus (DM) was significantly associated with increased risk of HCC as shown by a meta-analysis.[18] Among diabetics using metformin, HCC incidence was lower, but in diabetics using sulfonylurea a higher risk was indicated.[19] The cause for the increased risk of HCC in Type 2 DM is not fully understood and was attributed to the development of NAFLD, which would cause chronic liver injury and later the development of HCC.[20]

### Contraceptives

Oestrogen-progestogen contraceptives are classified by the International Agency for Research on Cancer (IARC) as a cause of HCC.[21] However HCC risk is not associated with the use of oral contraceptives.[22]

### Occupational exposures

Vinyl chloride has been classified by the IARC as a cause of HCC.[21] A follow-up study that included 1,600 male Italian autoclave workers had an almost 10-fold higher risk of HCC due to exposure to vinyl chloride gas compared to individuals who were not exposed.[23]

## PROTECTIVE FACTORS

Several studies have demonstrated the protective role of metformin. This medicine is considered as an anti-cancer and anti-oxidant agent for solid tumours (colon, prostate), as metformin decreases insulin resistance. It promotes a decrease in plasma insulin (which is known as a growth promoting factor with direct mitogenic effects) and a decrease in glucose levels in patients with Type 2 DM, which is a known risk factor of HCC. The ability of metformin to decrease the plasma insulin is the reason for its proposed anti-oncogenic mechanism.[24]

Several meta-analyses suggest that coffee, statins, and vegetables have an inverse relationship to the development of HCC.[25-27] Coffee has a protective effect in the prevention of HCC. A possible explanation could be related to the inclusion of paraxanthine. This metabolite inhibits the connective tissue growth factor found in the hepatic parenchyma and thus protects the liver from HCC. It has been shown that the intake of two cups per day reduces the risk of HCC development by 43%.[25] Another meta-analysis also indicates that one cup of coffee per day decreases the risk of HCC and there is an inverse relationship between the amount of cups consumed and the risk of HCC.[28] Statins are medications that inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which leads to the inhibition of myelocytomatosis oncogene (*Myc*) phosphorylation by having an anti-oxidant effect. These mechanisms lead to tumour suppression and decrease the risk of HCC.[26] Statins are also known to be used in the treatment of metabolic syndrome, also recognised as a risk factor that can lead to NASH. Particular interest is focussed on the impact of statins on the development and progression of neoplasms. Furthermore, statins have been shown to cause apoptosis in cultured cancer cells through this same method, specifically by inhibition of geranylgeranyl pyrophosphate production. Another mechanism proposed is that statins may prevent HCC through an indirect effect by prevention of HCV replication.[29]

Vegetables contain phytochemicals, such as isothiocyanates, glucosinolates, indole-3-carbinol, and flavonoids, that have anti-tumour effects, although the mechanism of protection is not completely clear. It was also reported that Vitamin E has been found to decrease the risk of HCC.[27] Consumption of fish has been shown to decrease the risk of HCC development due to their rich source of omega-3 polyunsaturated fatty acids, such as

eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, which have anti-inflammatory properties. They lead to a decrease in interleukin (IL)-1 and IL-6 pro-inflammatory activity, which is known to contribute to HCC development.[29-31]

## PATHOGENESIS

HCC pathogenesis is multifactorial, as different factors contribute directly or indirectly to hepatocarcinogenesis. Environmental, infectious, nutritional, and metabolic factors are all associated with development of HCC.[32] Several genetic pathways have been considered as a cause of HCC, including post-injury regeneration of hepatocytes, angiogenesis, and activation of signalling pathways.[33] Reactive oxygen species and reactive nitrogen species have also had an effect on the regenerative, respiratory, and energetic pathways in the liver cells.[34] Hepatocytes that were injured due to liver injury, such as viral hepatitis, NAFLD, or DM, have the ability to regenerate from stem cells. The occurrence of gene mutations in components of the Wnt/β-catenin signalling pathway, such as axin and β-catenin, takes place during the renewal of stem cells and results in the formation of HCC cells.[33,35,36]

### Role of Growth Factors and Angiogenesis in Hepatocellular Carcinoma

HCC has high vascularity, which contributes to the growth of the tumour. Several growth factors are attributed to the angiogenesis, cell proliferation, and metastatic activity. Among them vascular endothelial growth factor (VEGF), platelet-derived growth factor, epidermal growth factor, fibroblast growth factor (FGF), and insulin-like growth factor can be found in tumour cells as well as their surrounding cells.[33] Growth factors affect surrounding cells in both paracrine and autocrine fashion and contribute to the proliferation of the cells. Treatment for HCC can be associated with targeting these growth factors.[33] Thrombosis in the portal vein is a complication that can be seen in high expression of growth factors.[37]

### Role of Signalling Pathways and Genetics in the Development of Hepatocellular Carcinoma

Telomerase reverse transcriptase (TERT), VEGFA, FGF19, tumour protein p53, and epigenetic modifiers such as AT-rich interactive domain-containing protein 1 (ARID1) play a role in the formation of HCC.[38] In addition to these genetic modifications, signalling pathways were also associated with hepatocarcinogenesis, including, the Wnt/β-catenin pathway and receptor TK-activated pathways, including the Ras/Raf/MEK/ ERK pathway and the PI3K/AKT/mTOR pathway.[39]

The most frequent mutation that leads to HCC is associated with TERT promoter (≤60% of cases). TERT is a part of the telomerase complex together with the telomerase RNA component. Telomerase reactivation is a key factor in malignant transformation and it occurs in >90% of HCC, due to *TERT* promoter mutation, TERT amplification, or HBV insertion in the *TERT* promoter. In 6% of low grade dysplastic nodules and in 19% of the high-grade dysplastic nodules there are *TERT* promoter mutations. There are dramatic increase in *TERT* mutations in early HCC, while in advanced and progressed HCC, *TERT* mutations remain stable.[38]

The tumour protein p53 cell cycle pathway mutation is associated with up to half of HCC patients (12–48%) and is related mainly to aflatoxin exposure. Mutations in the retinoblastoma pathway, cyclin-dependent kinase inhibitor 2A (*CDKN2A*) deletion (2–12%), and RB transcriptional corepressor 1 (*RB1*) mutation (3–8%) were associated with HCC.[38]

SWItch/sucrose nonfermentable (SWI/SNF) chromatin remodelling complexes modify chromatin structure and nucleosome position. They modify the transcription fate of the cell in an indirect way. These complexes have tumour suppression effects and cause inactivation of the *ARID1A* and *ARID2A*, members of the SWI/SNF that are seen in HCC.[38]

VEGFA could have a double effect in the tumourigenesis of HCC, firstly by angiogenesis and secondly by inducing overexpression of hepatocyte growth factor. High levels of VEGFA are correlated with poor survival.[38] FGF19 is described in 5–14% of HCC cases, and high levels of expression are associated with poor prognosis of HCC.[38]

The Wnt/β-catenin pathway plays a role in intracellular signalling and cell-cell interactions. This pathway is often activated by a *CTNNB1* mutation (11–37%) and by an inactivating mutation of *AXIN1* (5–15%) or *APC* (1–2%). This leads to accumulation of β-catenin in the cytoplasm and leads to stimulation of genes associated with cell proliferation, angiogenesis, and anti-apoptotic effects that take part in malignant transformation.[38,39] The Ras/Raf/MEK/ERK signalling pathway has a role in extracellular signal transduction, cell growth, and survival. The continuous activation of this pathway by tyrosine

kinase ligands is significant in HCC development.[39] The PI3K/AKT/mTOR pathway has a key role in cellular proliferation and cell survival. PI3K activates AKT which in turn inactivates pro-apoptotic proteins such as Bcl-2-associated death promoter (BAD) and caspase-9; this leads to the survival of cancer cells. Considering mTOR function in regulation of cell translation, an examination of HCC cases found that mTOR had been upregulated.[39]

Telomerase is an enzyme which protects chromosomes. With each cell cycle the telomere length reduces, and when the telomere length is too short they signal for apoptosis. If inactivation in the protective mechanism occurs, the chromosomes become unstable which in turn leads to cell proliferation.[40] Telomerase activity in liver tissue of the normal population has either low or undetectable levels of this enzyme, while high levels of telomerase can be found in HCC patients.[41]

## PATHOLOGY

Gross morphology of HCC ranges from single mass to multi-nodular masses with diffusely scattered foci. Multi-centric and multi-nodular HCC can be attributed to cirrhotic and non-cirrhotic patients, respectively.[32] The multi-nodular type has a discernible capsule, is hypovascular, and does not show intrahepatic metastasis and portal vein invasion.[42] HCC is a soft tumour except for the fibro-lamellar type. The tumour can be grey-white, tan-brown, yellow due to fatty changes, or green due to bile production.[32]

### Histological Appearance

The International Consensus Group for HCC and the World Health Organization (WHO) proposed the following classifications:

1. Early HCC:
a) well-differentiated
b) small size (<2 cm)
c) poorly defined margins, vaguely nodular type

2. Progressed HCC:
a) >2 cm
b) small size (<2 cm), but moderately differentiated, distinctly nodular type[42]

Different cytological and architectural patterns can be seen in HCC. In the architectural patterns the most common is the trabecular, which resembles normal liver tissue. The pseudo glandular or acinar type is filled with fibrin or bile and has dilated bile canaliculus-like structures.[32,42] Another two types are scirrhous HCC, which is uncommon and shows fibrosis and the compact variant with sinusoid like blood spaces that are inconspicuous and slit-like, giving the tumour a solid appearance.[32,43] The cytological patterns include pleomorphic cell, clear cell, sarcomatous change, fatty change, mallory-hyaline bodies, globular hyaline bodies, pale bodies, and ground glass inclusions.[43] Pathologists use diagnostic tools, such as immunochemistry, for molecular pathology. This provides more accurate tumour phenotyping and will have a significant role in diagnostic and prognostic purposes.[42,44]

### Precancerous Lesions

Hepatocellular adenoma is a benign tumour composed of cells closely resembling normal hepatocytes and is a rare cause of HCC. It appears more commonly in females using oral contraceptives over 2 years,[42,43,45] but also in females with maturity onset diabetes of the young Type 3.[45] Other associated diseases with adenoma formation are glycogen storage disease and polycystic kidney.[45] The Wnt/β-catenin pathway plays a significant role in the transformation of hepatic adenoma to HCC.[38]

Dysplastic nodules are hepatocytes <1 mm in diameter with dysplasia but without histological criteria of malignancy.[46] The nodules show variable atypia with an increased cell density and are usually <1.5 cm in size with or without fibrous rim and normal nuclear-cytoplasmic ratios.

Large cell dysplasia is characterised by pleomorphic cellular enlargement and multi-nucleation of liver cells that occur in groups or occupy the whole cirrhotic nodule.[32,43] Small cell dysplasia is characterised by elevation in the nuclear-cytoplasmic ratio between the normal and diseased liver cell without multinucleation and large nucleoli. It has been assumed that small cell dysplasia has higher tendency of precancerous activity over large cell dysplasia.[32,43]

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# HEPATOCELLULAR CARCINOMA. PART 2: CLINICAL PRESENTATION AND DIAGNOSIS

**Lior Charach, Lior Zusmanovitch, *Gideon Charach**

*Department of Internal Medicine C, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
**Correspondence to drcharach@012.net.il*

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## ABSTRACT

Clinical presentation of hepatocellular carcinoma (HCC) can vary from asymptomatic patients to patients presenting variable symptoms such as pain, lethargy, jaundice, hepatic encephalopathy, anasarca, ascites, variceal bleeding, diarrhoea, paraneoplastic symptoms, cutaneous manifestations, and abnormal laboratory values. Diagnosis of HCC is based on computed tomography (CT), magnetic resonance imaging (MRI), and tumour markers. The most commonly used is alpha fetoprotein.[1,2] MRI is the imaging method of choice, although it has decreased sensitivity in detecting lesions <2 cm.[3] Other possibilities include biomarkers such as embryonic antigen, protein antigen, enzymes and isoenzymes, cytokines, and genetic biomarkers. Liver biopsy is used in selected patients who do not present typical features of HCC on CT or MRI. Surveillance by ultrasound is recommended every 6 months in cirrhotic patients. The Barcelona Clinic Liver Cancer (BCLC) scoring system has been proposed for staging of HCC, and numerous scoring systems have been developed to evaluate progression and determine treatment possibilities; they take into account the clinical as well as the laboratory and pathological criteria, biomarkers, biopsy, and imaging methods.

Keywords: Hepatocellular carcinoma (HCC), clinical presentation of HCC, diagnosis of HCC.

## CLINICAL PRESENTATION

Hepatocellular carcinoma (HCC) patients are frequently asymptomatic and the appearance of symptoms can signal the development of severe disease. Symptoms can appear early in patients with HCC due to chronic liver disease. In 90–95% of HCC patients a triad of right upper quadrant pain, palpable mass, and weight loss[4] manifest; other symptoms that can be mentioned are jaundice, hepatic encephalopathy, anasarca, ascites, variceal bleeding, diarrhoea, paraneoplastic symptoms, cutaneous manifestations, and abnormal laboratory values.[5-8] In non-cirrhotic patients, physical examination findings can be abdominal distention, anorexia, hepatomegaly, wasting, and right upper quadrant pain. A fatal complication of HCC is tumour rupture in which the patient experiences hypotension, irritation of the peritoneum, and severe abdominal pain.[5] One of the paraneoplastic symptoms manifested in HCC is bone pain associated with hypercalcaemia, which is caused by osteolytic metastasis; other symptoms include erythrocytosis, hypoglycaemia, and androgen insensitivity syndrome.[5,6] Erythrocytosis is due to an increase in the erythropoietin production in HCC patients.[9] Overproduction of vasoactive intestinal peptide and gastrin are common causes of watery diarrhoea, mainly in cirrhotic patients due to their secreting characteristics.[5-7] Cutaneous manifestations of HCC include pityriasis rotunda; Leser-Trélat sign, which is the appearance of multiple seborrheic keratoses; dermatomyositis; and pemphigus foliaceus. Porphyria cutanea tarda has been associated with HCC patients with chronic hepatitis C virus.[5,8]

## TUMOUR MARKERS

Embryonic antigen, protein antigen, enzymes, isoenzymes, cytokines, and genetic biomarkers all have a potential utility in the detection of HCC.[10]

## Embryonic Antigen

Alpha fetoprotein (AFP) is a glycoprotein that is normally produced during fetal life by the fetal liver and yolk sac. In adults, elevated AFP can indicate ongoing disease and patients with HCC often show elevation in serum concentration of AFP.[10] Another cause of elevated AFP can be related to chronic hepatitis C virus infection.[11] AFP has three glycoforms: AFP-L3 is the main isoform of AFP in the serum of HCC patients and can be detected in approximately one-third of patients with small HCC (<3 cm) when cut-off values of 10–15% are used.[2,12] This biomarker was found to be related to recurrence rate when it increases >10% or rises after normalisation with treatment. AFP-L3 was found to be associated with poorly differentiated and advanced HCC. However, when comparing between total AFP level and AFP-L3 it seems that the latter is more specific in diagnosing HCC.[2,12] It has been suggested that AFP-L3 can be used as a screening tool because it can appear up to 12 months before imaging methods detect HCC, but meta-analyses concluded that, due to poor sensitivity, it has limited function in screening studies.[2,13] Clinical studies showed that at a cut-off of 20 ng/mL, the sensitivity of serum AFP is 41–65% and the specificity is 80–94%. However, there are several disadvantages of AFP in regard to early diagnosis. Firstly, the positive rate of AFP in HCC is about 60–80%, which is not enough to make it a sensitive biomarker. Secondly, false positive results can be recorded during pregnancy and when the patient also has liver or gastrointestinal diseases. Thirdly, false negative results can occur.[10] Although the serum biomarkers discussed next may show similar or better sensitivity and specificity, AFP is still the most commonly used screening biomarker.

## Protein Antigen

Heat shock protein (HSP) is a molecule that forms in cells exposed to stress, including carcinogenesis. These proteins protect the cells and promote the cells to repair the damage from the anxious stimuli. HSP 70 and HSP 27 have been found in HCC tissues. HSP 70 correlates with portal vein invasion and tumour stage and size. HSP 27 has been seen in hepatitis B virus-infected patients with HCC. A previous study has shown that the sensitivity of HSP 70 is 57.5% and the specificity is 85%.[10,14]

Glypican-3 is a family of the heparan sulfate proteoglycans linked to the cell membrane by a glycosylphosphatidylinositol linkage.[15] Glypican-3 plays a role in cell growth regulation, migration, differentiation, and development. Levels of glypican-3 were increased in HCC patients, and no correlation to tumour size, stage, or AFP was indicated. A sensitivity of 77% and specificity of 96% was correlated to this marker. Squamous cell carcinoma antigen (SCCA) is a serine protease inhibitor that can be found in high levels in HCC patients and helps tumour cells evade apoptosis.[10,16] High levels of SCCA can indicate the early stages of HCC. Specificity and sensitivity are 46% and 84%, respectively; this finding makes SCCA a complementary marker for AFP.[10]

Golgi protein 73 and tumour-associated glycoprotein were elevated in patients with HCC and can be used as markers for detection of HCC.[10]

## Enzymes and Isoenzymes

Des-γ-carboxyprothrombin (DCP), γ-glutamyl transferase (GGT), and α-1-fucosidase (AFU) are supplementary markers to AFP in the detection of HCC.[10] DCP, also known as PIVKA-II, occurs due to the absence of vitamin K. It has better sensitivity for HCC lesions >5 cm in diameter. When combined with AFP, it gives a better prediction of HCC recurrence 6 months post-surgery. DCP enables better prediction of larger tumour and vascular invasion.[10] GGT has low serum levels in healthy adults; however, in patients with liver disease such as cholestasis, inflammation, or benign or malignant tumour, the level of GGT increases. The enzyme has relatively low sensitivity (43%) and can be used as a supplementary marker together with AFP.[10]

Alkaline phosphatase is a liver enzyme that increases in cases of several liver diseases and is known to play a role in the diagnosis and screening of HCC. It may also predict survival, with a cut-off >121 U/L, a sensitivity of 41.4%, and a specificity of 85.9%.[17] AFU is a lysosomal enzyme. It can be found in healthy adults and displays elevated levels in patients with HCC. When used alone, AFU has poor specificity; however, when combined with AFP it can greatly improve AFP sensitivity and specificity.[10]

## Cytokines

Transforming growth factor-β1 has been indicated to be a good supplementary marker in the diagnosis of HCC as it has higher sensitivity than AFP. Transforming growth factor-β1 has a role in cell regulation, including angiogenesis, differentiation, invasion, and cell proliferation.[10,18]

It has an immunosuppressive effect by inhibiting the proliferation of natural killer cells and cytotoxic T cells, which allows tumour cells to grow.[10] Vascular endothelial growth factor functions in endothelial migration, new vessel formation, invasion, and metastasis. The expression of vascular endothelial growth factor in HCC patients is upregulated and correlates to prognosis and recurrence of the tumour.[10] Interleukin (IL)-8 is a chemokine that has prognostic and diagnostic properties in HCC patients. IL-8 plays a role in chemotaxis, enzyme release, and expression of adhesion molecules in neutrophils as well as tumour proliferation and metastasis. When compared to healthy adults the levels of IL-8 in HCC patients were higher. IL-8 overexpression has been shown to be an indicator of tumour size, absence of tumour capsules, and invasion of the veins.[18]

### Genetic Biomarkers

microRNAs (miRNAs) are new biomarkers for HCC diagnosis. miRNAs are small non-coding RNAs that effectively block translation by promoting the degradation of target mRNAs or binding to complementary sequences in the 3' untranslated region. miR-29, miR-199a/b-3p, and miR-122 were downregulated in HCC cells. miR-21 was the only one that was upregulated in the HCC cells. A sensitivity of 87.3% and specificity of 92% were shown for miR-21.[10]

## IMAGING METHODS

### Ultrasound

Ultrasound (US) is a widely available non-invasive technique. It is free of radiation and has an important role in the surveillance of HCC in cirrhotic patients.[3,5] Some authors recommend US modality in combination with AFP in biannual screening of high-risk patients in order to reduce HCC mortality.[19] Nevertheless it has disadvantages, especially the difficulty in differentiating between benign and malignant tumours. When US is combined with AFP it aids in the diagnosis of HCC.[3]

HCC lesions <3 cm are usually hypoechoic relative to the surrounding tissues while lesions >3 cm are hyperechoic with a mosaic or infiltrative pattern and may have a thin capsule.[3] Non-contrast-enhanced US is not the method of choice in detecting HCC, and it has been replaced with computed tomography (CT) and magnetic resonance imaging (MRI) due to their higher sensitivity and positive predicting value.[3]

HCC lesions are characterised by high vascularity, and the contrast-enhanced US can display the intrahepatic vascular flow in HCC lesions and help in the diagnosis. In addition, contrast-enhanced US can differentiate between bland thrombus and tumour invasion.[5] Contrast-enhanced US has a higher accuracy of biopsy, which lowers the false negative rate of malignant lesions. Disadvantages of US include association with obesity and the requirement for necessary operator experience. Moreover, US has difficulties in differentiating between benign and malignant lesions in the context of nodular cirrhosis.[3]

### Computed Tomography

CT is a widely used radiological technique for diagnosis of HCC. The most commonly used type of CT is the multidetector CT, which has a higher quality, thin sections, and three-dimensional capabilities but is not contrast-enhanced. Intravenous contrast is used for better diagnostic results and can be divided into an arterial phase, a portal phase, and a delayed phase.[3,5] In the development of HCC, which is a hypervascular lesion, there is loss of portal venous blood supply and formation of collateral arterialisation. When contrast material is introduced intravenously there is an increase in arterial enhancement followed by a delay in the venous 'washout' phase. This pattern is a characteristic sign for diagnosis of HCC (Figure 1).[21] CT showed better sensitivity than US but poorer sensitivity than MRI.[22]

### Magnetic Resonance Imaging

MRI has properties similar to a CT scan and it is free of radiation but is an expensive imaging method. In contrast-enhanced T1 W with gadolinium, images in the hepatic artery, portal vein, and delayed phase show improved detection and characterisation of small HCC lesions and are superior to multi-phasic helical CT. Combined double contrast MRI, which includes gadolinium together with super paramagnetic iron oxide, is found to be highly sensitive (92%) in the detection of HCC >1 cm in size and better than either of them alone. T2 weighted images show hypo-intensity due to the uptake of super paramagnetic iron oxide or ferumoxide particles taken up by Kupffer cells.[23] MRI sensitivity is high; however, it causes difficulties in evaluating tumours <2 cm. Nevertheless, it is the imaging method of choice for HCC.[3,5]

Nuclear imaging positron emission tomography (PET) has limited use as a diagnostic tool in HCC.

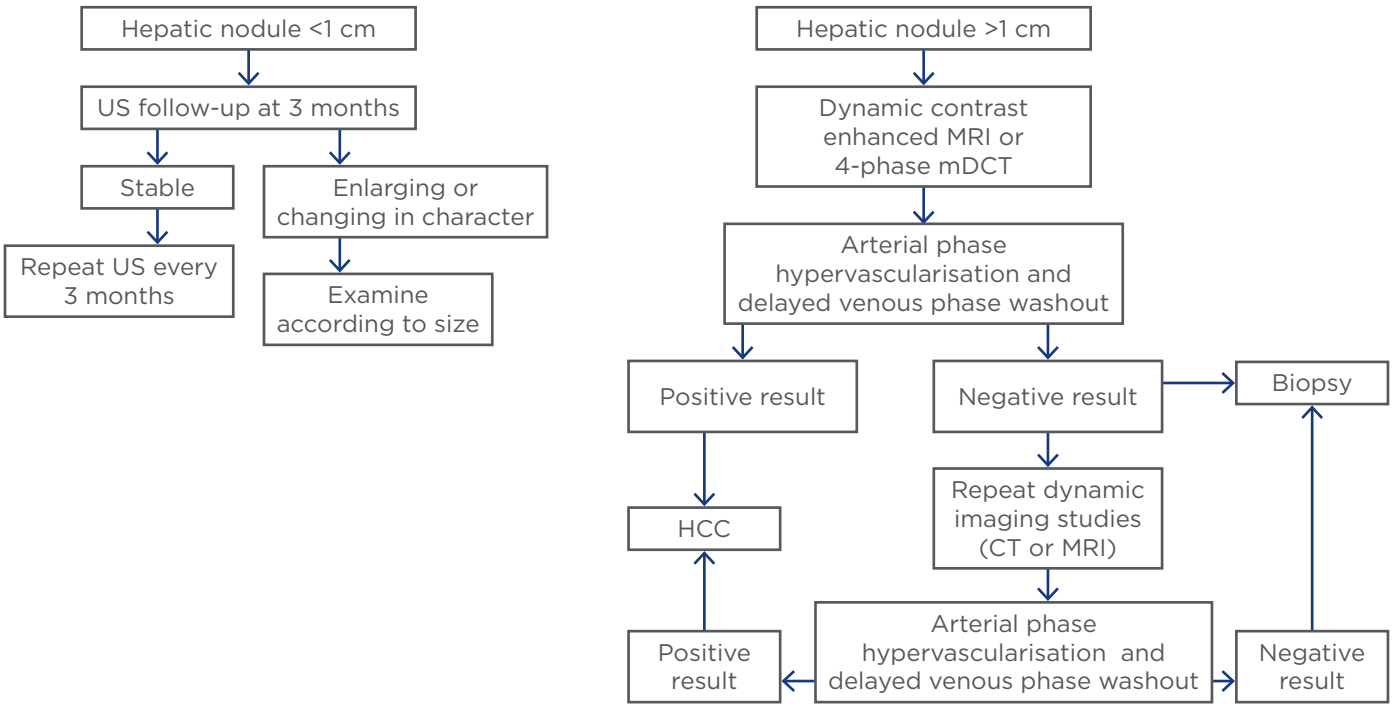


**Figure 1: American Association for the Study of Liver Disease (AASLD) practice guidelines for hepatocellular carcinoma surveillance and diagnosis.[20]**

US: ultrasound; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging; MDCT: multidetector computed tomography; CT: computed tomography.

It is instead used to evaluate the spread of HCC to tissues outside the liver. PET imaging is based on radiolabelled glucose (fludeoxyglucose [$^{18}$F-FDG]), which binds into neoplastic cells demonstrating increased metabolic activity. HCC tumours with good or moderate differentiation and metastasis properties may not produce a high level of metabolism requirements as compared to that of neighbouring tissues. $^{18}$F-FDG was first used in the diagnosis of HCC, but due to high expression of glucose-6-phosphatase in HCC cells this imaging was not sensitive enough. However, when $^{11}$C-acetate was introduced, the sensitivity and specificity were increased, but it still could not distinguish between HCC and benign lesions. Recent studies of dynamic PET with kinetic modelling show that the differentiation of HCC from benign liver tumours is possible.[3,5]

## Liver Biopsy

Confirmation of HCC can be done by means of percutaneous fine needle aspiration biopsy or core biopsy, guided by US, CT, or transjugular biopsy. It has a higher sensitivity and specificity than non-invasive techniques and can be used to detect HCC with characteristics that do not meet the radiological or laboratory parameters of HCC.[5,24] Complications are not common during biopsy but some can be seen such as infections or haemorrhage and the risk of tumour spread from the biopsy needle. Limitations for biopsy are platelet count <50,000 mm$^3$, or international normalized ratio (INR) >2. Transjugular biopsy overcomes these problems but results in non-targeted biopsy and therefore is usually appropriate for diffuse liver processes.[25] Mortality rates for biopsy procedures are low and range between 0.006% and 0.3%, although sampling errors and repeated biopsy are frequent clinical issues.[5,26]

## Surveillance

Surveillance of HCC has an important role in the detection of early tumours and treatment, thus it can lead to more effective treatment. AFP, US, CT, and MRI are examples of methods in which surveillance can be carried out.[19,27-30] According to the international guidelines of the American Association for the Study of Liver Disease (AASLD), screening of patients with cirrhosis and patients at high risk of HCC is mandatory, and US should be used every 6 months. AFP, which is the most widely used biomarker, has not been proven to be adequate for surveillance even when combined with US.

**Table 1: Barcelona Clinic Liver Cancer staging classification.[34,35]**

| Stage | PST | Tumour status | | Liver function studies |
|---|---|---|---|---|
| | | Tumour stage | Okuda stage | |
| Stage: Very early | 0 | <2 cm | I | Child–Pugh A |
| Stage A: early HCC | | | | |
| A1 | 0 | Single | I | No portal hypertension and normal bilirubin |
| A2 | 0 | Single | I | Portal hypertension and normal bilirubin |
| A3 | 0 | Single | I | Portal hypertension and abnormal bilirubin |
| A4 | 0 | 3 tumours <3 cm | I–II | Child–Pugh A–B |
| Stage B: intermediate HCC | 0 | Large multinodular | I–II | Child–Pugh A–B |
| Stage C: advanced HCC | 1–2* | Vascular invasion or extrahepatic spread | I–II | Child–Pugh A–B |
| Stage D | 3–4[1] | Any | III | Child–Pugh C |

Stage A and B, all criteria should be fullfilled; *: Stage C, at least one criteria should be fulfilled; PST 1–2 or vascular invasion/extrahepatic spread; 1: Stage D, one or more criteria should be fulfilled; PST 3-4 or Okuda stage III/Child–Pugh C. Small HCC <5 cm, large HCC >5 cm.[36]
PST: performance status test; HCC: hepatocellular carcinoma.

**Table 2: Treatment schedule proposed for hepatocellular carcinoma cirrhotic patients according to the Barcelona Clinic Liver Cancer classification system.[34,37]**

| Stage | Treatment intention | First/second choice |
|---|---|---|
| Stage: very early | | Surgical resection/RFA |
| Stage A: early HCC | | |
| A1 | Radical | Surgical resection/RFA |
| A2 | | Surgical resection → OLT/percutaneous treatment |
| A3 | | OLT/percutaneous treatment |
| A4 | | OLT/percutaneous treatment |
| Stage B: intermediate HCC | Palliative* | Trans-arterial embolisation (associated or not to percutaneous treatment)<br>Chemoembolisation |
| Stage C advanced HCC | Palliative* | New agents |
| Stage D: end-stage HCC | Symptomatic | Supportive treatment |

*In the setting of Phase II investigations or randomised control trials.
HCC: hepatocellular carcinoma; RFA: radiofrequency ablation; OLT: orthotopic liver transplantation.

Nevertheless, when combining AFP together with AFP-L3 and DCP, as in the GALAD model, the levels of sensitivity range between 79.3% and 99.2% with a specificity of 50–88.3%.[31] In Japan, the Japanese Society of Hypertension (JSH) recommendations for patients at extremely high risk of HCC (cirrhosis, hepatitis B virus) are to have US examination every 3–4 months, together with measurement of AFP, AFP-L3, DCP every 3–4 months.[1] The contrast-enhanced CT scan and MRI are the most common diagnostic tools in HCC detection. There is much debate regarding the cost-effectiveness of HCC surveillance;[32] however, US every 6 months in cirrhotic patients is the recommended and most cost-effective method for surveillance.[21,27]

## STAGING

### Barcelona Clinic Liver Cancer

Currently, the only validated staging is the Barcelona Clinic Liver Cancer (BCLC) staging, and it can be divided into five categories: very

early, early, intermediate, advanced, and terminal. The American Association for the Study of Liver Disease (AASLD) recommends BCLC staging for HCC (Table 1). This staging helps to predict the outcome and the appropriate treatment (Table 2).[34]

### Okuda Score

The Okuda score is a prognostic score that includes tumour size higher or lower than 50% of the liver, the presence of hepatic failure markers and factors such as presence or absence of ascites, and serum albumin and bilirubin levels. The score contributes to the diagnosis of HCC and can be divided into three stages. The survival rate of Stage I patients is 11.5 months, Stage II patients 3 months, and Stage III patients 0.9 months.[34]

## SCORES

Several different scores were dedicated to the evaluation of the progression of HCC. The Child–Turcotte–Pugh (CTP) classification, Model of End Stage Liver Disease (MELD), Okuda score, and Cancer of the Liver Italian Program (CLIP) score are all prognostic factors for the determination of survival and treatment of HCC patients.

### Child–Turcotte–Pugh System

The primary Child–Turcotte system included clinical parameters of ascites, encephalopathy, bilirubin levels, albumin levels, and nutritional status. In 1973, Pugh modified the nutritional status for prothrombin time (Table 3).[16] Although it has no HCC specific parameters, the CTP system is instrumental in grading liver function and is part of HCC evaluation. It also helps determine the proper treatment where surgery has the highest cure potential. The CTP system has some disadvantages, which include day-to-day fluctuation in key parameters and inter-laboratory variations. Nevertheless, the CTP score has been incorporated into other HCC-specific staging systems such as BCLC.[34]

### Model of End Stage Liver Disease

The MELD was originally developed for predicting survival of patients following transjugular intrahepatic shunt.[38] The model is a logarithmic score composed of serum creatinine, total serum bilirubin, and INR. Patients with HCC may be prioritised for curative orthotopic liver transplantation upon this scoring, but, while waiting for MELD score to move them up on the graft allocation priority list, a worsening in the condition of patients in early stage HCC may occur. This problem has been solved by giving 'extra' points to HCC patients.[34]

#### MELD scoring system

MELD Score = 9.57 x ln (serum creatinine in mg/dL) + 3.78 x ln (serum bilirubin in mg/dL) + 11.2 x ln (INR) + 6.43.[34]

### Cancer of the Liver Italian Program Score

The CLIP score is the most recently developed prognostic scoring system for HCC. It combines tumour stage and macroscopic tumour morphology, serum AFP levels, and the presence or absence of portal vein thrombosis with an index of the severity of cirrhosis to determine a prognostic score range 0–6 (Table 4). The score has several disadvantages. Firstly, it can discriminate between patients with a 0–3 score but cannot discriminate between patients with a score range of 4–6.

**Table 3: Child–Turcotte–Pugh score.[34]**

| Measurements | Score | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Encephalopathy | None | Mild | Moderate |
| Ascites | None | Slight | Moderate |
| Bilirubin (md/dL) | 1–2 | 2–3 | >3 |
| Albumin (mg/dL) | >3.5 | 2.8–3.5 | <2.8 |
| PT | <4 | 4–6 | >6 |

Stage A: 5–6 points; Stage B: 7–9 points; Stage C: 10–15 points.
PT: prothrombin time (seconds prolonged).

**Table 4: Cancer of the Liver Italian Program scoring system.[34]**

| Variables | Score | | |
|---|---|---|---|
| | 0 | 1 | 2 |
| Child–Pugh stage | A | B | C |
| Tumour morphology | Uninodular and extension ≤50% | Multinodular and extension ≤50% | Massive or extension >50% |
| AFP (ng/dL) | <400 | ≥400 | |
| Portal vein thrombosis | No | Yes | |

AFP: alpha fetoprotein.

Secondly, the system is not able to select the group that will benefit from curative and aggressive treatment. Thirdly, the stratification ability of this score is poor and approximately 80% will show a score of 0–2.[34,39]

## Chinese University Prognostic Index

The Chinese University Prognostic Index (CUPI) is another method by which HCC risk can be measured. A Cox regression model that determines the relationship between survival and several variables has been included in this index. The relation between tumour, node and metastasis (TNM) staging and 18 other clinical factors has been studied, and the outcome was a survival rate of 3 months. The model has confirmed TNM staging as a highly valuable predictor of 3-month survival. The model also identified presentation of asymptomatic disease, AFP level, total bilirubin, serum alkaline phosphatase, and clinical detection of ascites as significant prognostic factors. CUPI and CLIP score were the best models in prediction of survival in advanced HCC patients among 12 different systems analysed.[38,40]

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# HEPATOCELLULAR CARCINOMA. PART 3: SURGICAL AND MEDICAL TREATMENT

**Lior Charach, Lior Zusmanovitch, *Gideon Charach**

*Department of Internal Medicine C Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
**Correspondence to drcharach@012.net.il*

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## ABSTRACT

Hepatocellular carcinoma (HCC) treatment is variable and depends on the size, location, and presence of extra hepatic metastasis and vascular invasion. HCC treatment options have advanced significantly over the past few decades and include surgical and non-surgical methods. In the past, systemic chemotherapy was the non-surgical treatment and there was no significant increase in overall survival rate. Nowadays sorafenib, a molecular targeted drug, is the treatment of choice and has shown proven benefits in increasing survival time; other systemic therapies did not show longer statistical superiority. However, surgical treatments, such as liver transplantation and surgical resection, are still the only methods offering a curative opportunity; however, these are not free of adverse effects and recurrence of the tumour. Non-surgical techniques including ablative treatment, radiotherapy, transarterial chemoembolisation, and percutaneous ethanol injection also show some benefit in the survival of patients with HCC. Future molecular targeted drugs are currently under investigation in different stages of clinical trials, and there are positive expectations regarding their benefit in treating HCC.

Keywords: Hepatocellular carcinoma (HCC), surgical treatment of HCC, medical treatment of HCC.

## TREATMENT

### Surgical Resection

The selection of surgical liver resection takes into consideration the different scoring systems: Barcelona Clinic Liver Cancer (BCLC) scoring system, Milan criteria (MC) (one lesion ≤5 cm in diameter or ≤3 lesions ≤3 cm in diameter), Model of End Stage Liver Disease (MELD), and Child–Pugh. Resection is considered in Child–Pugh Class A and early B and MC and MELD score <9. In Asian countries, indocyanine green (ICG) clearance at 15 minutes with a cut-off >20% is regarded as a contraindication to resection.[1] Surgical resection is the treatment of choice for patients with solitary hepatocellular carcinoma (HCC), yet several contraindications are known, such as extra hepatic metastasis, vascular invasion, main bile duct involvement, and bilobar tumour. Patients without chronic liver disease show better long-term results, while patients with Child–Pugh Class A that are selected for surgery will still show postoperative liver decompensation, such as ascites. Continuous ascites for >3 months is a poor prognostic factor. Portal hypertension >10 mmHg is the best indicator of unresolved postoperative hepatic decompensation. Thus, surgical resection should be advised for patients with preserved liver function and without significant portal hypertension.[2,3] The extent of the resection should include the removal of all the malignant tissues with maximal preservation of tumour-free liver parenchyma. Limited resection, which tries to preserve liver parenchyma, has a less successful outcome due to tumour recurrence by local spreading; whereas anatomical resection, which is the resection of the vascular territory of the tumour of small solitary HCC lesions, has a higher survival rate than limited resections and is also the treatment of choice.[3] The resection goal is to preserve adequate future liver remnant (FLR). Post-hepatectomy liver failure is the most severe complication of hepatectomy and a major cause of death. Several methods have been used in order to assess FLR, and the current

method is based on computed tomography (CT) imaging. Hepatobiliary scintigraphy was shown to be superior and have higher prediction in patients with parenchymal disease, such as cirrhosis, cholestasis, steatosis, and chemotherapy injury. The disadvantages of these techniques include the potential discrepancy between the planned and actual transection planes; moreover, it is impossible to predict the functional contribution of liver parenchyma that is poorly perfused or has poor venous drainage after transection. ICG is used to evaluate preoperative liver function reserve and also as an early indicator of outcome following liver resection and orthotopic liver transplant (OLT).[4]

Small-for-size syndrome can occur in patients following liver resection when the liver regenerative capacity is impaired or as a result of extensive resection. Small-for-size syndrome and post hepatectomy liver failure present similar pathomechanisms, including the reduction of liver mass and portal hyper flow beyond a certain threshold.[5] In cirrhotic patients, non-anatomical resections were performed in order to conserve FLR; however, 'field changes' in the liver of these patients means they are more prone to tumour recurrence when compared to non-cirrhotic patients.[6]

According to the Liver Cancer Study Group in Japan, the prognosis of patients after hepatectomy shows 1, 3, 5, and 10-year survival rates of 85%, 64%, 45%, and 21%, respectively, in 6,785 patients followed up between 1988 and 1999.[3]

## Liver Transplantation

OLT in HCC is generally considered to be the best chance of curative treatment for patients with HCC liver dysfunction within the criteria. OLT is also significant in the prevention of postoperative complications associated with liver failure.[2,3] Indication in favour of liver transplant (LT) is based on the MC as seen in the Mazzaferro et al.[7] study, which showed a 4-year survival of 75% with a recurrence free survival of 83%. This prevents futile transplantation in patients likely to have microscopic extrahepatic metastasis.[7] The results of LT in unresectable tumours before the MC were used were poor, with a 3–5-year survival of 15% and a high rate of recurrence in the first months to years after transplantation. The application of the MC has dramatically increased the survival rate and made LT a first-line treatment option for patients with limited tumours.[3,8] Recently it has been implied that the MC may be too restrictive. The University of California San Francisco (UCSF) group (San Francisco, California, USA) and the BCLC tumour group, proposed widening the criteria and were supported by the Registry of Tumors in Liver Transplantation (Dallas, Texas, USA). The proposed criteria are one single lesion <6 cm or multiple lesions (no more than 4 lesions), with the largest being ≤5 cm. This expansion shows a 5-year survival of 72%.[1,8] Long-term survival after LT for HCC has been shown to decrease due to complications, such as long waiting lists, associated immunosuppressive therapy, graft rejection, and the expansion of the MC. At the time of transplantation, prolonged waiting time for LT has been associated with vascular invasion.[3] However, with fewer postoperative surgical complications, the use of marginal grafts, reduced tumour recurrence rates, and improved immunosuppressive regimes, the outcome has improved. Improved postoperative care allows for transplantation in a higher number of patients.[3]

HCC patients and patients with other underlying liver diseases have been waiting for LT, which makes the LT criteria very strict and competitive; thus, there is an increased interest in using living donors rather than only cadaveric donors.[1,3] The living donor liver transplant (LDLT) mortality risk is 0.1% for left hepatectomy and 0.5% for right hepatectomy.[1] LDLT increases the number of livers available for transplant, which allows for the extension of transplantations. In patients that do not meet UCSF or MC standards, LDLT should not be performed. Recent studies have shown the survival rate between the cadaveric and LDLT to be similar.[1] Comparison between LT and liver resection in HCC is dependent on the patients' clinical status. In patients with inadequate functional liver parenchyma, LT may be the only curative option. In patients with adequate liver function, liver resection can be the curative option.[1,9] The Rahman et al.[9] study showed a 5-year survival range of 40–70% in resection and 52–81% in transplantation. Tumour management, while waiting for LT, includes radiofrequency ablation (RFA) and transarterial chemoembolisation (TACE). Liver resection can be used as a bridge to transplantation.[3] Downstaging of HCC, using the aforementioned methods, can facilitate LT for patients outside the MC by decreasing the tumour burden. However, HCC recurrence rates after transplantation remain high after downstaging.[10]

## Percutaneous Ethanol Injection

Percutaneous ethanol injection (PEI) therapy has a wide range of anti-tumour effects, is inexpensive, and simple to use. The ethanol is injected by fine needle insertion directly into the mass on consecutive days under ultrasound (US) monitoring. The ethanol will lead to necrosis of the HCC via diffusion, causing thrombosis and ischaemia in the vessels of the tumour. The number of sessions for treatment of HCC depends on the size; for tumours <2 cm the number of sessions is 3–4. For tumour sizes from 2–3.5 cm the number of sessions is 8–12. Up to 4 sessions per week is recommended until arterial devascularisation of the tumour is reached. In non-compliant patients, single sessions are possible, but the injection volume should not exceed 70 mL, thus preventing serious side effects. Confirmation of the treatment efficiency can be detected by CT, angiography, or magnetic resonance imaging (MRI) 24 hours after the procedure.[11]

The disadvantage of ethanol injection is intense peritoneal pain. The pain can be avoided by slow injection and slow needle removal. Other complications, which occur rarely, are vascular thrombosis caused by ethanol entering the portal vein (PV) and dissemination of the tumour along the needle tract and haemoperitoneum. Small HCC tumours ≤3 cm show 90% necrosis with PEI while larger tumours show lower success rates and high recurrence rates.[11]

For treatment of larger tumours, it has been suggested to increase the ethanol injected or to use chemoembolisation in combination with PEI. The benefit of these methods is unknown.[11] Other methods, for example, the use of acetic acid or hot saline injections and placement of intra-tumoural microwave electrodes, have also been suggested to cause necrosis of the tumour. The advantages of hot saline and acetic acid methods are that they can enter the vascular system without causing damage and they need less volume to embed. Intra-tumoural placement of microwave electrodes shows complete tumour necrosis.[2,11] The survival rate with PEI is similar to those of surgical resection in tumours ≤3 cm. In larger tumours, depending on the size, the 1, 3, and 5-year survival rates were 81–97%, 42–82%, and 14–63%, respectively. Recurrence-free survival was not as successful, with rates of 60–83%, 51–82%, and 26–32%, respectively. In microwave ablation (MWA), the survival rate at 1, 2, 3, and 4 years was 45.9%, 26.9, 26.9, and 13.4, respectively. The disease-free survival time was 15.5 months.[12] A combination of PEI with TACE has been shown to have a better effect than monotherapy.[11]

## Radiofrequency Ablation

In the RFA method, an electrode is inserted in the tumour under US monitoring, in a percutaneous intercostal or subcostal approach, to start the ablation and cause coagulative necrosis.[13,14] RFA is indicated in patients who are not eligible for surgical resection without extra hepatic tumour, Child–Pugh Class A or B, single tumour size ≤5 cm in diameter or three with fewer tumours ≤3 cm in diameter and is considered the preferred method in these patients. RFA is not indicated in patients where the tumour is not visualised by US; the total bilirubin level ≥3 mg/dL; the platelet count is $<50\times10^9$/L; or prothrombin activity is <50%; there is enterobiliary reflux or adhesion between the tumour and the gastrointestinal tract; there are exophytic or capsular lesions due to complications such as intra-peritoneal bleeding and subcapsular haematomas.[15-18]

Tumours <3.5 cm, embedded in the hepatic tissue and far from the blood vessels, show the best results. The blood flow interrupts the ablation process by cooling the heating process which makes the tumour adjacent to blood vessels harder to treat.[11] Post-ablation US, CT, and MRI can be performed in order to detect residual tumours. The ability of contrast-enhanced US to detect residual tumours 1 day after RFA was 27%. Using positron emission tomography/CT, a residual tumour can be detected 1–2 days after ablation. Non-enhanced T1-weighted imaging (T1W) can show a hyperintense zone 2 days after RFA.[13,19,20]

Follow-up by US and CT was carried out every 4 months. Levels of serum alpha-fetoprotein (AFP), lectin reactive alpha-fetoprotein, and des-γ-carboxy-prothrombin were measured every month.[16] The incidence of complications associated with RFA measured in 2,982 patients was 2.2% per treatment and 1.5% per procedure. A summary of complications displayed in RFA patients is shown in Table 1.[16]

In a comprehensive study,[12] the survival rates at 1, 3, 5, 7, and 10 years in patients who were i) unsuitable for surgical resection, LT, or refused surgery; ii) free of extrahepatic metastasis or vascular invasion; iii) free of other malignancies that may determine the patient prognosis were 96.6%, 80.5%, 60.2%, 45.1%, and 27.3%, respectively.[16] For patients with

Child–Pugh Class A or B with MC fulfilled, the 5-year survival rate was 63.8%.[16]

The distant recurrence rate at 1, 3, 5, 7, and 10 years with no local tumour progression was 25.6%, 63.3%, 74.8%, 78.1%, and 80.8%, respectively. Meta-analyses showed the superiority of RFA over PEI and the correlation to better OS.[21] This may be explained by low recurrence and better effectiveness of RFA in necrosis of the HCC.[21] When comparing survival between RFA and surgical resection, no significant difference was found.[16]

## Percutaneous Microwave Ablation

MWA is a relatively new method with potentially faster ablation and potentially larger ablation areas. MWA and RFA are considered first-line treatments in HCC. Several advantages over RFA, such as greater penetration of energy into tissues and low sensitivity to physical tissue property variation and impedance, may suggest that MWA could be a better treatment for HCC than RFA.[22-26] At 3 months, 6 months, and 12 months post-ablation, patients were followed up by CT scan.[27] Successful treatment is the complete absence of contrast enhancement with homogenous hypo-density in the ablation zone.[22,27] In patients where MWA did not show favourable results, other techniques can be employed, such as RFA, TACE, PEI, or a combination of ≥2 techniques.[22] Complications associated with MWA were reported in Livraghi et al.[28] where major complications occurred in 2.9% of patients, including symptomatic pleural effusion, intra-peritoneal bleeding, ileal or colonic perforation, liver decompensation and liver infarction, haemothorax, hepatic abscess, and several other gastrointestinal and cardiac complications. Minor complications were also reported in 7.9% of the patients and included subcutaneous burns, asymptomatic pleural effusion, portal thrombosis, slight thickening of the gallbladder wall, and bradycardia.[28] Poggi et al.[22] researched patients with small HCC lesions, which showed 100% complete ablation. Intermediate lesions showed 90% complete ablation and large lesions showed 69% complete ablation. Local tumour progression was observed in 5% within a 2-year medium follow-up.

## Trans-Arterial Chemoembolisation

TACE is a method in which a chemotherapeutic agent is administered to the hepatic artery with or without lipidol, an iodinated ester that serves as a carrier for chemotherapeutic agents into the tumour, increasing the level of the drug in the tumour, leading to vascular ischaemia and occlusion.[14,15]

Cisplatin, mitomycin C, and doxorubicin are the most common chemotherapeutic agents used in combination with TACE in HCC, not prioritising one drug over the other.[29] TACE is indicated in HCC patients not suitable for resection and with sufficient liver function without extra hepatic metastasis or vascular invasion. Several studies have suggested that TACE may still be used in selected cases with PV thrombosis.[30-32] It is also used as a bridge to OLT and prior to, or post, RFA.

**Table 1: Types of complication in 2,982 patients on treatment of radiofrequency ablation for hepatocellular carcinoma.[16]**

| Complication | Percentage of complications and number of patients (n) |
|---|---|
| Neoplastic seeding | 0.8% (24) |
| Liver abscess | 0.2% (6) |
| Haemoperitoneum | 0.4% (12) |
| Haemothorax | 0.16% (5) |
| Symptomatic pleural effusion | 0.03% (1) |
| Massive hepatic infarction | 0.2% (6) |
| Gastrointestinal perforation or penetration | 0.16% (5) |
| Haemobilia | 0.06% (2) |
| Skin burn | 0.03% (1) |
| Pneumothorax | 0.1% (3) |
| Gallbladder injury | 0.03% (1) |
| Cerebral infarction | 0.03% (1) |

Contraindications to TACE include: Child–Pugh Class B or higher, renal insufficiency, severely decreased PV flow, and technical difficulties in hepatic intra-arterial treatment.[30] Child–Pugh is used as a prognostic factor for survival of patients with unresectable HCC who are treated with TACE.[30] Common complications associated with TACE treatment were fever, abdominal pain, vomiting, ascites, and gastrointestinal bleeding.[17] Other complications perceived in TACE treatment are liver injury, namely liver abscess, acute hepatic failure, liver infarction; extra hepatic lesions including severe cholecystitis, splenic infarction, pulmonary embolism, gastrointestinal mucosal lesion; iatrogenic injury due to catheter insertion and perforation of the celiac artery and its branches.[30] Recently, doxorubicin eluting beads (DEB) were used together with TACE in order to control the drug release duration. DEB can carry several chemotherapeutic agents whilst showing a lower toxicity level to surrounding liver tissue and lower recurrence when compared to TACE alone.[29,33]

TACE has been shown to increase survival rate in HCC patients.[34] The 1, 3, 4, and 5-year survival in a cohort study using TACE-DEB shows 89.9%, 66.3%, 54.2%, and 38.3% survival rates, respectively, with a median of 48.6 months.[35] When comparing TACE to surgical resection, no significant difference was shown,[36] whereas another study in which sorafenib, a multikinase inhibitor acting on the vascular endothelial growth factor (VEGF) receptor, was combined with TACE showed a superior outcome over TACE alone.[37]

The Assisted Reproductive Technology (ART) index was developed in order to select the patients that may benefit from retreatment with TACE and those who will not gain any benefit. In this score, patients with a BCLC Stage B and ART score >2.5 points had a poor prognosis, while a score of <2.5 points had a better prognosis of 22.5–28 months OS. Patients with Child–Turcotte–Pugh Class B scoring 7 or 8 points and an ART score between 0–1.5 had a good prognosis as well; 14.5 months OS. This result indicates that patients with an ART index >2.5 may not benefit from TACE retreatment.[38] The Hepatoma Arterial-embolisation Prognosis (HAP) score is an index composed of 4 stages developed to select the patients that may benefit from TACE/transarterial embolisation.

The majority of the patients in the study had Child–Pugh Class B and MELD >10. Patients with HAP C and D are unlikely to benefit from TACE. The median survival for the groups A, B, C, and D was 27.6, 18.5, 9.0, and 3.6 months, respectively.[39]

Stereotactic Body Radiation Therapy (SBRT) is a method established >20 years ago. This method can be used in the treatment of patients with a local tumour who are not suitable for RFA, TACE, chemotherapy, or surgery. The appropriate candidates for this therapy are patients with lesions located in a central portal area, regions near to the great vessels, biliary ducts, or metastases located below the diaphragm or at the surface of the liver. The toxicity of Stereotactic Body Radiation Therapy is mainly associated with liver dysfunction, yet other complaints including pain, fatigue, nausea, and vomiting have been reported.[40-42]

## Radioembolisation

Radioembolisation using $^{90}$Yttrium (Y90) is a type of brachytherapy which can be used in patients with HCC who fulfil the MC and are in the transplant list. Its effect is mainly to slow down the tumour progression and to decrease the number of patients enlisted in the transplant programme.[29,43] It is also useful in patients outside the transplant list and with advanced HCC, by down-staging HCC. In advanced HCC, it is possible to use Y90 due to its low embolic effect. Radioembolisation demonstrates superiority over TACE in advanced stages by downstaging HCC.[29,43] Complications are due to irradiation of surrounding tissue and not as a result of the microembolic effect. The most common adverse effect is post-radioembolisation syndrome characterised by nausea and vomiting, fever, fatigue, abdominal discomfort, and anorexia. Other side effects include hepatic dysfunction, biliary damage, gastrointestinal ulceration, lymphopenia, and radiation pneumonitis.[43,44] Radioembolisation shows similar survival rates as in the treatment with sorafenib.[43] In intermediate stage patients, BCLC-B, the survival rate was 15.4–16.6 months. Finally, in the advanced stage, median OS ranged from 6–10 months.[44]

## Systemic Treatment

Systemic treatment is carried out in two parts. Firstly, by systemic chemotherapy and secondly, by molecular targeted therapy.

### Systemic chemotherapy

Systemic chemotherapy monotherapy with doxorubicin in advanced HCC was the most common therapy before the usage of sorafenib. Other cytotoxic agents, including cisplatin, were

given but no favourable outcome was seen and the survival rate was not increased.[45,46] Combination therapy of cisplatin with epirubicin, 5-fluorouracil, or doxorubicin, interferon alpha and 5-fluorouracil showed higher responses, but without a higher survival rate.[45,46] Hormonal therapies with tamoxifen, octreotide, and interferon were suggested, but a meta-analysis that was conducted did not reveal a significant survival benefit.[45] Due to the futility of systemic chemotherapy in improving survival rates, it is not recommended for the treatment of HCC.[45,46]

**Molecular targeted therapy**

Molecular targeted therapy is the standard treatment in advanced stage HCC.[45] The following are some of the treatments being used.

### Sorafenib

Sorafenib is a multikinase inhibitor of RAF kinase and VEGF receptors that are involved in the angiogenesis around the tumour. The efficiency of sorafenib was examined in two studies: the SHARP trial[47] and Asia-Pacific trial.[48] In both studies, survival time in patients with advanced stage HCC was prolonged; the median survival time was <12 months in both studies.[45,49]

Sorafenib is regarded as safe. Severe adverse effects can be associated with sorafenib, including hand-foot skin reaction, which is the most common side effect, diarrhoea, fatigue, and anorexia.[29]

### Other molecular targeted therapies

Nivolumab, programmed-death-1 (PD-1) inhibitor, gains attention as a future therapy with promising results. Nivolumab demonstrated two complete and seven partial responses with an overall response rate (ORR) of 19%.[51] Other molecular therapies with sunitinib, brivanib, linifanib, vandetanib, nintedanib, dovitinib, and a combination therapy of sorafenib with doxorubicin or erlotinib were also studied. The results of the studies showed no benefits in survival rate or time, as compared to sorafenib treatment alone. Sorafenib combination with doxorubicin was the only study to show an increase in median time to progression, OS, and progression-free survival as compared to doxorubicin monotherapy. Synergism between doxorubicin and sorafenib was studied but there was no evidence of synergism.[45,52]

Other combinations were also studied including: gemcitabin and pegylated doxorubicin, gemcitabin with oxaliplatin and cetuximab, bevacizumab with oxaliplatin and capecitabin, sorafenib and TACE, sorafenib with hepatic resection, and sorafenib with RFA. In the combination of gemcitabine with pegylated liposomal doxorubicin, gemcitabine was administered first because of studies that showed an increase in topoisomerase II expression which leads to an increase in the cytotoxicity.[53-56] The treatment was well tolerated by the patients and the outcome showed an ORR of 24%, a disease control rate (DCR) of 58.5%, a progression-free survival of 5.8 months, and a median survival time of 22.5 months.[55] Gemcitabine in combination with oxaliplatin, otherwise known as GEMOX, with addition of cetuximab was studied in HCC patients. GEMOX treatment benefits HCC patients because of the lack of liver and kidney side effects. This combination is relatively safe, but some side effects were still documented, such as skin rash, myelosuppression, and neurotoxicity. The outcome of this combination showed an ORR of 20% and DCR of 60%, a median PSF of 4.7 months and an OS of 9.5 months.[57,58] Research was conducted to reveal the effect of bevacizumab and capecitabine, in combination with oxaliplatin. The results showed that the combination was well tolerated and the outcome of the results showed a partial response of 20% and DCR of 78%, progression-free survival of 6.8 months and OS of 9.8 months.[59]

TACE in combination with sorafenib proved to be a useful method of treating HCC. In research undertaken to compare the benefit of sorafenib post-TACE, it was found that for patients administered sorafenib after TACE, the median time to progression did not improve and there was no proven benefit in administering sorafenib after TACE.[60] Combination of sorafenib as adjuvant to RFA, or following resection, has not shown any favourable results in terms of recurrence, free survival, time to recurrence, and OS.[61]

The best supportive treatment for palliative therapy includes pain control, mainly by opiates and with supporting medications against constipation, pruritus, and anti-emetics. Glucocorticoids may be added to alleviate symptoms such as fever, pain, nausea, fatigue, and anorexia. Ascites removal together with albumin supplementation is important in assisting wellbeing. Palliative radiation therapy can be used for distant invasion to lymph node, brain, and especially to the bone. Percutaneous cementoplasty can also be used in case of painful bone metastasis. Nutritional and psychological support together with the methods aforementioned may improve the quality

of life in these patients but does not improve life expectancy.[62] In order to improve the quality of life a multidisciplinary approach is often needed involving a hepatologist, an oncologist, a pain control specialist, a dietician, a psychologist, and a social worker.

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# A LOOK AT PLATELET COUNT IN CHRONIC HEPATITIS C INFECTION

***Romeo-Gabriel Mihăilă**

*Faculty of Medicine, "Lucian Blaga" University of Sibiu; Hematology Department, Emergency County Clinical Hospital Sibiu, Sibiu, Romania*
**Correspondence to romeomihaila@yahoo.com*

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## ABSTRACT

A complete blood count performed in chronic hepatitis C virus (HCV) infected patients can identify thrombocytopenia or an increased number of platelets, the cause of which must be established. Most of these patients are predisposed to develop thrombocytopenia as the disease progresses due to a lower thrombopoietin production, increased platelet pooling in the spleen, viral bone marrow suppression, or interferon-based therapy. However, a severe thrombocytopenia can have an autoimmune aetiology, which is very probable at values <15x10$^3$/mm$^3$. Thrombopoietin analogues are useful both in patients with primary immune thrombocytopenia and in those who will begin the treatment with pegylated interferon and ribavirin before surgery. The common causes of an increased number of platelets in chronic HCV infected patients are splenectomy, ribavirin treatment, liver transplantation, and hepatocellular carcinoma. However, thrombocytosis can also be hereditary, reactive, or malignant, especially in essential thrombocythaemia or other myeloproliferative diseases that can be associated. A hepatic blood flow obstruction present in chronic HCV infected patients must draw attention to a possible associated myeloproliferative disorder (which frequently manifests in thrombocytosis) that represents its aetiology in two-thirds of cases and which can evolve with a constant or an intermittent increase in platelet count. The role of the JAK-STAT signalling mechanism is presented in both chronic hepatitis C patients and in those with essential thrombocythaemia. It was suggested that *STAT3* could have a role in HCV RNA replication. In addition, the HCV core protein is involved in the modulation of fibrogenetic gene expression in hepatic stellate cells through a *JAK2-STAT3* dependent pathway. Ruxolitinib (a *JAK1/JAK2* inhibitor) can have beneficial effects in essential thrombocythaemia and is a subject of research in chronic hepatitis C. The discovery of the aetiology of thrombocytopenia or an increased number of platelets can contribute to a more complete diagnosis and appropriate treatment. The identification of associated disorders in chronic HCV infected patients is of vital importance for them.

Keywords: Essential thrombocythaemia, hepatitis C virus (HCV), thrombocytopenia, thrombocytosis.

## INTRODUCTION

Many complications can occur during the evolution of chronic hepatitis C virus (HCV) infection. Some of them can be related to thrombocytopenia or thrombocytosis. In addition, some patients may have associated diseases that can evolve with thrombocytopenia or thrombocytosis. The identification of thrombocytopenia or thrombocytosis aetiology and of associated disorders in chronic HCV infected patients can be vital for them. A literature review was carried out in OvidMD Advantage, Ovid MEDLINE® Daily, Ovid MEDLINE, and PubMed using hepatitis C, thrombocytopenia, thrombocytosis, and essential thrombocythaemia as search terms.

## THROMBOCYTOPENIA IN CHRONIC HEPATITIS C INFECTION

Thrombopoietin (TPO) is a growth factor produced in the liver that binds to the c-Mpl receptor present on megakaryocytes and platelets.[1] The activation of the JAK-STAT mechanism occurs after TPO binds to its receptor and results in platelets production stimulation.[2] The platelet count rise

occurs after a latency period of 5 days and reaches a peak after 10–12 days.[2] A decreased platelet count leads to an increase of free TPO levels, which induces a higher platelet production by bone marrow megakaryocytes.[1]

Chronic liver disease patients (including those with HCV aetiology) are predisposed to develop thrombocytopenia as the disease progresses. Its aetiology is complex. The most common causes are the lower TPO production and the higher platelet destruction due to hypersplenism.[3] The thrombocytopenia associated with hypersplenism is caused by increased platelet pooling in the spleen.[4] So-called hypersplenism has little clinical effect. In addition, there is no proof showing that correcting the hypersplenism has consequences on patient survival.[5]

HCV itself could also produce bone marrow suppression.[6,7] In addition, it was found that HCV viraemia was independently associated with lower platelet count after adjustment for liver fibrosis.[8] There are also reports on the improvement of thrombocytopenia after interferon (IFN)-based therapy obtaining sustained viral response.[9-11] The maximum increase in platelet count was observed after 6 months of antiviral treatment.[9] The patients without virological response to IFN presented with decreased platelet counts.[10]

Treatment-related thrombocytopenia is another cause. Conventional antiviral therapy containing pegylated interferon (PEG-IFN) frequently reduces platelet count in HCV patients. The pooled incidence of clinically significant thrombocytopenia is around 8.8–10.0%.[12] It was observed that a baseline platelet count <100x10$^3$/mm$^3$ and a rapid early platelet diminution (>30% decrease in the first 2 weeks) are significantly associated with severe thrombocytopenia, defined as <50x10$^3$/mm$^3$, requiring reduction of PEG-IFN dose.[13] A large multicentre study analysed the bleeding risk due to thrombocytopenia in HCV-infected patients with biopsy-proven advanced liver fibrosis (Ishak score 4–6) during IFN-based therapy. Sixteen percent of patients with a platelet count between 75x10$^3$/mm$^3$ and 149x10$^3$/mm$^3$, and 30% of them with a platelet count <75x10$^3$/mm$^3$, required IFN dose reductions, and 3% and 16% of them, respectively, discontinued the IFN due to severe thrombocytopenia. On-treatment bleedings were generally mild despite the advanced fibrosis.[14] Fortunately, current antiviral regimens (IFN-free) comprising direct-acting antiviral agents not only produce excellent sustained virological response rates (>90%) but also carry a very low risk for the development of thrombocytopenia (≤1%, according to prescribing information sheets of daclatasvir/asunaprevir, sofosbuvir, the association between dasabuvir, ombitasvir, paritaprevir, and ritonavir, and the combination of ledipasvir and sofosbuvir). In other words, the barrier of PEG-IFN-based antiviral therapy in thrombocytopenic HCV patients has been overcome.

But, if thrombocytopenia is severe (<15x10$^3$/mm$^3$) in patients with HCV-related liver cirrhosis then an autoimmune aetiology is very probable. A high titre of platelet-associated immunoglobulin G was found in 40 out of 41 such cirrhotic patients and was significantly lower in splenectomised patients compared to those with an intact spleen, as a result of a lower CD4/CD8 ratio in the first group, followed by diminished autoantibody production.[15] Immune thrombocytopenic purpura and thrombocytopenia present in chronic hepatitis C have a common treatment: the c-Mpl receptor agonists, such as eltrombopag, which is a small oral molecule[2] that acts as a thrombopoietic agent able to increase platelet count in thrombocytopenic patients with chronic hepatitis C.[16] Eltrombopag produced a dose-dependent increase of platelet count in HCV-related cirrhosis patients. The antiviral treatment could be initiated in 45 out of 56 of them and some patients completed their 12 weeks of antiviral therapy under concomitant eltrombopag treatment in the study of McHutchison et al.[17] ENABLE-1 and ENABLE-2 are two Phase III randomised, controlled studies that included 1,520 thrombocytopenic patients with HCV and advanced fibrosis and cirrhosis. They were treated with eltrombopag in order to reach a predefined minimal threshold for the initiation of antiviral treatment with PEG-IFN-α and ribavirin. More patients treated with eltrombopag maintained >50x10$^3$ platelets/mm$^3$ during the anti-HCV-treatment, could receive higher PEG-IFN-α doses, and reached significantly higher rates of sustained virological response, compared to placebo. Liver decompensation and thrombotic events were more frequently present in the eltrombopag group of ENABLE-2.[18]

Romiplostim, a second-generation c-Mpl receptor agonist, contains four TPO agonist peptides inserted in an immunoglobulin (IgG) heavy chain and is effective in the treatment of primary immune thrombocytopenia.[6] If eltrombopag is the agent widely studied in patients with HCV infection,

only a few reports and one observational study up to the present described the use of romiplostim before PEG-IFN/ribavirin treatment or before surgery in these patients. Romiplostim was given to a splenectomised patient with immune thrombocytopenic purpura before PEG-IFN/ribavirin treatment; the antiviral treatment started at a value of $65x10^3$ platelets/$mm^3$ and led to an early virological response followed by a sustained virological response; in this case, romiplostim was effective and safe.[19] Romiplostim also allowed the treatment of hepatitis C in a patient coinfected with HIV.[20] A severe thrombocytopenia produced during the antiviral treatment of two HCV-related cirrhosis patients was successfully treated with romiplostim (with a platelet count >$50x10^3$/$mm^3$), which allowed continuation and completion of the IFN protocol without dose reduction; both patients obtained a sustained virological response.[21] A group of 35 thrombocytopenic patients with HCV-related liver cirrhosis received romiplostim at a dose of 2 µg/kg/week for a maximum of 1 month in order to increase the platelet count. Of this cohort, 33 achieved a number of ≥$70x10^3$/$mm^3$ and became eligible for surgery. The maximum peak of platelet count was between $73x10^3$/$mm^3$ and $24x10^4$/$mm^3$. They had no postoperative bleeding or thrombotic events.[22] TPO also affects the liver. Researchers have previously investigated whether, as well as stimulating liver regeneration, TPO also stimulates hepatocellular carcinoma cell proliferation. Until now, the answer to this question has been no, in both *in vitro* and *in vivo* studies.[23]

It is useful to look at platelet count in chronically HCV-infected patients who developed hepatocellular carcinoma. Chronic HCV infection is an important aetiological factor for this type of cancer. It was shown that patients with a pretreatment platelet count <$118x10^3$/$mm^3$ have a low risk for extrahepatic metastasis after treatment, while a platelet number >$212x10^3$/$mm^3$ was associated with a higher risk for this type of metastasis.[24] This observation may help to improve the therapeutic strategy in patients at high metastatic risk. It is known that tumours can contribute to an increase of platelet production and activation; activated platelets can contribute to tumour growth and metastasis.[25] But it seems that HCV-related cirrhotic patients have no activated platelets (assessed by flow cytometry) during hepatocellular carcinoma development or recurrence; they have also an increased level of von Willebrand factor and of ADAMTS13 activity.[26]

## THE INCREASE OF THE PLATELET COUNT IN CHRONIC HEPATITIS C

Common causes of the increase of the platelet count in chronically HCV-infected patients are splenectomy, ribavirin treatment, and liver transplantation (LT). But clinically evident thrombocytosis, usually defined as >$45x10^4$/$mm^3$, is rare in HCV patients receiving splenectomy or ribavirin monotherapy.

A platelet count augmentation can be observed after splenectomy in HCV-chronic infected patients and this increase persists for a long time.[27] An increase of platelet count can be found in patients with chronic hepatitis C treated with ribavirin, which induces haemolytic anaemia followed by a rise in serum erythropoietin. A higher endogenous erythropoietin stimulates not only the erythrocytes production but also that of platelets. Such an augmentation was shown after 4 weeks of ribavirin monotherapy (from $14.0x10^4$ to $15.8x10^4$/$mm^3$) while TPO did not increase.[28] IFN-related thrombocytopenia diminished in patients treated not only with IFN, but also with ribavirin, due to its thrombocytotic response.[29] It was shown that rs1127354 and rs7270101 (two functional variants in the ITPA gene) produce ITPase deficiency and defend against ribavirin-induced haemolytic anaemia. However, a platelet count reduction appeared in these patients.[29] A reactive thrombocytosis (platelet count >$45.0x10^4$/$mm^3$ for at least 7 days), which begins within 8 weeks after LT, was observed especially when LT was made after a seronegative fulminant hepatic failure and was negatively associated with HCV-related liver cirrhosis. This thrombocytosis had a median duration of 25 days and did not raise the hepatic artery thrombotic risk.[30]

Thrombocytosis may also occur in hepatocellular carcinoma patients. Fifty-two of 634 biopsy-proven hepatocellular carcinoma patients had a platelet count >$40.0×10^4$/$mm^3$. The patients with thrombocytosis were younger and had a larger tumour size, less cirrhosis,[31] higher serum level of alpha-fetoprotein, and an increased risk of main portal vein thrombosis. They were also less able to receive therapy than those without thrombocytosis and had shorter survival.[32] In addition, they had a significantly higher mean serum TPO level than those without thrombocytosis. Thrombocytosis is considered to be a paraneoplastic syndrome in these patients and is due to the overproduction of TPO by hepatocellular carcinoma cells.[32]

Thus, although TPO may not have direct effects on cancer cell proliferation, platelets most certainly do, and TPO agonists may therefore, at least in theory, have adverse effects on HCV-infected patients who develop hepatocellular carcinoma.

Unfortunately, no associated diseases pathology has been written at present; such an attempt would be difficult. But it is useful to point out a possible association of two chronic diseases: chronic hepatitis C and essential thrombocythaemia, as they have a common pathway and a possible common treatment. It is estimated that the incidence of essential thrombocythaemia in the European Union (EU) is between 0.38 and 1.7 per 100,000 people per year.[33] When hepatitis C coexists with essential thrombocythaemia, plateletpheresis is indicated if the patient has thrombocytosis (e.g. 1.3 million/mm$^3$) and should be subjected to a surgery procedure (e.g. a cardiopulmonary bypass as a treatment modality for an aortic insufficiency).[34] The common pathway present in these two diseases is represented by the signalling mechanism JAK-STAT. About 53% of patients with essential thrombocythaemia present with the mutation *JAK2* V617F[35] (that was discovered in 2005), which is responsible for JAK2 enzyme activation and is involved in the control of several vital cell functions, such as survival, differentiation, and proliferation.[36] It is not entirely clear at present how mutations in the pseudokinase domain (JAK homology 2 domain or JH2 domain) can increase the *JAK2* activation but some progress has been made.[37] A rigidification of alphaC-helix contributes to a hyperactivation of the JH1 domain in patients with a *JAK2* mutation.[38] The heterozygous *JAK2* V617F mutation stimulates megakaryopoiesis and patients often have essential thrombocythaemia, while a homozygous *JAK2* V617F mutation increases erythropoiesis and decreases megakaryopoiesis, often leading to polycythaemia vera.[39] But the essential thrombocythaemia patients can also have other mutations. About 3%[40] have a gain-of-function mutations in the gene that encodes the Mpl receptor (discovered in 2006): another pathway to activate *JAK2*.[41] Other patients (˜32%)[35] have mutations in exon 9 of the calreticulin gene (discovered in 2013) that can also hyperactivate the JAK2-STAT pathway,[42] or are triple negative (˜12% of them).[35] The occurrence of disease-initiating mutations in haematopoietic stem cells could be the consequence of genomic instability present in these patients.[43] Each of the three mutations activates the JAK2-STAT signalling mechanism. An important remark must be made: serum TPO levels are normal or slightly elevated in essential thrombocythaemia patients as the c-Mpl receptor is poorly expressed and the uptake and catabolism of TPO is defective; an inverse correlation was found between serum TPO levels and platelet mass.[44]

What pathophysiological implications does the JAK-STAT pathway have in chronic HCV infected patients? STAT3 is activated by non-structural proteins present in HCV structure through oxidative stress mediation; activated *JAK2* also influences this process.[45] It was suggested that STAT3 could have a role in HCV RNA replication.[45] HCV core is involved in increasing expression of IFN-γ receptor 2, which can explain the up-regulated JAK-STAT pathway produced by HCV core. In contrast, JAK1/2 and STAT3 activation and STAT3-mediated transcription were impeded by HCV core in the presence of interleukin (IL)-6 stimulation.[46] Blocking the IFN mechanism of action through the inhibition of STAT1 phosphorylation by JAK1 favours a possible hepatitis E virus infection but not with HCV.[47] In addition, HCV core protein is involved in the modulation of fibrogenetic gene expression in hepatic stellate cells through a JAK2-STAT3 dependent pathway.[48] E2 protein found in the structure of HCV is implicated in increasing fibrosis production in hepatic stellate cells by upregulation of collagen alpha(I) synthesis and oxidative stress, via a JAK related pathway.[49] Platelets can decrease collagen production by inactivating hepatic stellate cells and accelerating liver regeneration, so it is estimated that platelet transfusions could improve liver function in chronic liver disease patients by increasing the platelet count.[3] A high expression of JAK2 found in the normal tissue fragments located around a resected hepatocellular carcinoma signifies a poor prognosis.[50]

Apart from IFN-α, a medication used for essential thrombocythaemia treatment is useful also in chronically HCV-infected patients: ruxolitinib, approved by the US Food and Drug Administration (FDA) for the treatment of intermediate or high-risk myelofibrosis. A *JAK2* V617F allele burden decrease with >50% was obtained in 23.5% of the 22 essential thrombocythaemia patients treated with ruxolitinib, an oral *JAK1* and *JAK2* inhibitor, but without complete molecular remission.[51,52] *JAK2* inhibitors proved to be useful for the treatment of patients with myeloproliferative neoplasms.[36] The chronic *JAK* inhibitor treatment leads sometimes to cell persistence by transphosphorylation of *JAK2* through other *JAK* kinase family members.[53]

Tofacitinib (a pan-*JAK* inhibitor)[54] could be a solution for the patients who are resistant to ruxolitinib (a selective inhibitor of *JAK1/2*). But it should be noted that no *JAK* inhibitor to date has proved beneficial in treating HCV infection. This is only one direction for future research, such as that published by Ma et al.[55]

Chronically HCV-infected patients may have other causes of the increase of the platelet count. A thrombocytosis found in them is rarely hereditary (as a result of mutations of the TPO or MPL genes, or of the JAK2 gene apart from V617F and that of the gelsolin gene)[56] and, more often, can be present in a disease or situation that evolves with reactive thrombocytosis (various infectious or inflammatory diseases, blood loss, iron deficiency anaemia, or just iron deficiency), in prefibrotic myelofibrosis, chronic myeloid leukaemia,[57] BCR positive thrombocytosis,[58] or some types of myelodysplastic syndromes,[59] such as the 5q deletion (5q-syndrome). The MPL Baltimore (Lys39Asn) mutation that manifests with thrombocytosis has to be mentioned, as it can be found in about 7% of African Americans.[60]

An extreme thrombocytosis found in chronic HCV-infected patients (that is often the expression of essential thrombocythaemia or associated with other myeloproliferative diseases)[61] may be clinically suspected not only in patients with various located thromboses (that occurs at a platelet count between $40x10^4$ and $10x10^5$ platelets/$mm^3$) or bleeding (possible at >$10x10^5$ platelets/$mm^3$, when acquired von Willebrand disease can occur) but also in those with erythromelalgia, which is the expression of chronic microvascular arterial occlusive disease.[62] There is increasing evidence on the role of thrombotic risk factor for *JAK2* V617F mutation.[63] The thrombotic risk is much higher in patients with myeloproliferative neoplasm (including those with essential thrombocythaemia) who also have some inherited thrombophilic single nucleotide polymorphisms.[64]

A hepatic blood flow obstruction present in chronically HCV-infected patients must draw attention to a possible associated myeloproliferative disorder (which frequently manifests as thrombocytosis), that represent its aetiology in two-thirds of cases and which can evolve with a constant or an intermittent increase in platelet count.[65] A Budd–Chiari syndrome or a portal cavernoma (secondary to a single or repeated portal vein thrombosis) can also be a consequence of a myeloproliferative disorder, which can occur with thrombocytosis. The *JAK2* 46/1 haplotype enrichment is associated with myeloproliferative neoplasm occurrence and with a high risk of splanchnic vein thrombosis in them.[66] The risk of complications is much lower in reactive thrombocytosis, excepting the cases with arterial disease or prolonged immobilisation.[61] A differential diagnosis between reactive thrombocytosis and essential thrombocythaemia can be made using lag time (a parameter useful for thrombin generation studying) and procoagulant phospholipids ratio; high values for these parameters were associated with a high negative predictive value for an essential thrombocythaemia diagnosis.[67]

An important issue present sometimes in patients with high platelet count (including in those splenectomised) is pseudohyperkalaemia. A plasmatic ionogram (not only a serum one) is indicated in such situations in order to make a differential diagnosis between it and a real hyperkalaemia;[68] plasmatic potassium level is normal in these patients. The platelet indices can also be useful. Chronic hepatitis C patients with high liver fibrosis evaluated by transient elastography have higher values of mean platelet volume, platelet distribution width, and platelet large cell ratio compared to those with less expressed liver fibrosis.[69]

## CONCLUSIONS

The most common causes of thrombocytopenia are the lower TPO production and the higher platelet destruction due to increased platelet pooling in the spleen. If thrombocytopenia is severe (>$15x10^3$/$mm^3$) in patients with HCV-related liver cirrhosis, an autoimmune aetiology is very probable. TPO analogues are useful both in patients with primary immune thrombocytopenia and those who will begin the treatment with PEG-IFN and ribavirin or before surgery. An increase of platelet count found in chronic hepatitis C patients can be due not only to splenectomy, ribavirin treatment, and LT, but also to an associated disease; it can rarely be hereditary. An associated myeloproliferative disorder (which frequently evolves with thrombocytosis) can produce hepatic blood flow obstruction. The JAK-STAT signalling mechanism is presented both in patients with essential thrombocythaemia and in those with chronic HCV infection. Ruxolitinib (a *JAK1/JAK2* inhibitor) has beneficial effects in the first disorder and it is a subject of research for the last.

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*6th–8th September 2017*

*Tehran, Iran*

This collaboration between the European Association for the Study of the Liver (EASL) and the Iran Hepatitis Network (IHN) focusses on the theme: 'Best of EASL', with the aim of improving understanding of hepatology in Iran and the surrounding regions. The conference promises to be high in quality, featuring lectures from academics at the forefront of hepatology research from across Europe on topics including viral hepatitis and the management of liver diseases in Iran.

## Acute on Chronic Liver Failure: Is it Ready for Clinical Practice?

*15th–16th September 2017*

*Chicago, Illinois, USA*

This 2-day extravaganza will explore all facets of acute on chronic liver failure in a series of sessions focussing on topics including pathophysiology, predisposing and precipitating factors, treatment and prevention, and transplant versus palliation, as well as a consideration of clinical experience in organ failures throughout the world and individual extra-hepatic organ failures. Reduced 'early bird' prices are available until 17th August.

## British Association for the Study of the Liver (BASL) Annual Meeting 2017

*20th–22nd September 2017*

*Coventry, UK*

The biggest event in the British hepatology calendar, the BASL annual meeting, will this year take place in the heart of the Midlands at the University of Warwick Conference Centre. The conference is designed for anyone with an interest in hepatology, including clinicians, nurses, scientists, and more. Topics of inflammation, malignancy, and transplantation will be covered in poster and abstract presentations.

## 24th International Symposium on Hepatitis C Virus and Related Viruses (HCV 2017)

*25th–28th September 2017*

*Hyannis, Massachusetts, USA*

Attendees at HCV 2017 can expect an exciting programme packed with the latest in hepatitis C virus research as well as advances in related diseases. The congress organisers invite you to join the very top tier of international researchers as well as the rising stars of the field to discuss topics including basic molecular virology, viral-host interactions, pathogenesis, immunology, and vaccine development.

## European Association for the Study of the Liver (EASL) – American Association for the Study of Liver Disease (AASLD) Joint Meeting on Alcoholic Liver Disease and Alcoholic Hepatitis 2017

*30th September–1st October 2017*

*London, UK*

This will be the second joint meeting of EASL and AASLD and will concentrate on the topic of alcoholic liver disease. The event aims to unite experts within the field, from across the two continents, to combine their expertise, research, and energy to advance the understanding of this all-too-common condition and translate the recent advances in understanding into disease definition, meaningful endpoints, and determinants of future clinical studies.

## European Association for the Study of the Liver (EASL) First NAFLD Summit

*9th–11th November 2017*

*Rome, Italy*

Hosted in the historic city of Rome, Italy, the EASL's first summit to focus exclusively on non-alcoholic fatty liver disease (NAFLD) promises to be an extensive examination of this prevalent condition, which affects nearly 40% of the population in Europe and the USA. Organisers hope to see plenty of audience participation in the joint sessions between academics and industry leaders, in order to further enhance the collaboration of these two sectors to fight NAFLD.

## 13th International Conference on Clinical Gastroenterology and Hepatology

*7th–8th December 2017*

*Madrid, Spain*

This collaborative meeting is guaranteed to include plenty of topics sure to interest hepatologists and gastroenterologists from across the board. The buzzing metropolis of Madrid is the perfect backdrop to this event, which features abstracts and presentations centred on advancing therapeutic options and treatment procedures for gastrointestinal disorders. There is also an opportunity for young researchers to network and learn more about this speciality.

## The International Liver Congress™ (ILC) 2018

*11th–15th April 2018*

*Paris, France*

After the huge success of the meeting this year, the ILC returns next April to the City of Love, Paris, France. The 5-day event promises to be the most spectacular one yet, featuring abstracts from the most pre-eminent researchers and lecturers. It will include debates and symposia on the most up-to-the-minute research into all things hepatology-related. Registration opens in October, with reduced ticket prices available until 31st December for those eager early birds.