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Spencer Gore, CEO

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+ ARTICLES

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Front cover and contents photograph: Vienna, Austria, home of ILC 2019. © jakobradlgruber / 123rf.com

EMJ Hepatol. 7.1

Welcome

A warm welcome to all our readers to this year's edition of *EMJ Hepatology*, a journal packed full of cutting-edge advancements and exciting new research. This edition's content includes peer-reviewed articles, abstract presentations, and the highly anticipated coverage of the International Liver Congress (ILC) 2019. Everyone here at EMJ relishes the opportunity to play a part in sharing the advancements made in the hepatology field through our open access platform.

We were lucky enough to attend this year's ILC in the beautiful Austrian capital, Vienna, alongside the 9,000 hepatology experts in attendance. The fantastic range of content on offer demonstrated once again why the ILC is such a popular event for hepatologists. New treatments, shortcomings in the field, and improvements in patient care were among the countless topics presented and discussed. There was a strong focus on collaboration, something that will be key in working on the challenges that the field is facing. For everyone who was unable to attend ILC, we present our informative congress review, which provides all you need to know about the event. We are already looking forward to ILC 2020, taking place in London, UK!

Once again, we were extremely impressed with the range of abstract presentations on offer at the ILC. Some stand-out abstracts are featured in this edition, summarised by the presenters themselves to give our readers a first-hand account of the research. Topics this year included the risk of environmental exposure in the development of autoimmune liver diseases, and progress in the treatment of acute hepatic porphyria and polycystic liver disease. For hepatology experts, our Abstract Reviews section is not to be missed.

The congress review is not all that *EMJ Hepatology 7.1* has to offer; the journal also includes a brilliant collection of peer-reviewed papers on the hot topics this year, including clinical updates in liver fibrosis and primary sclerosing cholangitis. In addition, Somaya Albhaisi's paper "Non-Invasive Imaging Modalities in Nonalcoholic Fatty Liver Disease: Where Do We Stand?" offers a fascinating look at the diagnostic modalities for nonalcoholic fatty liver disease, considering research in the search for an alternative to costly and invasive liver biopsies. These are just a handful of examples from the brilliant range of papers we have feature in this year's issue of *EMJ Hepatology*.

I would like to finish by thanking everyone who contributed to this edition of our hepatology journal. We are thrilled to be able to contribute to a field of medicine that has such passion for improving treatments and care for patients. Here at EMJ, we are always looking for new contributors and submissions, so please do not hesitate to get in touch if you want to work with us to keep expanding the world of hepatology. Here's to another year of industry-leading innovations, ground-breaking research, and open access knowledge sharing!

Spencer Gore

Chief Executive Officer, European Medical Group

Foreword

Dear colleagues,

Allow me to welcome you to this year's edition of *EMJ Hepatology*.

Pathological conditions that share clinical manifestations, symptoms, and diagnostic criteria can be a major hindrance when it comes to selecting the appropriate treatment. The article by Nayagam et al. delves into this topic in detail regarding the cross-over between autoimmune hepatitis and primary sclerosing cholangitis, and in doing so highlight a novel hepatic condition that is under-represented in the literature. Nonalcoholic fatty liver disease (NALFD) is rapidly becoming the predominant cause of advanced stage liver disease in many parts of the world, and with the expected advent of new drugs to treat this condition as it is evolving, timely diagnosis becomes a major issue. Despite the unique benefits that invasive measures have for the diagnosis, imaging, and staging of hepatic conditions, they often have significant setbacks regarding cost, scalability, and morbidity and mortality. Dr Somaya Albhaisi provides an important review of existing and newly emerging non-invasive measures used to image NAFLD, addressing the challenges we must face along the way to providing optimal patient care.

Other valued additions to the journal include a detailed review by Bouchecareilh et al. on alpha-1 antitrypsin deficiency (AATD)-mediated liver disease. AATD-mediated liver disease is suddenly entering the limelight for two reasons: for one, AATD is increasingly recognised as an important co-factor for disease progression in other types of liver disease, and with a heterozygous prevalence of 2% in the general population, this becomes a relevant contributor to progression of ALD or NAFLD. At the same time, novel molecular treatment approaches like siRNA targeting faulty proteins in monogenic disease are entering clinical development and show great promise of success, so these once impossible-to-treat genetic traits become will become treatable diseases before too long.

Furthermore, Dr Ahmed M. Elsharkawy gives a first-hand account of experiences regarding the management and treatment of hepatitis C virus in patients who inject drugs and a study by Hanafy et al. aims to assess potential markers of fibrosis progression in hepatitis C virus-related nephropathy In the second feature, Dr Dhiraj Tripathi provides a critical analysis of the transjugular intrahepatic portosystemic stent-shunt.

Hepatic pathologies are influenced by a broad range of genetic and environmental factors, making them a global burden that needs to be tackled through international collaboration. This collaboration is certainly on display throughout this peer-reviewed selection of papers, and I am sure you will enjoy reading them as much as I did.

Yours sincerely,

Prof Markus Peck-Radosavljevic

Klinikum Klagenfurt am Wörthersee, Austria


ANFAHRT RECHTS

Congress Review

Review of the European Association for the Study of the Liver (EASL) International Liver Congress (ILC) 2019

Location: Reed Messe Wien Congress and Exhibition Centre – Vienna, Austria

Date: 10th–14th April 2019

Citation: EMJ Hepatol. 2019;7[1]:10-25. Congress Review.

Vienna is often referred to as the 'City of Dreams' owing to it being the birthplace of psychoanalyst Sigmund Freud. Much in the way that Freud was a pioneer in the field of psychoanalysis, encouraging discussion across academia that continues to this day, this year's International Liver Congress (ILC) held in this beautiful city garnered a large, international ensemble of hepatology experts, each of whom had inspiring aspirations for the field. Innovation is an essential facet of all medical research, and this was clearly on show across 5 engaging days in the Austrian capital.

Profs Dominique Valla and Tom Hemming Karlsen welcomed the approximately 9,000 delegates from 125 countries, with the former offering some sound advice to the audience: "Seek out knowledge that is missing in your everyday practice and understanding of liver disease, and have the curiosity to go beyond your individual field, as there is much to be learned at the meeting points between different disciplines." This collaborative approach taken towards tackling problems in the hepatology field was emphasised across some of the key talking points broached at the congress, including the need for encouraging positive change in public health and also the partnership with international bodies and patient organisations.

With 2,500 abstracts and 1,500 poster presentations, there was a wealth of information for our team to absorb during our time in Vienna. As always, we have compiled some of the abstracts we were most impressed by and included them in this edition of *EMJ Hepatology 7.1*. Additionally to these sessions, various topics of discussion were appropriately divided into six specialities and presented throughout each day:

1. Liver Tumours
2. Cholestasis and Autoimmune
3. Viral Hepatitis
4. Metabolisms, Alcohol, and Toxicity

5. General Hepatology
6. Cirrhosis and Complications

The steps needed for eliminating hepatitis C virus infection by 2030 were reiterated in one informative session, detailing the strategic approach needed regarding approaches to investment, screening and diagnosis, and prevention of reinfection. A further discussion revolved around how declining healthcare resource utilisation was leading to worse manifestations of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis in the USA and European populations, and how the development of tools to inform patients and help physician decision making could help with the short and long-term management of this life-threatening disease. Several of the sessions followed in this vein: firstly, by acknowledging our shortcomings in the field and the problems facing us, before discussing proactive and implementable solutions that can potentially improve quality of life for countless patients.

There was an impressive variety in the types of session provided at ILC 2019, among which the educational sessions are worthy of particular recognition. There was clearly an emphasis on maximising the dissemination of ideas and information, as attendees were able to make the most of seminars on critical reflections on landmark papers, 'solve the case' sessions, research think tanks, basic science seminars, and meet the expert sessions. Targeted sessions were conducted to address some of the aforementioned key talking points, including those involving nurses and allied health professionals, as well as other public health sessions. Seeing first-hand experts from across the healthcare spectrum come together to discuss collaborative solutions to global problems was inspiring, and we are sure the optimism surrounding such discussions will carry on to ILC in the future.

There were several momentous developments in the field that were brought to our attention at the congress, which we have presented in this congress review for your reading pleasure. Perhaps the most exciting could be the first findings from the clinical trials of two investigational drugs used for the treatment of acute hepatic porphyria and polycystic liver disease associated with autosomal dominant polycystic kidney disease, two rare and poorly managed liver diseases. It is encouraging to see equal attention given to rare conditions such as these and the more common hepatic diseases discussed at this congress, including nonalcoholic fatty liver disease and viral hepatitis: these conditions are a major burden on the healthcare system, and it is to all our betterments to develop effective therapeutic strategies together. Witnessing the innovation of potentially life-changing treatments is always exciting for the scientific and clinical community, making our attendance at this year's ILC all the more worthwhile.

"...have the curiosity to go beyond your individual field, as there is much to be learned at the meeting points between different disciplines."

There was an impressive variety in the types of session provided at the ILC 2019, among which the educational sessions are worthy of particular recognition.

It would appear that ILC 2019 was a resounding success, both for the research and medical communities, and indeed the European Medical Journal! Based on our experiences this year, we are very much looking forward to next year's congress in our backyard of London, where we are certain that the hepatological advancements made this year will have developed even further to improve the overall management and treatment of liver disease. Until then, we hope you enjoy reading our review of this year's congress highlights.

ILC 2019 REVIEWED →

Encouraging Progress in the Treatment of Two Rare Liver Diseases

PROMISING findings have emerged from the clinical trials of two investigational agents regarding two rare and poorly managed liver diseases: acute hepatic porphyria (AHP) and polycystic liver disease (PLD) associated with autosomal dominant polycystic kidney disease (ADPKD). The results were presented in a EASL ILC press release at this year's ILC.

The AHP study (ENVISION) involved the use of an RNA interference agent termed givosiran. Givosiran selectively knocks down the hepatic delta aminolevulinic synthase 1 (ALAS1) enzyme responsible for the accumulation of toxic intermediates characteristic of deficient heme synthesis. Heme is vital for haemoglobin function, and its inhibition can manifest into serious neurovisceral attacks and other chronic, morbid symptoms.

In a cohort of patients experiencing these attacks, givosarin reduced the mean annualised rate of attacks by a significant 74% compared to placebo ($p=6.04x10^{9}$), and 50% of these patients remained attack-free compared to the control (16.3%). Accumulation of the prognostic toxic intermediates was also stifled.

Prof Manisha Balwani, Department of Genetics and Genomic Sciences and Department of Medicine, Icahn School of Medicine at Mount Sinai, New York City, New York, USA, and principal investigator of the study, commented: "Givosiran represents a novel approach to the treatment of this rare liver disease, for which there is a considerable unmet need."

The second study investigated the use of the somatostatin analogue lanreotide for treating ADPKD-associated PLD. This drug targets the characteristic enlargement of the liver caused by the formation and accumulation of cysts. Previous studies had been unable to demonstrate long-term volume-reducing effects for lanreotide.

In the study cohort of 305 ADPKD patients (175 of which had PLD), the lanreotide-treated group exhibited a 1.99% decrease in height-adjusted total liver volume following 120 weeks, compared to a 3.92% increase in the control group. Importantly, this effect was still observed 4 months following the final injection, demonstrating the drug's long-term effect.

Dr René van Aerts, Radboud University Medical Centre, Nijmegen, Netherlands, was enthusiastic about the findings: "This study has provided the robust evidence we needed that lanreotide is associated with sustained reductions in liver growth in patients with PLD due to ADPKD."

Considering the difficulties associated with facilitating clinical trials for rare genetic conditions, the significant findings presented here for both conditions could impact the field in ways that, although too early to ascertain their magnitude, could help improve the quality of life for countless people.

Real-World Studies Demonstrate Glecaprevir/ Pibrentasvir Effectiveness for HCV Treatment

INFECTING an estimated 71 million people worldwide, hepatitis C virus (HCV) remains a serious problem for global healthcare; however, despite this the increased availability of direct-acting antivirals (DAA) has greatly improved the scope for therapeutic intervention. Glecaprevir/ pibrentasvir (G/P) is one such DAA, a fixed-dose pan-genotypic combination tablet. Following its approval in July 2017, the results from two large real-world studies have emerged detailing high rates of sustained virological response (SVR) in HCV patients following G/P treatment. These observations were presented during a EASL ILC press release at this year's ILC in Vienna, Austria.

The first study, carried out at Hannover Medical School, Hannover, Germany, included 1,698 HCV patients from the German Hepatitis C-Registry (DHC-R) who had received G/P treatment. Notable comorbidities that often lead to deferred HCV treatment were present in the cohort, including the receiving of opioid substitution therapy (26%), presence of psychiatric disease (15%), and significant alcohol (6%) and drug (3%) abuse. In the intent-to-treat population, there was a reported 97% SVR rate 12 weeks following treatment cessation (SVR12), and mental and physical component scores were greatly improved in the patients with comorbidities.

Prof Markus Cornberg, who presented the findings, commented: "We found G/P treatment to be safe and highly effective, and to lead to significant improvements in reported physical and mental wellbeing."

An additional study by researchers in the USA studied data from 1,131 patients who had started G/P treatment between August 2017–April 2018. A SVR12 rate of 93% was reported for the G/P group, with additional findings from a sofosbuvir/ velpatasvir treated cohort showing a 90% SVR12 rate. These results appear to support the findings of the Hannover team, and collectively attest to the success of this particular DAA treatment regimen for tackling the problem of global HCV infection.

"We found G/P treatment to be safe and highly effective, and to lead to significant improvements in reported physical and mental wellbeing."

Hepatitis B Virus Treatment Tenofovir Could Lower Risk of Hepatocellular Carcinoma

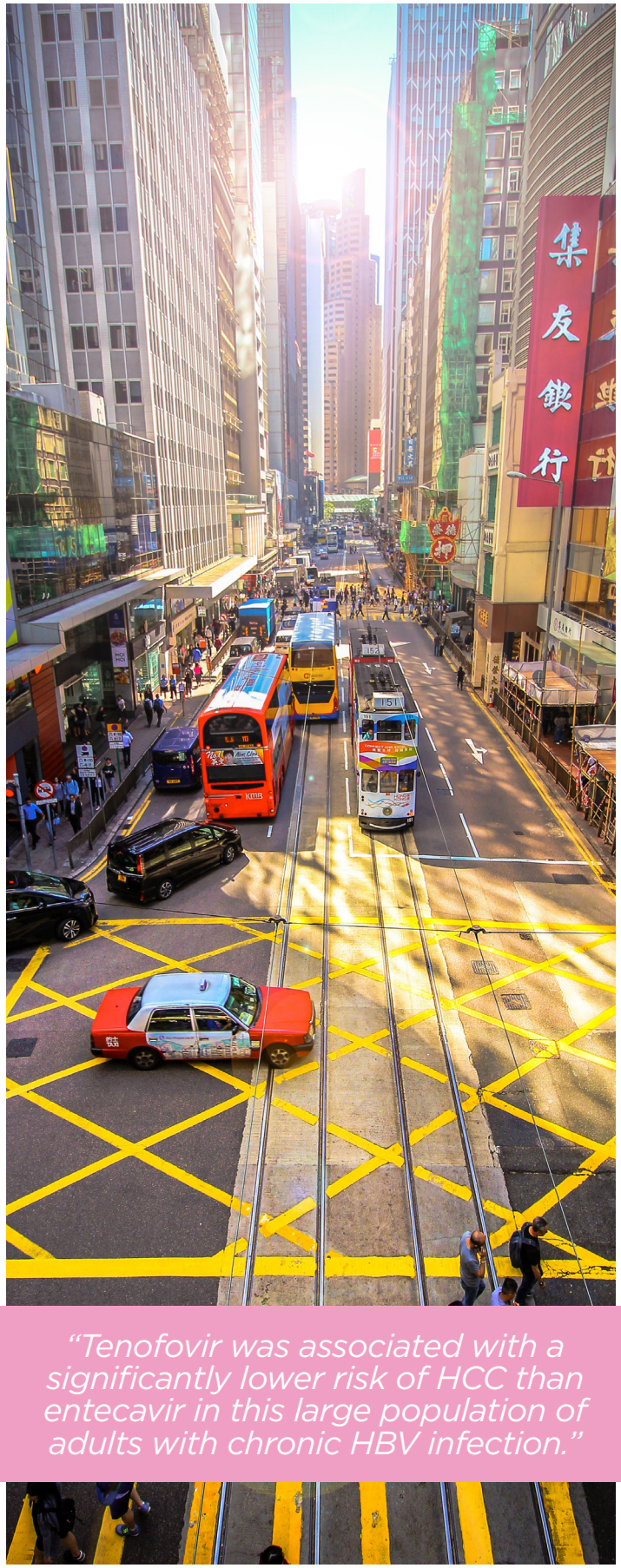
TENOFOVIR could lower the risk of hepatocellular carcinoma (HCC) when used to treat hepatitis B virus (HBV), as found by a study presented at this year's ILC and reported in an EASL ILC press release dated 13th April 2019. The study found that chronic HBV treated with tenofovir (TDF), as opposed to entecavir (ETV), lowers the risk of HCC by at least one third.

Approximately 290 million people worldwide are HBV-infected, which can lead to the development of HCC. TDF and ETV are recommended as first-line treatments for HBV, but current guidelines do not specify a preference between the two antiviral drugs. A previous South Korean study found that HCC risk was lower in patients who received TDF as treatment rather than ETV. Following this, further research was required to explore these findings.

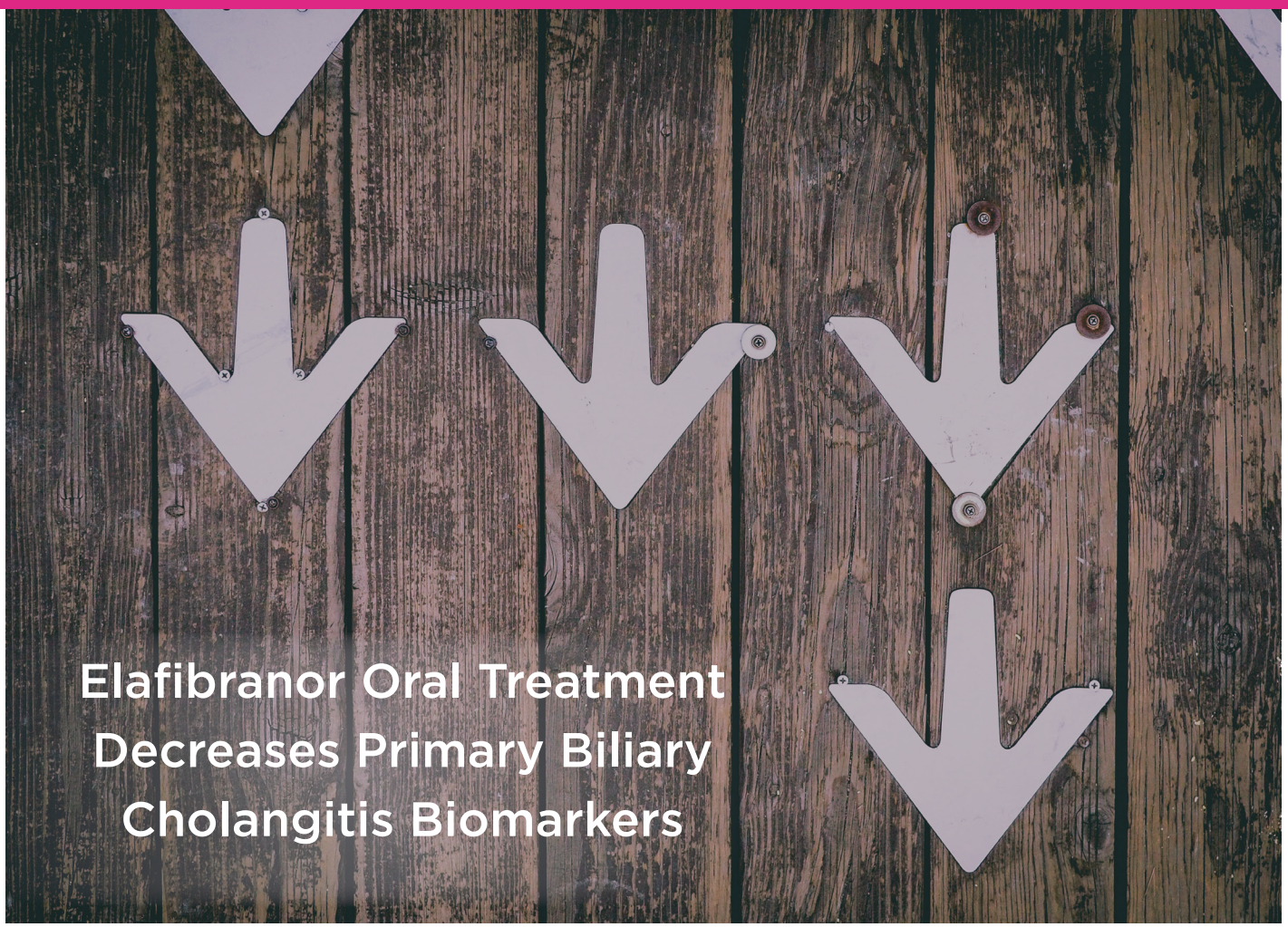
The observational study included 29,123 adults who had received initial treatment of ETV or TDF for chronic HBV for ≥6 months from 2008–2018. The participants were located using public hospital and clinic databases across Hong Kong. The sample had a mean age of 53.7±13.3 years and was 63.5% male. TDF was initially used to treat 1,227 of the patients (4.2%), whereas the remaining 27,896 (95.8%) initially received ETV.

At the study follow-up of 3.3 years (interquartile range of 1.6–5.0 years) 9 TDF-treated patients (0.7%) and 1,468 (5.3%) ETV-treated patients had developed HCC. The 5-year cumulative incidences were, for ETV-treated individuals, 7.5% (95% confidence interval: 7.1–7.9%) and, for TDF-treated individuals, 1.3% (95% confidence interval: 0.6–2.6%).

Dr Terry Yip, The Chinese University of Hong Kong, Hong Kong, China, presented the conclusion: "Tenofovir was associated with a significantly lower risk of HCC than entecavir in this large population of adults with chronic HBV infection." The researchers did recognise the limitations of their study due to its observational nature, but found the results promising as they were consistent with the South Korean research findings.



"Tenofovir was associated with a significantly lower risk of HCC than entecavir in this large population of adults with chronic HBV infection."



Elafibranor Oral Treatment Decreases Primary Biliary Cholangitis Biomarkers

"12 weeks of elafibranor treatment was well tolerated and produced marked improvements in alkaline phosphatase."

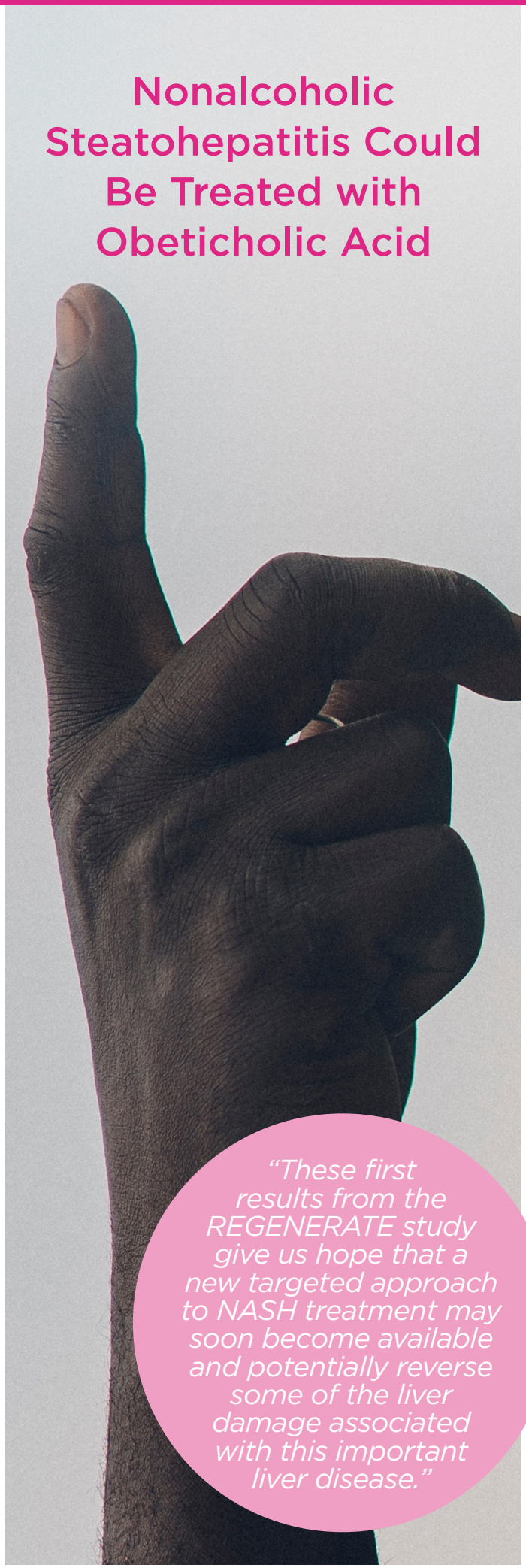
ELAFIBRANOR has significant anticholestatic efficacy in patients with primary biliary cholangitis (PBC), according to researchers who presented their findings at ILC 2019 in Vienna, Austria, as reported in a EASL ILC press release from 13th April 2019. Dr Velimir Luketic, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA, presented the study: "12 weeks of elafibranor treatment was well tolerated and produced marked improvements in alkaline phosphatase (ALP)."

The chronic autoimmune disease PBC causes destruction of the bile ducts, impeding bile flow, which leads to cirrhosis and liver disease. Elevated levels of liver enzymes such as ALP are a biomarker of PBC. There is a need to develop new treatments for PBC as the current options are limited and, when not intolerable, elicit a limited response in the patient.

The researchers studied 45 PBC patients without cirrhosis who had received the current recommended treatment, ursodeoxycholic acid, but had not demonstrated an adequate response. Elafibranor, an oral treatment with an anti-inflammatory effect, was used in this Phase II study. Participants were randomised to a 12-week period in one of three groups: add-on oral elafibranor at 80 mg per day, 120 mg per day, or placebo. At the end of Week 12, patients were reviewed for a change in ALP.

Results showed a significant decrease of ALP in both groups that received an elafibranor dose compared with placebo ($p<0.001$): 48% decrease in the 80 mg group, 41% decrease in the 120 mg group, and a 3% increase in the placebo group. Lipid and inflammatory markers also saw significant improvements, and pruritis was also decreased. Dr Luketic concluded: "These results suggest the treatment has substantial anticholestatic efficacy that we hope will translate into long-term benefits for patients."

Nonalcoholic Steatohepatitis Could Be Treated with Obeticholic Acid



OBETICHOLIC acid is an effective treatment option for nonalcoholic steatohepatitis (NASH) with fibrosis, as seen in a study presented at ILC 2019 and reported in a EASL ILC press release dated 11th April 2019. The Phase III REGENERATE study showed that 25 mg per day of obeticholic acid (OCA) improved fibrosis in nearly 25% of participants.

NASH has a global prevalence estimated to be from 1.50–6.45%, but there are no approved medications specifically for NASH treatment in Europe or the USA. Dr Zobair Younossi, Professor and Chairman of the Department of Medicine, Inova Fairfax Medical Campus, Falls Church, Virginia, USA, presented the study and outlined its necessity: "There is an urgent need for effective treatment regimens for NASH, a common liver disease which can lead to cirrhosis, liver failure, and need for transplant."

The researchers studied 931 individuals diagnosed with NASH and significant or severe fibrosis. These participants were randomised into three groups: 10 mg OCA per day (n=312), 25 mg OCA per day (n=308), and placebo (n=311). The primary endpoints were either improvement of fibrosis with no worsening of NASH or resolution of NASH without deterioration of fibrosis.

Results showed the 25 mg group to have been the most successful: they met the endpoint of improvement of fibrosis without worsening of NASH in 23.1% of participants (p=0.0002 versus placebo). NASH resolution was not achieved, but some symptoms were reduced in this group: hepatocellular ballooning by 35.1% (p=0.0011 versus placebo) and lobular inflammation in 44.2% (p=0.0322 versus placebo).

The most common adverse event, pruritus, was reported by 51% of the 25 mg OCA group, 28% of the 10 mg OCA group, and 19% of the placebo group. The 10 mg and placebo groups saw <1% withdrawal due to pruritus, compared with 9% of the 25 mg group. Aside from this, the results are promising, as Dr Younossi concluded: "These first results from the REGENERATE study give us hope that a new targeted approach to NASH treatment may soon become available and potentially reverse some of the liver damage associated with this important liver disease."

"These first results from the REGENERATE study give us hope that a new targeted approach to NASH treatment may soon become available and potentially reverse some of the liver damage associated with this important liver disease."

The Future of Liver Disease in People with HIV

A GLIMPSE into the potential future of liver disease in people living with HIV was provided by the results of two studies presented at ILC 2019. These studies suggested that nonalcoholic fatty liver disease (NAFLD) could eventually be the most common liver disease affecting people with HIV. Additionally, it was highlighted that there were: "...significant proportions of patients with HIV infection at risk of NAFLD and progressive liver disease," according to Dr Sila Cocciolillo, Royal Victoria Hospital, McGill University Health Centre, Montreal, Canada, an author of one of the studies. The studies were reported in a EASL ILC press release dated 11th April.

These findings are of importance as they suggest that the healthcare system may need to adapt its practices. For instance, Dr Cocciolillo recommended that there should be dedicated monitoring of patients with HIV. In regard to NAFLD becoming an increasingly common cause of liver disease in people with HIV, Prof Philip Newsome, Vice-Secretary, EASL declared: "This reinforces the need to study therapeutic agents in patients with NAFLD and HIV, an area which is seldom examined."

The first study presented at ILC, which was USA-based, saw the examination of the records of >47,000 people with HIV. Of these people, approximately >10,000 had liver disease. Over a 10-year period ending in 2016, there were several headline findings:

- The prevalence rates of viral hepatitis declined from 27.75 to 24.17 per 100,000 people (p=0.009).
- Prevalence rates of NAFLD increased to 11.62 from 5.32 per 100,000 people (p<0.001).
- Mortality rates related to viral hepatitis decreased from 3.78 to 2.58 per 100,000 people (p=0.006).
- Mortality rates related to NAFLD increased from 0.18 to 0.80 per 100,000 people (p=0.041).

The second study presented, which was a multinational collaboration, focussed on two cohorts of adults with HIV who did not have viral hepatitis coinfection and were not heavy drinkers. The researchers used elevated alanine aminotransferase levels and/or significant fibrosis to identify those who were at risk of progressive liver disease. Based on this, they estimated that 25.2% of patients with NAFLD were at risk of progressive liver disease, whereas 18.4% of patients without NAFLD were at risk of progressive liver disease. As already discussed, these two studies in conjunction suggest a potential trend in the future of liver disease for those living with HIV. This suggests the need for healthcare systems to prepare for such a future, and also the need for further studies into this trend.

"This reinforces the need to study therapeutic agents in patients with NAFLD and HIV..."

Could This Novel Molecule Be the Holy Grail for Hepatitis B Virus-Infected Livers?

COVALENTLY closed circular DNA (cccDNA) exhibits a mechanism in hepatitis B virus (HBV) infection that currently inhibits treatment of the infection. However, researchers from Shanghai, China, have discovered a new molecule that can be administered orally that, in their study, removed all traces of cccDNA in human hepatocytes and mouse models. The molecule, named ccc_R08, was evaluated by the researchers in two studies, the first on human hepatocytes and the second on mice that had been infected with circular DNA to mimic the mechanism of HBV infection in humans.

In the first study, the molecule was administered 2 days following infection in the hepatocytes. The researchers noted a significant drop in the levels of cccDNA in the cells, and simultaneously, there was no problematic effect on the mitochondrial DNA and cellular toxicity was not detected.

Following on from this, the researchers looked at the effect of the molecule on mouse models transduced with circular DNA that mimicked HBV infection in humans. Just like in the earlier study, treatment with ccc_R08 led to a drop in serum levels of HBV DNA, pre-genome RNA, hepatitis B surface antigen, and hepatitis B e-antigen which was maintained in the post treatment follow-up period. Following the end of this period, the levels of the circular DNA molecules in the liver sat below the lower limit of quantification in the subgroup that was treated. By comparison, the control group receiving entecavir showed no change on cccDNA levels.

Dr Lu Gao, Roche Innovation Centre, Shanghai, China, commented: "We were encouraged to see that this agent had the potential to reduce pre-existing cccDNA from the liver in this animal model of HBV replication, even to undetectable levels." He added: "We think this type of molecule is well worth exploring further to evaluate its potential to cure chronic HBV infection in humans."

"this agent had the potential to reduce pre-existing cccDNA from the liver in this animal model of HBV replication[...]"

"Oral faecal microbiota capsules are an interesting innovation to modulate the gut microbiota in cirrhosis and could represent a novel treatment strategy to reduce the burden of recurrent hepatic encephalopathy."

Faecal Microbiota Transplant Reduces the Burden for Hepatic Encephalopathy Patients

HEPATIC encephalopathy (HE) is a burdensome neurological syndrome that affects up to 40% of people with cirrhosis. The standard of care for HE is treatment with lactulose, but this, along with the use of other antibiotics, can greatly impact the gut microbiota, disposing patients to additional incidents of HE, cognitive impairment, and systemic inflammation. Now, new research from a randomised, patient-blinded, placebo-controlled study, presented in a EASL ILC press release, has shown the use of oral capsule faecal microbiota transplantation (FMT) to be effective at reducing hospitalisations and dysbiosis, as well as improving cognitive function.

Researchers identified 20 patients with cirrhosis and recurrent HE who were already receiving lactulose plus rifaximin; these patients were then randomised 1:1 to receive either 15 FMT capsules (prepared from the same donor) or placebo. Duodenal/sigmoid biopsies, cognitive function assessment (with the EncephalApp and the psychometric hepatic encephalopathy score [PHES]), and stool analysis were performed both pre-treatment and 2–4 weeks post-treatment. Follow-up was 5 months.

Results were promising for this new treatment: 6 patients in the placebo group died versus one in the treatment group (p=0.05), and the placebo group also experienced more hospitalisations than the treatment group (9 versus 1, respectively; p=0.02). A significant increase in duodenal mucosal microbial diversity was reported in the FMT group after treatment, including an increase in *Ruminococcaceae* and *Bifidobacteriaceae* and a decrease in *Streptococcaceae* and *Veillonellaceae.* Cognitive function also showed a marked improvement versus placebo (p=0.02).

While further studies are needed to verify these findings in a larger cohort, the future is bright for this exciting treatment modality. Discussing the study, Annalisa Berzigotti, University of Berne, Switzerland, and EASL governing board member, concluded: "Oral faecal microbiota capsules are an interesting innovation to modulate the gut microbiota in cirrhosis and could represent a novel treatment strategy to reduce the burden of recurrent hepatic encephalopathy."

Large Numbers of Teenagers and Young People are at Risk of Undiagnosed Nonalcoholic Fatty Liver Disease

"These data highlight the impact of the obesogenic environment, and in particular, its role in the development of NAFLD in a much younger sector of the population."

Nonalcoholic fatty liver disease (NAFLD), the accumulation of lipids in the liver (steatosis) unrelated to alcohol consumption, is the most common form of chronic liver disease among both adults and children with a global prevalence of 20–30%. Large numbers of young adults have features suggestive of NAFLD, and 1 in 40 have already developed fibrosis. These findings were presented by Dr Kushala Abeysekera to attendees of the ILC and reported in a EASL ILC press release dated 12th April 2019.

A team of researchers from Bristol, UK, analysed the ultrasound scans of 4,021 young adults from the Avon Longitudinal Study of Parents and Children (ALSPAC). Prevalence of NAFLD in the cohort was 2.5%. These individuals were revisited as young adults (mean age: 24 years) to assess steatosis and fibrosis using transient elastography.

After excluding those with excessive alcohol consumption, 76 (2.4%) of the 3,128 individuals had some degree of fibrosis and 8 (0.3%) had fibrosis evaluations equivalent to stage 4 (F4) fibrosis. Steatosis (indicative of NAFLD) was identified in 680 (20.8%) individuals, with half of them (331) staged as severe (S3). Furthermore, when liver enzymes (alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase) were analysed, a positive correlation with their increase and the increase of fibrosis (F) ($p \leq 0.002$) and controlled attenuated parameter (CAP) scores ($p<0.001$) was observed, indicative of liver damage. Finally, BMI rose significantly with both F and CAP scores ($p<0.001$ for both).

Prof Phillip Newsome, Vice-Secretary, EASL, said: "These data highlight the impact of the obesogenic environment, and in particular, its role in the development of NAFLD in a much younger sector of the population. This requires swift changes in public policy if we are to defuse the ticking time bomb of obesity and NAFLD."

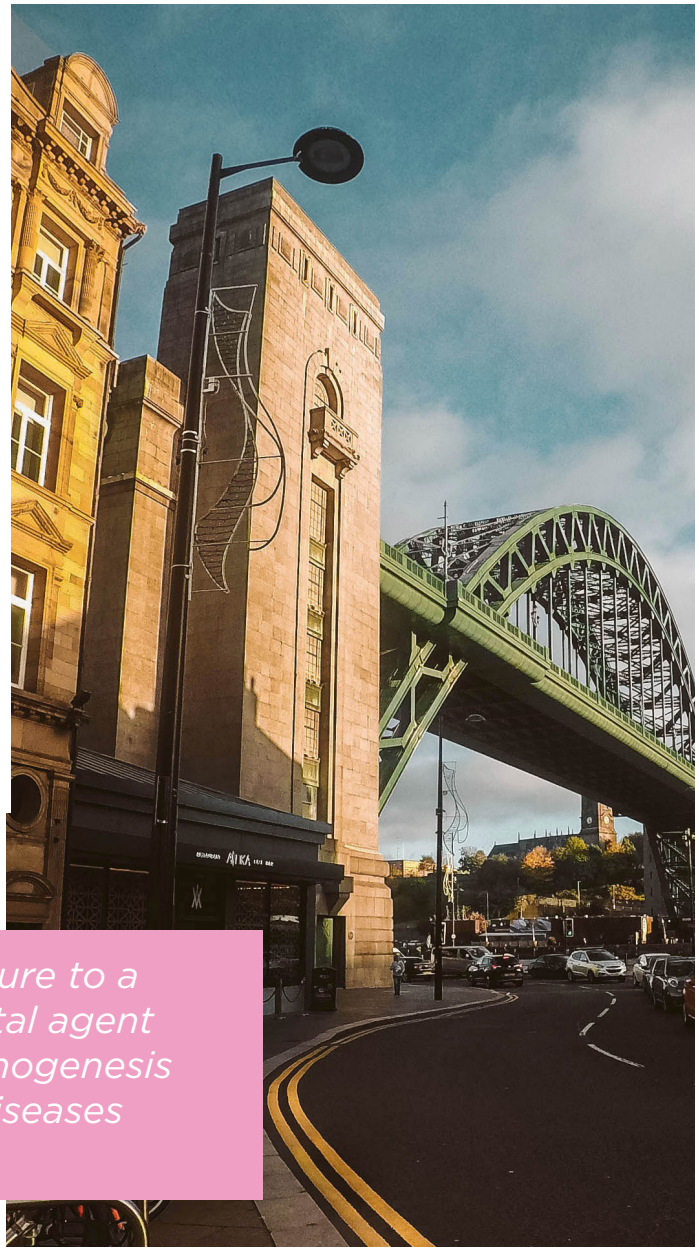
Environmental Exposure and the Risk of Autoimmune Liver Disease Development

ENVIRONMENTAL exposure could be contributing to the development of certain autoimmune liver diseases, as shown by a study presented at the ILC and reported in a EASL ILC press release from 11th April 2019. The study, carried out in northern England, found significant clustered cases of primary biliary cholangitis (PBC), autoimmune hepatitis (AIH), and primary sclerosing cholangitis (PSC), which are relatively rare conditions, pointing to the involvement of an environmental agent.

The study was carried out by a group of researchers from Newcastle, UK and was supported by the National Institute for Health Research Newcastle Biomedical Research Centre. Participants were from the north-east of England and north Cumbria and had one of the three autoimmune liver diseases: PBC (n=2,150), AIH (n=963), and PSC (n=472). Postal addresses were used to carry out spatial point analysis to investigate clustering, along with spatio-temporal analyses.

Higher than expected prevalence of the three autoimmune diseases were found at approximately 1.0–2.0 km. Extra clusters appeared for AIH and PSC at approximately 10 km and for PBC at approximately 7.5 km. Dr Jessica Dyson, Associate Clinical Lecturer at Newcastle University and Consultant Hepatologist, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle, UK, discussed the findings: "This study suggests that exposure to a persistent, low-level environmental agent may have played a role in the pathogenesis of all three autoimmune liver diseases studied, not just PBC."

While the study did identify clusters of these diseases, the environmental trigger is still unknown and further research is needed. Prof Marco Marzioni, Università Politecnica delle Marche, Ancona, Italy, and an EASL Governing Board Member discussed the research: "Triggers are as yet unknown. Environmental factors have been considered, but no solid data have emerged so far. The study presented today has sufficient scientific rigour to reinforce the idea that environmental exposure may play a major role in triggering autoimmune diseases of the liver."



"This study suggests that exposure to a persistent, low-level environmental agent may have played a role in the pathogenesis of all three autoimmune liver diseases studied, not just PBC."

Georgia's Road to Hepatitis C Virus Elimination

On the morning of Saturday 13th April in Vienna, Austria, the EASL International Liver Foundation (EILF) declared the accreditation of the first Centre of Excellence in Viral Hepatitis Elimination in Georgia for the country's leading work in hepatitis C virus (HCV) eradication.

The centre's objectives were clear: "To build and showcase the exemplary efforts of national viral hepatitis programmes, creating a technical assistance hub for other countries, and, in addition, to act as a catalyst for continued excellence and necessary expansion within the country," as Jeffrey Lazarus, Vice Chairman of the Board, EASL International Liver Foundation, explained.

Globally, it is estimated that 71 million individuals live with HCV and, despite having the biomedical tools available to eliminate the disease, nearly 400,000 affected individuals die each year, mostly from cirrhosis and hepatocellular carcinoma.[1] At the World Health Assembly in 2016, 194 countries pledged to eliminate the threat of viral hepatitis by 2030, but only 12 of those countries are currently on track towards this target.[2] New initiatives are clearly needed to achieve this lofty goal and engage the global community.

One of the countries to embrace this project was Georgia. In 2015, 7.7% of the Georgian population were positive for HCV, with 5.4%

needing treatment (around 150,000 people);[2] Georgia recognised the unmet need and embarked on the world's first national HCV elimination programme, which entailed improving the surveillance, prevention, screening, and treatment of the disease.

"Our public health stands on three pillars: quality, access, and cost."

Georgia set itself a 90-95-95 target for 2020: to diagnose 90% of those living with HCV, to treat 95% of those diagnosed, and to cure 95% of those treated.[2] "We are already exceeding targets with our treatment and cure rates hitting 98.2%," said Dr David Sergeenko, Minister for the Ministry of Internally Displaced Persons from the Occupied Territories, Labour, Health, and Social Affairs of Georgia, who was present in Vienna to accept recognition of behalf of Georgia's exemplary centre. To date through the programme, >1.4 million adults have been screened for the virus and >55,000 are currently in treatment or have been treated.[2]

Dr Sergeenko announced: "It is a great pleasure and honour for us to be granted the status of the first EILF Centre of Excellence in Viral Hepatitis Elimination. Our unprecedented HCV Elimination Programme in Georgia is a direct result of a successful public–private partnership, which originated from our close co-operation with the U.S. Centers for Disease Control (CDC) and Prevention and the pharmaceutical company Gilead Sciences."

Having support from a strong network of stakeholders was a main driver behind the ambitious goals set by the country and a key to their success; working with the CDC has provided scientific support, and partnership with Gilead meant Georgia has received donations of direct-acting antiviral HCV medications. In addition to these collaborations, the country's experience in the diagnoses of other diseases, such as HIV, and the government's strong commitment to healthcare has made Georgia an ideal place for launching the programme.

"Our public health stands on three pillars: quality, access, and cost. We are very fortunate that our partnerships handled our quality and cost. Our focus was access: improving access by location, by quality, and by capacity," explained Dr Sergeenko.

Additional findings and improvements in healthcare were experienced as a result of this elimination programme, including safer blood transfusions and improved infection control.[2]

Georgia's commitment to HCV elimination will surely kick-start and enhance other programmes. These centres of excellence are in place to represent distinguished places of thought leadership and act as a hub of knowledge to nurture exchanges of research, training, good practices, and lessons learnt.

"Through universal access to HCV diagnostics and treatment, HCV burden in Georgia is being gradually eliminated. Beyond its immediate public health impact at national level, the programme could serve as a model for other countries by generating valuable data and sharing best practices to support implementation of elimination programs in different parts of the world," said Dr Sergeenko.

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"Through universal access to HCV diagnostics and treatment, HCV burden in Georgia is being gradually eliminated."

Congress Interview

Professor Dina Tiniakos

National and Kapodistrian University of Athens, Greece

We met with *EMJ Hepatology* Editorial Board member, Prof Dina Tiniakos, at the ILC, to discuss her career path and her experience at the congress itself. From the latest research in nonalcoholic liver disease to the battle against gender discrimination in the workplace, this interview contains insights into many key areas for burgeoning hepatologists.

Q1 After finishing your medical degree, you continued with research by doing two PhDs. What made you choose this path?

I did a PhD at the University of Athens in 1993, before doing a PhD at Newcastle University in 1998. I had always wanted to do research and decided that choosing pathology as speciality would give me this opportunity. Pathology lies between basic science and clinical medicine, and therefore gives the possibility to do research on both sides of the spectrum.

Q2 One of your research foci is fatty liver. What about this topic really interests you, and what excites you about this topic looking to the future?

Nonalcoholic fatty liver disease (NAFLD), is the most common chronic liver disease today. Until recently, viral hepatitis was the main focus in hepatology, but now almost 25% of the population (or even as high as 30% in some countries, like the USA) are experiencing the negative effects of fatty liver due to obesity and/or diabetes.

The presence of fat in the liver, simple steatosis, can be diagnosed non-invasively by imaging. However, currently, liver biopsy is required to diagnose nonalcoholic steatohepatitis, the progressive form of NAFLD. Clinical research in NAFLD is focussing on the development of biomarkers to non-invasively diagnose steatohepatitis and fibrosis. Hepatopathologists are working closely with their clinical colleagues in this field for both nonalcoholic and alcohol-related fatty liver disease.

"...almost 25% of the population (or even as high as 30% in some countries, like the USA) are experiencing the negative effects of fatty liver due to obesity and/or diabetes."

"AI is also entering the field of gastroenterology, where machines can be taught, for example, to look for and identify polyps during endoscopic procedures."

Q3 You are chairing a parallel session on NAFLD staging and prognosis on Sunday. Are there any speakers that you are particularly excited to hear from, and what do you think will be gained from their talks? Do you think there will be any challenging topics brought up in discussion?

Most of these oral presentations will focus on non-invasive techniques for staging fibrosis in NAFLD. Liver biopsy is currently the gold standard for evaluating the accuracy of these methods. Combinations of serum markers with clinical data (algorithms) and transient elastography are common non-invasive techniques for assessing liver fibrosis. Non-invasive methods are sensitive and specific for excluding advanced fibrosis and cirrhosis, but they cannot assess intermediate stages of fibrosis, resulting in grey areas in about 15% of the cases. Simple algorithms could be useful in primary practice to identify patients who should be further evaluated by a hepatologist.

Q1 You have published many academic papers on a variety of topics. Is there a particular paper or research project that is particularly memorable and that you are most proud of?

This is a difficult question. It is hard to choose because so much work is devoted to each project. During one of the European Union (EU)-funded research projects that were led by Newcastle University, EPoS (Elucidating Pathways of Steatohepatitis), genetic factors associated with NAFLD development were identified. Through my involvement doing the central histological review of the NAFLD biopsies and ensuring homogenous scoring of morphological features, we have been able to show that the TM6SF2 rs58542926 gene polymorphism influences hepatic fibrosis progression and can identify NAFLD patients who may be more prone to developing advanced fibrosis independent of confounding factors, such as age, BMI, diabetes mellitus, and PNPLA3 genotype. The research work performed at Newcastle University in fatty liver disease and hepatic fibrosis is world-class and my involvement is something that I am very proud of.

Q5 As a pathologist, how directly do you interact with clinicians and other roles in your typical work?

In clinical practice, the pathologist diagnoses the disease and offers predictive and prognostic information affecting treatment decisions. In hepatology, expert liver pathologists provide or confirm diagnosis and its aetiology, highlight possible concurrent liver disease, and score disease activity and fibrosis, among other inputs. Pathologists play important roles in oncology as members of multidisciplinary teams managing patients with cancer by supplying information about tumour histological type/subtype, differentiation, extent of invasion, lymph node involvement, response to treatment, and patient outcome.

Q6 What is your view regarding digital pathology?

This is a very exciting area in pathology, because digitised histopathological slides can be stored on the cloud, where they can be accessed and evaluated from anywhere using a PC, laptop, or even a smartphone, as if using a light microscope. Artificial intelligence (AI) is also entering the field. This technology allows, among other contributions, objective and accurate quantification of liver fibrosis and detailed evaluation of the extent of steatosis, thus giving to the hepatopathologist more time for the intellectual work: the assessment of the pattern of liver injury and interpretation of tissue findings in the clinical context leading to the correct diagnosis. Pathologists may be thinking that AI could replace them in the future, but it is envisaged that this technology will have an auxiliary role and we are happy to facilitate its development and validate its results. AI is also entering the field of gastroenterology, where machines can be taught, for example,

to look for and identify polyps during endoscopic procedures.

Q7 Is there a specific session here at the ILC that you have enjoyed the most, or perhaps a certain technique or advancement that you were most intrigued by?

Actually, the oral free paper session I have just attended was very interesting. Possible mechanisms underlying the decline of cognitive function in primary biliary cholangitis (PBC), described as "brain fog" by the affected patients, were demonstrated in mice and the symptoms were shown to be reduced by using obeticholic acid, a farnesoid X receptor agonist, that could directly regulate the blood–brain barrier during cholestasis. To date, there is no study to clarify how chronic cholestasis, which is the main biochemical abnormality, may affect the brain function of PBC patients.

Q8 You have worked in the UK, the USA, and Greece. Can you talk about how the research environment differs in these countries?

In the USA and UK, there is more organisation and clerical support compared to Greece, but of course there is also the financial factor: there are a lot more resources in the UK or USA due to the fact that Greece is still struggling with nationwide financial problems. There is much less financial support for the health sector today compared to several years previously. Nevertheless, the level of research in Greece is high and Greek researchers are very competitive when applying for EU funding.

Q9 Who has inspired you in the past or continues to inspire you today?

I believe in the value of mentoring throughout the life of a medical doctor, as we look up to and learn from different mentors throughout different stages in our careers. I have had several mentors in my career. Prof Alastair Burt, an expert liver pathologist, was the supervisor of my first research study when I did a student elective in pathology at the University of Glasgow, Glasgow, UK in 1985, and he is now my collaborator in academia. As a visiting fellow at Saint Louis University, St. Louis, Missouri, USA in 1995, I met Prof Elizabeth Brunt, another expert liver pathologist, who has influenced the way I diagnose liver biopsies, and we have co-authored several research and review articles, as well chapters in liver pathology. In Greece, Prof Ioanna Delladetsima provided a role-model for me, not only in liver diagnostics but also as an academic. Finally, my father, George Tiniakos, was also a liver pathologist, and so he was my first mentor. I am the 'apple under the apple tree' in this regard. Not only did I become a medical doctor, but also a pathologist; and not only did I become a pathologist, but also a liver pathologist! This had many positive aspects but was also intriguing and challenging sometimes.

Q10 Finally, what would be your advice to young hepatologists attending the ILC for the first time?

Firstly, due to the high number of parallel scientific sessions, they need to be very well-organised and plan in advance in order to make the most of their time during the congress. Attending basic science sessions will help them understand in-depth the pathophysiology of liver diseases. The physician of the future will be a physician-scientist who will not only know the clinical aspects of human disease but will also understand the basic science and molecular background. In hepatology, learning takes place in the clinics but, during ILC, focussing on basic science sessions gives additional value to attending the meeting.

Of course, making the most out of the ILC involves a combination of session types. There are very interesting clinical sessions every morning, which I know young trainees are keen to attend. Finally, the postgraduate course held in the first 2 days, is always very well attended and a 'must' while at the congress.

It is exciting to see the ILC growing; my first ILC, held in Athens in 1994, was attended by 500 delegates. Now, we were informed that 9,000 hepatologists and 2,500 clinical scientists have registered this year. The growth in attendance has been exponential in the last 25 years.

Procedural Risk of Bleeding: Does the Management of Thrombocytopenia in Chronic Liver Disease Patients Make a Difference? A Debate

This satellite symposium took place on 12th April 2019 as a part of the International Liver Congress™ (ILC) in Vienna, Austria

Chairpeople: Nezam Afdhal,[1] Markus Peck-Radosavljevic[2]

Speakers: Edoardo G. Giannini,[3] Mark Thursz[4]

1. Harvard Medical School and Beth Israel Deaconess Medical Centre, Boston, Massachusetts, USA
2. Klinikum Klagenfurt am Wörthersee, Klagenfurt am Wörthersee, Klagenfurt, Austria
3. Department of Internal Medicine, University of Genoa, Genoa, Italy
4. Imperial College London, London, UK

Disclosure: Prof Afdhal reports consultancy and membership of the respective advisory boards for Shionogi, Merck, Gilead, Echosens, and Ligand. Prof Giannini reports consultancy and membership of the respective advisory boards and is a speaker for Shionogi and GSK. Prof Thursz reports consultancy and speaker fees for Shionogi, Novartis, Altimmune, and Afimmune; he has also received grant funding from the Medical Research Council UK, National Institute for Health Research UK, Novartis, Gilead, GSK, and Norgine. Prof Peck-Radosavljevic is an investigator for Shionogi, AbbVie, ArQule-Daiichi, Bayer, BMS, Boehringer-Ingelheim, ImClone, Lilly, MSD, Novartis, and Roche; he is a speaker and advisor for Shionogi, AbbVie, Bayer, BMS, Boehringer-Ingelheim, Eisai, Ipsen, Lilly, MSD, and Roche. He has also received grant support from AbbVie, ArQule-Daiichi, Bayer, MSD, and Roche.

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Meeting Summary

Prof Nezam Afdhal provided a background to thrombocytopenia (TCP) in chronic liver disease (CLD). He explored the causes of TCP and discussed what are considered acceptable platelet levels. He described the delicate balance between thrombosis risk and bleeding risk that puts CLD patients with TCP at risk of complications, particularly when they require invasive procedures.

Through a series of case studies, the faculty highlighted current management dilemmas and novel approaches to TCP management. Prof Edoardo Giannini presented the case of a patient with hepatocellular carcinoma (HCC) (platelet count of $<50x10^9$/L) who was given a platelet transfusion prior to radiofrequency thermal ablation (RFTA). The patient's increase in platelet count was not clinically significant; therefore, the procedure was cancelled. Prof Giannini noted that radiology guidelines state that for procedures with a moderate risk of bleeding (such as RFTA), platelet transfusion is recommended for counts $<50x10^9$/L.

Prof Mark Thursz presented a case of a nonalcoholic steatohepatitis and refractory ascites, in which the patient had a number of large-volume paracentesis procedures. He then presented paracentesis studies highlighting that bleeding events are often unrelated to patients' platelet levels. Prof Giannini described a study in patients with acute-on-chronic liver failure (AoCLF) who underwent paracentesis and in whom the bleeding rate was 3%.

Following these case presentations, Prof Markus Peck-Radosavljevic discussed the role of thrombopoietin (TPO) in TCP in CLD. He then examined the pivotal trials of various TPO-receptor (TPO-R) agonists which have been studied in CLD patients with TCP undergoing invasive procedures. Clinical studies of the TPO-R agonist lusutrombopag included a large proportion of high-risk bleeding patients and therapy with this agent has been shown to elevate platelet count levels for up to 2 weeks, allowing a window in which to schedule invasive procedures.

Introduction

Professor Nezam Afdhal

Prof Afdhal opened the symposium, which took the format of a debate on the management of TCP in CLD.

TCP in CLD is common. Many factors can lead to the development of TCP in CLD, but there are two primary modalities: portal hypertension with associated hypersplenism, leading to both the sequestration and destruction of platelets, and the decreased levels or activity of TPO. Depending on the aetiology of the liver disease, other co-factors may also be at play; for example, HCC and chemotherapy, autoimmune disease (common in hepatitis C [HCV]), and antiviral therapy can also induce TCP.[1,2]

Decreased platelet production is usually due to low TPO levels, which results in reduced bone marrow production of platelets. Various clinical factors can impact this; alcohol, for example, is a well-known suppressant of platelet production.[3]

Chronic TCP is usually related to a slow decline in platelet production (i.e., the sequestration and destruction that occurs with progressive fibrotic cirrhotic liver disease). Platelet levels decrease as the liver progresses to cirrhosis. Similar changes over time can be seen in TPO levels.[4,5]

Coagulation is one of the most important functions of the liver. Many of the proteins and co-factors necessary for adequate haemostasis may be decreased in cirrhosis, including decreased production of procoagulants, coagulation factors, fibrinogen, and platelets, as well as increased levels of von Willebrand factor. Some diseases are associated with alterations in anticoagulants, such as proteins C and S and antithrombin 3, resulting in an increased risk of thrombosis.[1,6,7]

Consequently, in liver disease the balance between risk of bleeding and risk of thrombosis is disturbed.[6,8]

Impact of Thrombocytopenia on Bleeding Risk

The HALT-C trial in HCV was a 5-year study that examined the ability of low-dose interferon to prevent the progression of cirrhosis. Multiple repeated liver biopsies were performed on patients and bleeding risk was 0.6%. This percentage increased to 5.3% in patients with a platelet count of $\leq 60 \times 10^9$/L,[9] reaching levels that begin to define TCP.

A platelet count of ~60×10^9/L platelets will maintain thrombin generation at the 90th percentile of normal in patients with cirrhosis. Below ~60×10^9/L, thrombin generation is impaired (Figure 1).[10]

In conclusion, the balance between bleeding and clotting is extremely important in treating patients with cirrhosis. The causes can be multifactorial and must be evaluated by the clinician.

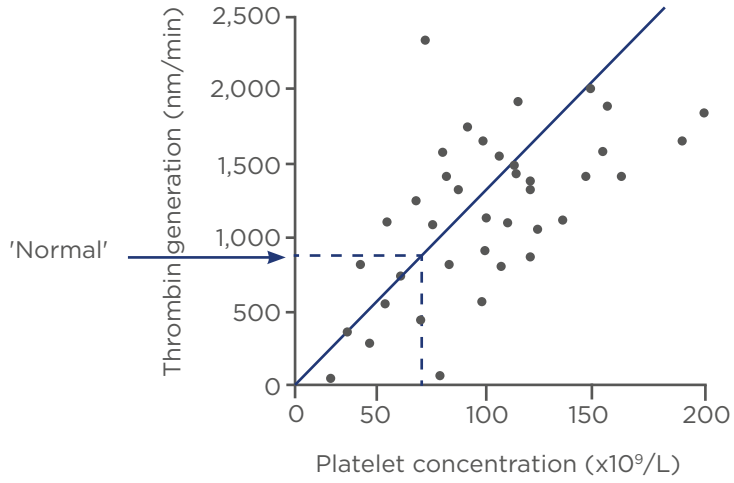

Figure 1: Platelet count of ~60x10⁹/L maintains thrombin generation at the 90th percentile of normal.

Adapted from Tripodi et al., 2006[10]

Debating the Management of a Chronic Liver Disease Patient with Thrombocytopenia Undergoing a Procedure: Case 1

Professor Edoardo Giannini

Prof Giannini presented the case of a 69-year-old male with a history of diabetes and arterial hypertension. In 2002, he was diagnosed with HCV and, following a liver biopsy, he was diagnosed with advanced fibrosis. Treatment with pegylated interferon was ineffective. In 2015, he presented with ascites and was treated with spironolactone. Endoscopy revealed small oesophageal varices. The patient was Child–Pugh Class B. He was treated with sofosbuvir/daclatasvir/ribavirin for 24 weeks and had a sustained virological response.

During follow-up, diuretics were withdrawn. In 2018, a 2.1 cm liver focal lesion (S5) was identified. A MRI scan diagnosed HCC. The patient's liver function was preserved but he had slightly altered renal function, mainly due to hypertension and diabetes. His alpha-fetoprotein was slightly altered (14 ng/mL) and his international normalised ratio (INR) was slightly prolonged (1.35). His platelet count did not improve after antiviral therapy (41x10⁹/L). His model for end-stage liver disease (MELD) score was 14 and he was Child–Pugh Class A. The multidisciplinary team discussed his case and decided to proceed with RFTA.

Prof Giannini referred to a study in which 4,133 RFTA procedures were performed in patients (mainly diagnosed with HCC) with a platelet count >50x10⁹/L. In this study, 1.5% of patients experienced bleeding and TCP was deemed to be a bleeding risk factor.[11] Another study in HCC patients where 1,843 RFTA HCC procedures took place highlighted that 10 platelet packs were transfused in patients with a platelet count <50x10⁹/L. The proportion of patients who bled in this study was 0.5%.[12]

Prof Giannini discussed the prevailing expert opinion in pre-procedure prophylaxis. Opinions include that platelet counts "below <50x10⁹/L may be associated with a higher risk of bleeding" and "thrombopoietin agonists may have a role in pre-planned procedural prophylaxis."[13] In a Spanish survey, 88.8% of healthcare professionals stated they would correct haemostatic abnormalities, based on platelet counts, if there was a moderate (3–10%) risk of bleeding. In total, 77.3% of responders thought that 26–50x10⁹/L was the appropriate platelet count range in which to take action to decrease the risk of bleeding.[14] He noted that, in an Italian study, platelet count did not increase in a clinically significant manner following platelet transfusion.[15]

Returning to the clinical case, Prof Giannini said that there was a modest increase in platelet

count following platelet transfusion, but levels did not increase sufficiently to allow RFTA.

Prof Giannini summarised by stating that RFTA in HCC carries a moderate risk of bleeding. Severe TCP may be associated with an increased bleeding risk and can result in delayed or cancelled procedures. Prophylactic platelet transfusions are commonly used, although they are controversial, and a threshold of >50x10^9/L pre-procedure is generally accepted.

Case 1 (Rebuttal)

Professor Mark Thursz

Prof Thursz began his case rebuttal by reviewing current guidelines on platelet levels and prophylactic approaches to managing bleeding risk. He noted that no guidelines exist for RFTA. For liver biopsy, the American Association for the Study of Liver Diseases (AASLD) states that platelet transfusion should be considered when levels are <50–60x10^9/L, the British Society of Haematology (BSH) says the range is <50–60x10^9/L, and the British Society of Gastroenterology (BSG) states that biopsy can be performed safely if platelet levels are >60,000/mm^3.[16,17]

A case series was presented in which, counterintuitively, the rates of post-procedural bleeding were higher in patients with platelet counts of >50x10^9/L compared to patients with ≤50x10^9/L.[18]

Prof Thursz noted that platelet level may not be the only parameter to consider, as platelet function may also be impaired in liver cirrhosis.[19] Clinicians have to weigh up procedural risk (by analysing bleeding time), consider platelet level and function, and assess potential response to platelet transfusion versus the risk.

Case 1 (Response)

Professor Edoardo Giannini

Prof Giannini pointed out that in the case series Prof Thursz presented, the 13% of patients with severe TCP were diluted into the series and this series also included patients who had a very low risk of bleeding. While he agreed that platelet function is an important consideration, he stated that it is not possible to assess it in a meaningful way.

The Society of Interventional Radiology (SIR) guidelines state that, in procedures with a moderate risk of bleeding (such as RFTA), platelet transfusion is recommended for counts <50x10^9/L. Prof Giannini noted that the risks and limitations of platelet transfusions include refractoriness, high cost, limited availability, risk of transmission of infection, limited efficacy, and transfusion-associated lung injury.

Debating the Management of a Chronic Liver Disease Patient with Thrombocytopenia Undergoing a Procedure: Case 2

Professor Mark Thursz

Prof Thursz presented the case of a 74-year-old male who presented in 2016 with abdominal swelling, a past history of poorly controlled diabetes, and a metallic aortic valve replacement. The patient had hypertension and hyperlipidaemia and was taking warfarin, metformin, candesartan, and atorvastatin.

The patient had features of CLD and features of liver failure (gross ascites and ankle oedema). He was anaemic, his platelet levels were 121x10^9/L, and his INR was raised, possibly due to warfarin.

The patient was screened for hepatitis. His ultrasound (US) and CT scans both showed gross ascites, an irregular liver edge, cirrhosis, abdominal varices, an enlarged portal vein, an enlarged spleen, and no focal lesions.

He had decompensated cirrhosis (MELD score of 18; Child–Pugh score of 8 [Class B]) and probable nonalcoholic steatohepatitis as the underlying diagnosis. The patient underwent a successful large volume paracentesis and was discharged on diuretic therapy.

A few months later he was re-admitted with encephalopathy, hyponatraemia, and diuretic-resistant ascites. His haemoglobin and platelet

levels (79x10^9/L) had dropped. Renal function had deteriorated slightly. There was no evidence of bacterial peritonitis.

The patient met the European Association for the Study of the Liver (EASL) diagnostic criteria for refractory ascites.[20] The patient underwent a large volume paracentesis, warfarin was reversed, and he received fresh frozen plasma (FFP). The patient deteriorated quickly after the procedure, requiring surgical repair of lacerated-wall varices, and diuretics were discontinued. The patient was discharged and another large volume paracentesis was planned. Three months later, he was re-admitted with tense ascites, an INR of 2.5, and a platelet count of 35x10^9/L. Six weeks later, the patient developed fatal portal vein thrombosis.

Prof Thursz presented data from a number of studies in CLD patients requiring paracentesis, one of which examined the haemorrhage risk of US-guided paracentesis (3,116 procedures).[21] Haemorrhage occurred in 6 (0.19%) procedures and was not related to INR or platelet count. Using this study as an example, Prof Thursz noted that not correcting coagulopathy could have saved 1,125 units of FFP and 366 units of platelets at a cost of $816,000. He noted that thromboelastography-guided transfusion made no difference to the risk of bleeding in patients receiving invasive procedures.[22] In 4,729 patients who underwent paracentesis, 9 (0.19%) had a haemorrhage after the procedure. Most of these patients had reasonable platelet levels and INR.[23]

Prof Thursz therefore concluded that paracentesis is a low-risk procedure in which the main cause of bleeding is typically procedural trauma.

Case 2 (Rebuttal)

Professor Edoardo Giannini

Prof Giannini felt that Case 2 was an example of a patient in which a pendulum was swinging between thrombosis and bleeding (Figure 2). Factors such as infection, alcohol use, or other external factors that had not been identified could tip the balance either way.

He described a study that analysed the bleeding rate and risk factors following paracentesis in patients with AoCLF; bleeding rate was 3%, over 10-times higher than that described in the study Prof Thursz presented. In this study, the haemostatic parameters seemed to play a role.[25]

Prof Giannini also described a recent study in which 60% of AoCLF patients at admission had a hypocoaguable profile compared to 30% of acute decompensated patients. In a secondary analysis of data, patients with a hypocoaguable profile were more frequently bleeding at admission, and more often had to receive transfusion of red blood cells, FFP, and platelets; these are factors that appear to be associated with a decreased survival of patients and an increase in mortality.[26] Prof Giannini added that unstable patients may tend towards a hypocoaguable state, meaning their bleeding tendency may be increased.

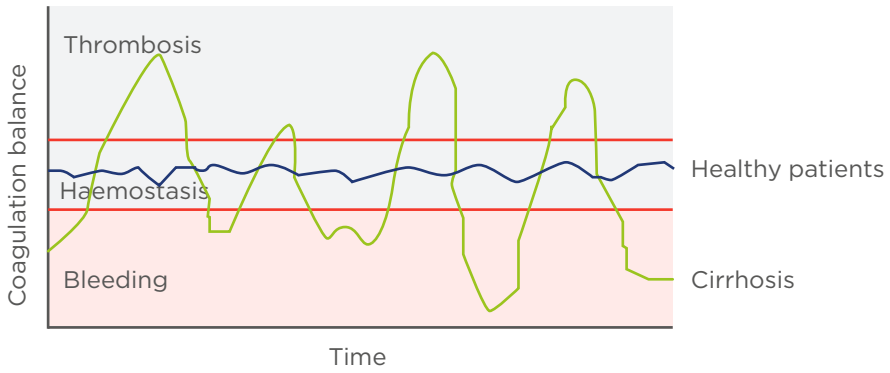

Figure 2: The 'ups and downs' of coagulation in a patient with cirrhosis.

Adapted from Tapper EB et al., 2013[24]

Case 2 (Response)

Professor Mark Thursz

Prof Thursz responded that the data set he cited related to several thousand patients, while Prof Giannini only referred to a study in several hundred patients. He restated that in paracentesis, the bleeding risk is very low and is not associated with the platelet count, but with the procedure. He noted that platelet transfusions are difficult to arrange when patients present as emergencies. He also believed that the safety concerns of platelet transfusions are underestimated.

Emerging Thrombopoietin-Receptor Agonists for the Management of Thrombocytopenia in Chronic Liver Disease Patients Undergoing a Procedure

Professor Markus Peck-Radosavljevic

TPO is the predominant endogenous thrombopoietic growth factor and is produced in the liver. While several cytokines are involved in thrombopoiesis, TPO plays a role across the platelet production pathway and is the most crucial and specific growth factor for platelet production. Reduced TPO production is a major factor in TCP in CLD and cirrhosis patients.[27]

Small molecule TPO-R agonists are capable of binding to the TPO receptors which activate the downstream signalling cascade to stimulate platelet production.[28] The first TPO-R agonist to be studied in liver disease was eltrombopag. In a study of 292 patients with cirrhosis with platelet counts of <50x10^9/L, treatment with eltrombopag increased platelet count; however, due to an excess of portal vein thrombosis in the treatment group versus the control group, the study was terminated early, and this drug is not used in the management of TCP in CLD.[29]

Another TPO-R agonist, avatrombopag has been evaluated in two Phase III trials (ADAPT-1 and ADAPT-2)[30] in patients with cirrhosis undergoing invasive procedures. The studies assessed two different doses: one for very severe TCP patients (platelet levels <40x10^9/L) and one for patients with 40–50x10^9 thrombocytes/L. Treatment was given for 5 days and the procedure was performed at Day 10. Both studies included a high number of patients undergoing low-risk bleeding procedures, such as endoscopy (52%). Some 12.7% of patients underwent moderate-risk procedures (e.g., chemoembolisation for HCC), and 9.6% and 7.8% underwent high-risk dental procedures and RFTA, respectively. Both trials had positive outcomes.

There have also been two Phase III studies of lusutrombopag: L-PLUS 1 and 2. Both trials had similar designs and patients' baseline platelet count was <50x10^9/L. Treatment was given for up to 7 days, depending on platelet count level at Day 5. The trials included additional safety checks: the portal vein was analysed via US or CT scan before and after the drug was given. No other TPO-R agonist drug trials have this much detailed information about non-clinically apparent portal vein thrombosis. Platelet transfusions were administered in patients who did not reach a platelet count of <50x10^9/L.[31,32]

In L-PLUS 1, a high proportion of patients underwent procedures that had a significant risk of bleeding, such as RFTA/microwave coagulation therapy and transarterial chemoembolisation. The L-PLUS 2 study had a high proportion of procedures, such as endoscopy and dental extraction. Overall, the risk of bleeding in the lusutrombopag trials was higher than in other trials. In L-PLUS 1, the primary endpoint (proportion of patients not requiring platelet transfusion prior to invasive procedure) was achieved by 79.2% of lusutrombopag patients compared to 12.5% of placebo patients. In L-PLUS 2, the primary endpoint (proportion of patients not requiring transfusion prior to invasive procedure, and no rescue therapy for bleeding, from randomisation through 7 days after invasive procedure) was achieved by 64.8% of lusutrombopag patients compared to 29% of placebo patients (Figure 3).[31,32]

Lusutrombopag therapy resulted in up to 2 weeks of elevated platelet counts, providing an opportunity for repeat procedures. There were almost twice as many bleeding events

in the placebo group (27.1%) compared to the lusutrombopag group (14.6%). In both studies, there was no difference in thrombotic events between lusutrombopag (3 events) and placebo (3 events).[31,32]

Discussion

During the discussion, the faculty answered questions from the audience, considering clinical circumstances that can affect a patient's coagulation status and bleeding risk. For example, Prof Giannini noted that renal dysfunction and infection in AoCLF may tip the balance in favour of a hypocoagulable state and make patients more prone to bleeding. Prof Afdhal explained that elevated creatinine can be associated with a higher risk of bleeding in patients undergoing paracentesis.

Prof Peck-Radosavljevic described how he would manage a patient with Budd–Chiari syndrome who has underlying thrombophilia. He would begin with anticoagulation therapy to improve portal hypertension. He said paracentesis would then often not be required. A transjugular intrahepatic portosystemic shunt could then be inserted; this will usually remove the issue of repeat paracentesis. He would not give a platelet transfusion or an agent that stimulates platelet production in a case with a distinct procoagulatory state.

In terms of the impact of drug therapies in bleeding risks, Prof Thursz suggested that newer anticoagulant drugs can have bleeding risks similar to that of warfarin. He said that monitoring thrombin generation in patients with very advanced liver disease may not be helpful.

Prof Afdhal highlighted the importance of the duration of the effect achieved by platelet transfusion therapy. Cirrhotic patients who undergo polypectomy have a high risk of secondary bleeding. Prof Peck-Radosavljevic explained that secondary bleeding can usually happen up to Day 7, which means this time period is covered with a TPO-R agonist but would never be covered by a platelet transfusion.

Prof Thursz thought that in pre-planned procedures, TPO-R agonists should be used in RFTA, in procedures done by endoscopists, such as a polypectomy, and dental procedures.

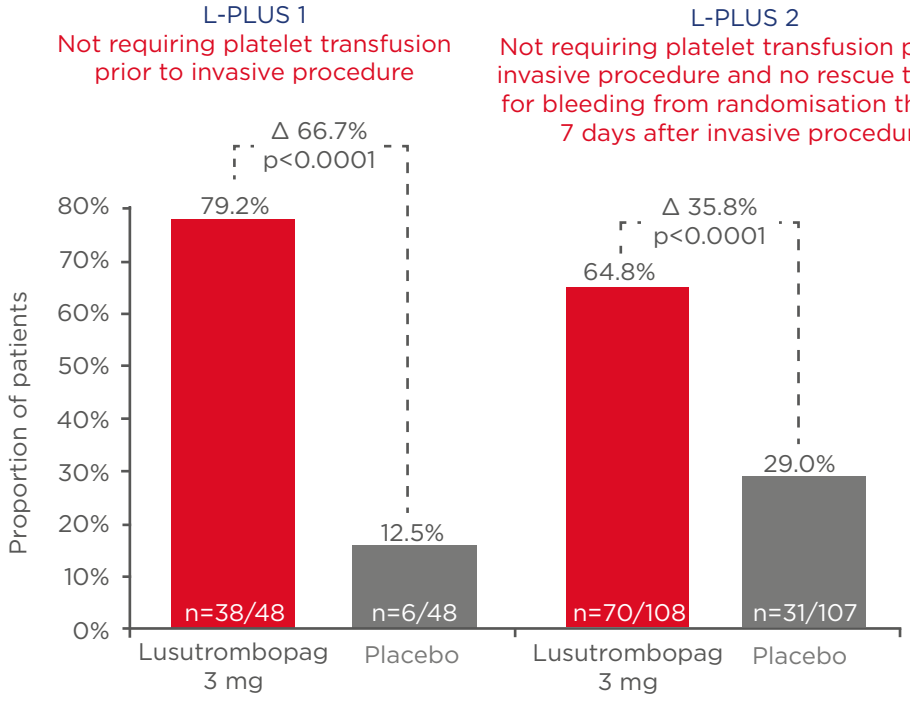

Figure 3: Primary endpoints in the L-PLUS 1 and L-PLUS 2 trials.

Adapted from Hidaka et al., 2018[31] and Peck-Radosavljevic et al., 2019[32]

He noted that dental sepsis can be an issue for patients awaiting liver transplant. Dental extractions have a high risk of bleeding because of the infection around the root of the tooth. Prof Afdhal stressed that the ability to increase platelet levels without a transfusion makes the process for dental extraction much more straightforward. Prof Giannini thought that closed procedures, in which no practical haemostasis can be performed, are where TPO-R agonists can be used most effectively. He also thought that dental procedures for patients on the liver transplant list, where several teeth need to be extracted, benefit from the long duration effect of TPO-R agonists. He also agreed that TPO-R agonists allow repeated procedures to be scheduled, without the need for repeated platelet transfusions.

Closing Remarks

Prof Peck-Radosavljevic concluded that the short duration of an increase, if any at all, in platelet count with platelet transfusions is concerning. With the new TPO-R agonist agents, platelet counts are reliably increased and these agents are extremely useful as the procedure can be pre-planned. Platelet transfusions are still commonly performed, without considering the superiority of the TPO-R agonist alternatives. Increased awareness of the benefits of TPO-R agonist therapy will increase patient safety during elective invasive procedures.

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Abstract Reviews

Written by the presenters themselves, *EMJ Hepatology's* Abstract Reviews section brings you all the action from the ILC poster presentations

The Role of Adipose Tissue in Metabolic and Cardiovascular Complications After Liver Transplantation

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Keywords: Adipose tissue, cardiovascular, complications, liver, metabolic, obesity, transplantation.

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BACKGROUND AND AIMS

Metabolic and cardiovascular complications are an important burden in patients after liver transplantation (LT) for many reasons, including as a result of immunosuppressive therapy.[1-3] Adipose mass is an independent predictor of morbidity in the general population and in other diseases,[4,5] but little is known in the transplant setting.[6] Therefore, the aim of this study was to evaluate the role of adipose mass at the time of LT, in regard to the outcomes of morbidity and mortality after LT.

METHODS

We enrolled 173 patients who received LT for end-stage liver disease between 2000 and 2015 and were subsequently monitored in our outpatient clinic with periodical controls. Patients with a minimum follow-up time of 3 years after LT were included. Nutritional assessment before LT was derived by the analysis of an available CT scan (L3-L4 slice), performed within a year before LT. In all patients, the authors evaluated the area (cm^2) of visceral, subcutaneous, and intramuscular adipose tissue; all measurements were indexed by patients' height (cm^2/m^2) to obtain subcutaneous adipose tissue (SAT),

visceral adipose tissue (VAT), and intramuscular adipose tissue (IAT) levels. Total fat area (TAT) was derived from the formula: TAT=SAT+VAT+IAT.

RESULTS

The most common aetiology of liver disease was viral hepatitis (43%), followed by alcohol abuse (16%) and nonalcoholic steatohepatitis (13%). At the time of LT, 42% of patients were obese or overweight according to BMI (corrected for ascites), 10% had experienced cardiovascular diseases before LT (myocardial infarction, cerebral stroke, or haemodynamically significant stenosis in a major arterial vessel), 26% had a diagnosis of diabetes, 17% had arterial hypertension, and 20% had dyslipidaemia.

During the observation period after LT (120±50 months), patients who experienced cardiovascular complications significantly increased (from 10% to 39%; p=0.03) and more patients developed diabetes (from 26% to 45%; p=0.02) and arterial hypertension (from 17% to 51%; p<0.01).

TAT, IAT, and SAT, detected by CT scan before transplantation, were significantly associated with cardiovascular complications after LT (p<0.01), and were also selected by a multivariate analysis, including diabetes, arterial hypertension, and dyslipidaemia. VAT and BMI before transplantation failed to correlate with cardiovascular complications after LT.

CONCLUSION

Cardiovascular complications are increased in patients after LT. Adipose tissue, quantified by CT scan before LT, is an independent predictor of cardiovascular complications. Pre-transplant estimate of adipose tissue could be useful in identifying patients at cardiovascular risk and in performing preventive treatments. BMI, although corrected for ascites, is likely to be a less specific estimate for adipose tissue amount.

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Efficacy of Oral Thrombopoietin Receptor Agonist Lusutrombopag in Chronic Liver Disease by Underlying Disease Aetiology

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Keywords: Chronic liver disease, thrombocytopenia, thrombopoietin.

Citation: EMJ Hepatol. 2019;7[1]:40-42. Abstract Review No. AR2.

BACKGROUND AND AIMS

Among patients with chronic liver disease (CLD), thrombocytopenia (TCP) is a frequent complication.[1,2] Before invasive procedures and surgery in CLD patients with TCP, platelet transfusions (PT) have been used to increase platelet counts, but their use is limited by several factors, including the short lifespan of transfused platelets, alloimmunisation, and various haemolytic, allergic, and other secondary reactions, which can lead to hospitalisations.[3]

Lusutrombopag is an oral thrombopoietin receptor agonist that has been approved in Japan (2015) and the USA (2018) for treatment of TCP, and in Europe for severe TCP (2019), and is associated with CLD in patients undergoing a planned invasive procedure.[4-6] In L-PLUS 1 (Japan) and L-PLUS 2 (global), two similarly designed, Phase III, multicentre, randomised, double-blind, placebo-controlled studies, patients with CLD and platelet count <50x10^9/L scheduled for an invasive procedure were randomised 1:1 to lusutrombopag 3 mg or placebo and dosed orally once daily for up to 7 days. Results showed that a higher proportion of lusutrombopag-treated than placebo-treated patients did not require a PT.[3,7]

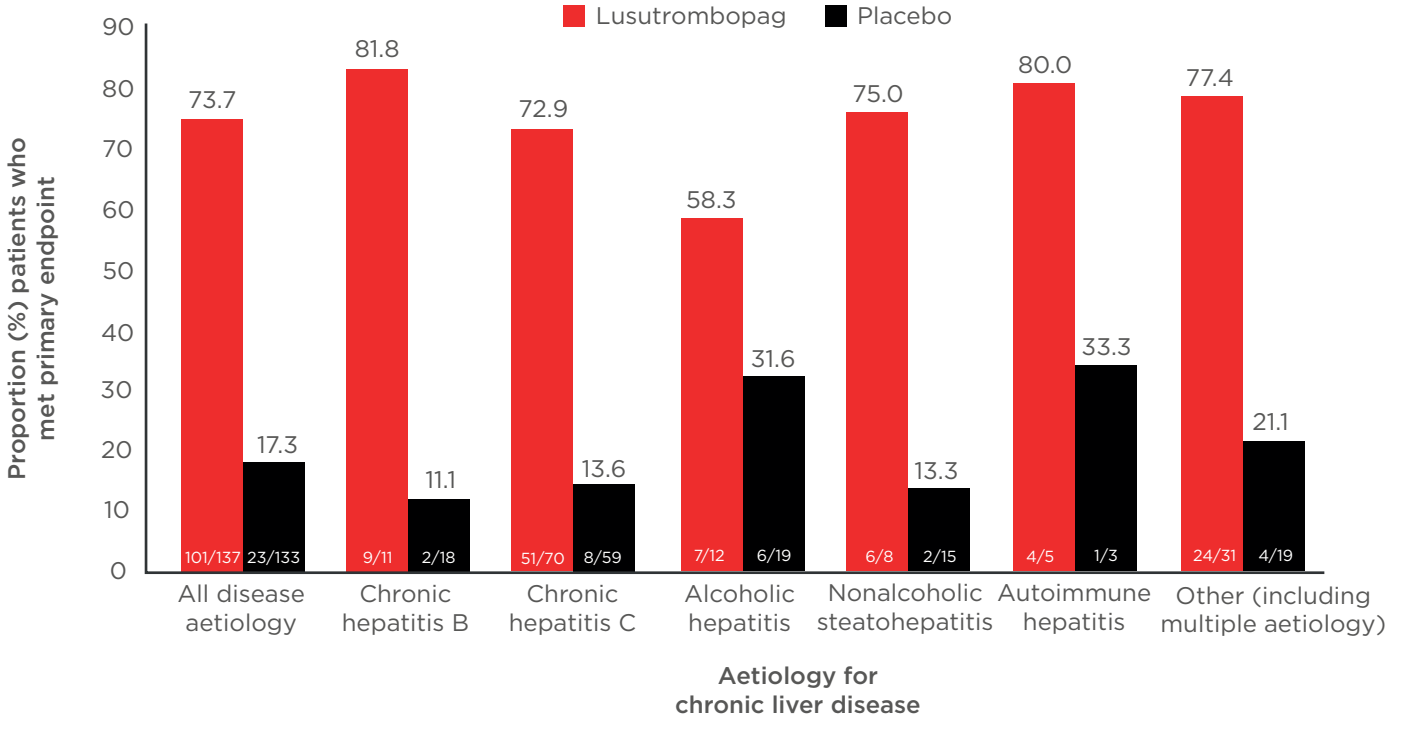

Figure 1: Proportion of patients who met primary endpoint by underlying aetiology for chronic liver disease.

Certain CLD aetiologies, such as alcohol abuse and hepatitis C virus, may cause bone marrow suppression and low thrombopoietin production.[8] Accordingly, the authors undertook the pooled analysis described herein to evaluate the efficacy of lusutrombopag in patients with CLD by underlying disease aetiology.

METHODS

For the current analysis, data from the L-PLUS 1 and L-PLUS 2 per-protocol (PP) patient populations (defined as all randomised patients with no major protocol violations) were pooled and assessed by various underlying CLD disease aetiologies. The primary efficacy endpoint was the proportion of patients who required no PT prior to the invasive procedure and no rescue therapy for bleeding from randomisation throughout 7 days after the procedure. Treatment-emergent adverse events (TEAE) were also assessed by disease aetiology subgroup.

RESULTS

Of the 312 patients randomised, 270 were in the PP population (lusutrombopag: n=137; placebo: n=133). Underlying CLD aetiologies were present in the PP population in the following proportions: chronic hepatitis B: 10.7% (29/270); chronic hepatitis C: 47.8% (129/270); alcoholic hepatitis: 11.5% (31/270); nonalcoholic steatohepatitis: 8.5% (23/270); autoimmune hepatitis: 3.0% (8/270); and other (including multiple aetiology): 18.5% (50/270). The underlying aetiologies for CLD were generally similar between the two treatment arms. Overall, 73.7% (101/137) of lusutrombopag patients met the primary endpoint versus 17.3% (23/133) placebo patients (difference of proportion: 55.8 [95% confidence interval: 46.6–65.0]; p<0.0001). Similarly, in each disease aetiology subgroup, more patients met the primary endpoint in the lusutrombopag versus placebo arm (Figure 1). Proportion of patients experiencing ≥1 TEAE were 61.9% for lusutrombopag and 64.5% for placebo; 6.5% and 9.0% of events, respectively, were deemed to be treatment-related. TEAE were generally similar between the two treatment arms across the underlying aetiologies. Thrombosis and thromboembolism-related TEAE occurred in 1.9% (3/155) of patients in the lusutrombopag arm (all deemed serious adverse events) and in 1.9% (3/155) of patients in the placebo arm (all deemed non-serious).

CONCLUSION

Regardless of underlying disease aetiology, lusutrombopag was found to be efficacious compared to placebo in avoiding the need for PT in patients with CLD-TCP scheduled to undergo invasive procedures. Furthermore, TEAE were generally similar between the treatment arms across disease aetiologies.

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A Comparison of Currently Available Direct-Acting Antiviral Hepatitis C Virus Therapy in Canada: On the Path to Elimination

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Keywords: Direct-acting antivirals (DAA), Hepatitis C virus (HCV), multidisciplinary care, people who use drugs (PWUD).

Citation: EMJ Hepatol. 2019;7[1]:43-44. Abstract Review No. AR3.

BACKGROUND AND AIMS

As many as 250,000 Canadians are living with hepatitis C virus (HCV) infection,[1] and specific populations, such as people who use drugs (PWUD), make up a large proportion of prevalent infections.[2] PWUD are largely excluded from receiving HCV care, in part due to provider concerns regarding adherence and therefore treatment efficacy in this population.[3] The World Health Organization (WHO) has outlined goals for the elimination of HCV as a public health concern by 2030.[4] To achieve this, attention must be paid to these vulnerable populations. This analysis was conducted to document the efficacy of the three most-prescribed all-oral HCV treatment regimens in Canada (elbasvir/grazoprevir [EG], sofosbuvir/ledipasvir [SL], and sofosbuvir/velpatasvir [SV]) among a large population of PWUD, to further support campaigns for the diagnosis and treatment of HCV infection in this priority population.

METHODS

The study was a retrospective analysis of all HCV-infected PWUD (positive urine drug screen <6 months) initiating HCV treatment at our centre between June 2015 and February 2019. All subjects were enrolled in a multidisciplinary model of care, addressing medical, psychologic, social, and addiction-related needs. The primary outcome was achievement of sustained virological response 12 weeks after treatment (SVR12) (i.e., undetectable HCV RNA ≥12 weeks after the completion of HCV therapy).

RESULTS

A total of 265 individuals initiated therapy with one of the regimens of interest, 218 of whom had reached sufficient follow-up for SVR12 analysis. In the EG cohort (n=61), 11% were HIV positive, 57% were on opiate substitution therapy (OST), and 3% were cirrhotic. In the SL cohort (n=74), 16% were HIV positive, 31% were on OST, and 20% were cirrhotic. Finally, in the SV cohort (n=130), 12% were HIV positive, 58% were on OST, and 17% were cirrhotic. Of those eligible for intention-to-treat SVR analysis to date, SVR12 rates are EG: 82% (42/51), SL: 91% (62/68), and SV: 87% (86/99). There was one virologic failure (in the SL group) and two deaths (one in each of the EG and SL groups); both deaths were related to opioid overdose. A total of 25/218 (11.5%) did not present for SVR12 evaluation. All are still alive, and active processes are underway to ascertain the outcome of HCV therapy and to re-engage them in long-term care. Thus, when excluding losses to follow-up and deaths (modified ITT analysis), SVR rates are EG: 100% (42/42), SL: 98% (62/63),

and SV: 100% (86/86). These results can be seen in Figure 1.

CONCLUSION

The authors found that the currently available regimens for the treatment of HCV appear to be highly successful among PWUD populations in this multidisciplinary care setting. Virologic failure has only been documented in one case, attesting to the efficacy of the therapeutic options, as well as the robust nature of the model of care during therapy. This might include, as needed on an individual basis, the daily dispensing of HCV medications with OST, weekly delivery of pills to shelters, and other strategies to enhance adherence. Despite these measures, about 10% of the cohort was 'lost to follow-up' with respect to SVR12 determination. This is not only important to ascertain outcome (although 17/25 were HCV RNA negative at last measure, including 9 at 1–11 weeks after the end of therapy), but also for long-term follow-up among cirrhotic patients and those at risk of reinfection. Going forward, the authors plan to enhance their model of care to address this important issue. These results support provision of HCV treatment to PWUD for the achievement of the WHO's global elimination targets.

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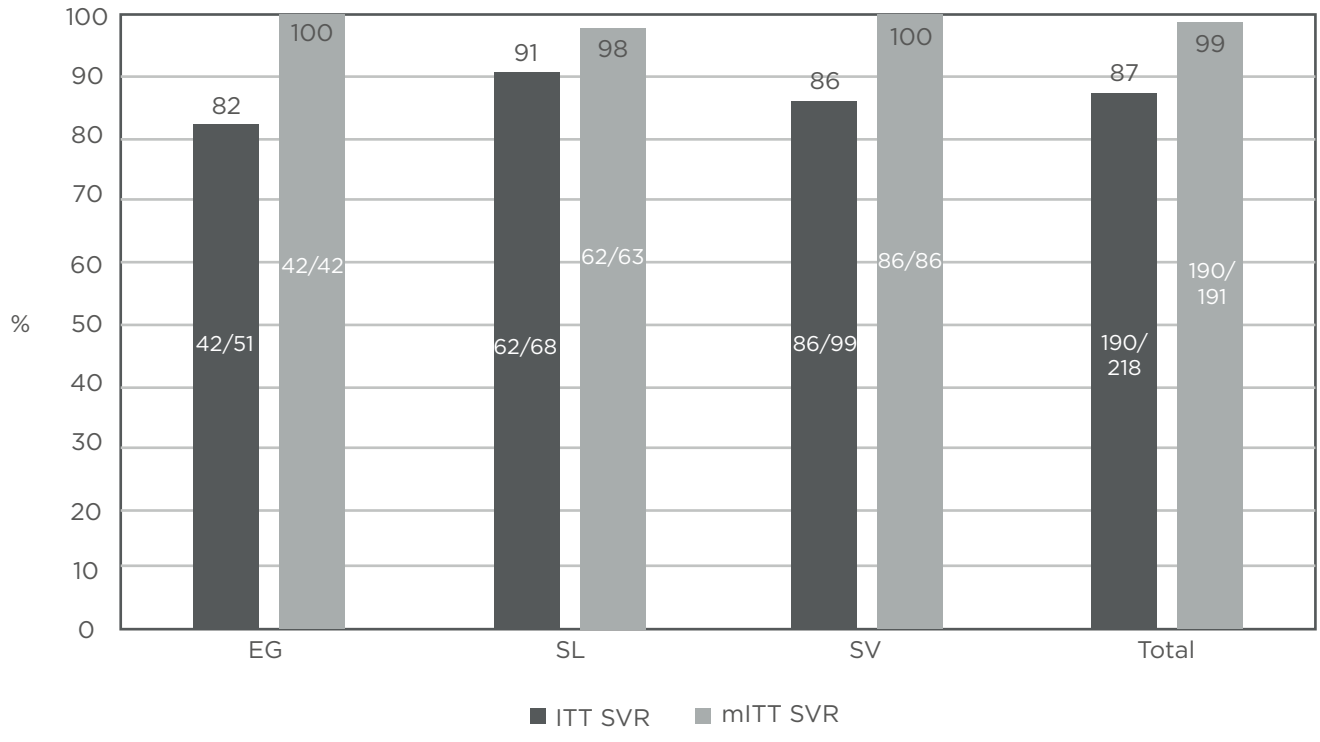

Figure 1: Hepatitis C virus treatment outcomes.

EG: elbasvir/grazoprevir; ITT: intention-to-treat; mITT: modified intention-to-treat; SL: sofosbuvir/ledipasvir; SV: sofosbuvir/velpatasvir; SVR: sustained virologic response.

The NIACE Score: A Prognostic Indicator in Hepatocellular Carcinoma

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Hepatobiliary, hepatocellular carcinoma (HCC), liver cancer, NIACE, prognosis, survival.

Citation: EMJ Hepatol. 2019;7[1]:45-46. Abstract Review No. AR4.

BACKGROUND AND AIMS

The Barcelona Clinic Liver Cancer (BCLC) classification is the reference system to stage and prognosticate hepatocellular carcinoma (HCC). The NIACE score[1] (tumour nodularity, infiltrative nature of the tumour, serum alpha-fetoprotein level, Child–Pugh score, and ECOG [The Eastern Cooperative Oncology Group] performance status) uniquely considers tumour characteristics but is yet to be validated in an Australian context. It is suggested that the NIACE score may offer prognostic clarity within BCLC classes, which encompass heterogenous tumours matched with single management options. The aim of this study was to compare the survival prognostic value of the NIACE score within the BCLC staging system.

METHODS

Data for this study was retrospectively analysed from a cohort of 2,202 patients with HCC collected across six metropolitan hospitals in Victoria, Australia, from January 2000–August 2018. Patients were included in the study if all markers were available to calculate prognostic scores, including tumour nodularity (0 if <3, 1 if ≥3), tumour infiltration (0 if no, 1.5 if yes), serum alpha-fetoprotein (0 if <200, 1.5 if ≥200 ng/mL), Child–Pugh score (0 if A, 1.5 if B), and ECOG status (0 if 0, 1.5 if ≥1). Baseline characteristics including age, sex, country of birth, ethnicity, aetiology of chronic liver disease, and the presence of cirrhosis were recorded. Survival time was measured from the date of diagnosis to date of death (or censored at last follow-up). Transplant-free survival (TFS) was used as the endpoint for analysis.

RESULTS

A total of 366 patients (86% male, 14% female; median age at diagnosis was 63±12 years; 103 [28.1%] Australian born) were included in the analysis. Aetiology of liver disease was hepatitis B virus (64 [17.4%]), hepatitis C virus (157 [42.8%]), alcohol-associated (143 [39.0%]), and other (108 [29.4%]). The mean serum alpha-fetoprotein level for the cohort was 7,839 ng/mL. Over a median follow-up time of 16 months, 185 patients (50.4%) were found to be deceased. The median TFS for BCLC 0 (n=21), A (n=108), B (n=82), C (n=113), and D (n=39) were 64, 29, 24, 12, and 4 months, respectively (p<0.001). The TFS for NIACE score <2.5 (n=169) versus ≥2.5 (n=197) was 34±3 months versus 12±2 months, respectively (p<0.001). Furthermore, NIACE had prognostic value within BCLC subclasses, with the TFS of patients within BCLC-A (36±7 months versus 20±3 months; p<0.01) and BCLC-B (31±7 months versus 19±4 months; p<0.005) being significantly different for scores of <2.5 versus ≥2.5, respectively (Figure 1). There was no significant discriminating ability amongst later stage BCLC-C or BCLC-D.

CONCLUSION

Accurate prognostication in HCC is of vital importance to allow for appropriate care planning and optimisation of treatment options. The NIACE score is an easy-to-calculate prognostic score, and this study demonstrated that NIACE can distinguish between two subgroups with different prognosis within early (BCLC-A) and intermediate (BCLC-B) stage HCC. The broader implications of these findings are that they may assist clinicians in improving

the accuracy of survival prognostication, thereby helping to define subgroups of patients who may benefit from an intensified treatment strategy. This is the first validation of the score in an Australian population. Further prospective validation of the NIACE score in HCC patients and comparison with other simple scores, such as Albumin-Bilirubin grade, is warranted to confirm its prognostic value and use in guiding decision making in conjunction with the BCLC staging system.

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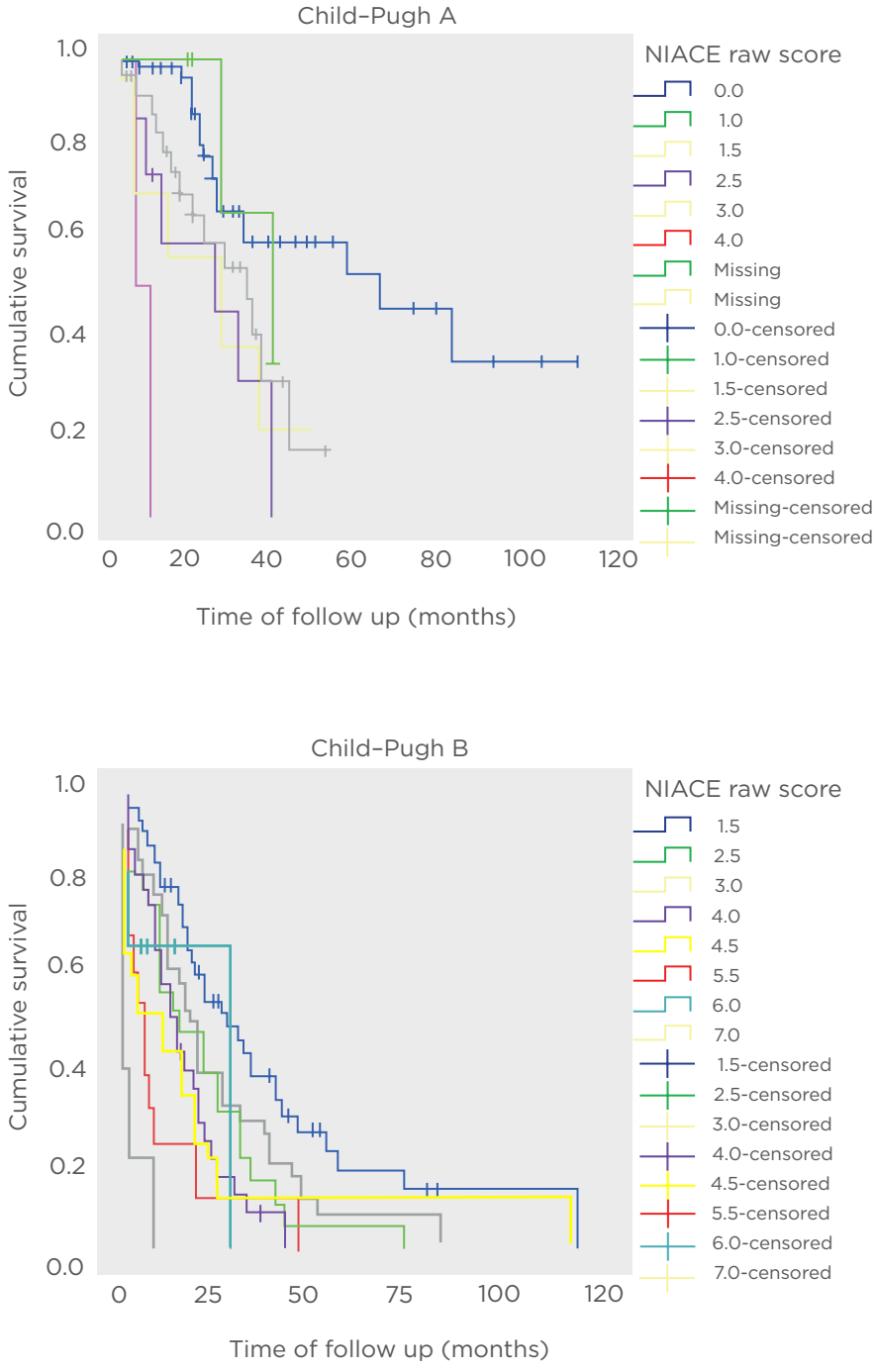

Figure 1: Kaplan–Meier curves showing survival of Child–Pugh A and B hepatocellular carcinoma patients by NIACE raw score.

L-carnitine Could Be Beneficial in Nonalcoholic Fatty Liver Disease Patients by Improving Choline Metabolism and Reducing Liver Biomarkers

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Keywords: Alanine transaminase (ALT), aspartate transaminase (AST), choline, choline deficiency, fatty liver disease, L-carnitine, liver metabolites, metabolomics, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH).

Citation: EMJ Hepatol. 2019;7[1]:47-48. Abstract Review No. AR5.

Nonalcoholic steatohepatitis (NASH) patients have reduced levels of choline in the liver.[1] Choline metabolism is critical in maintaining normal liver function[2] and is important in the synthesis of very-low-density lipoproteins from triglycerides (TAG) in the liver.[3] Choline is also important for normal kidney and mitochondrial function.[4] Choline deficiency will lead to an accumulation of liver TAG levels, resulting in elevated lipid levels. Furthermore, choline can be oxidised to betaine, which is an important methyl donor and participates in the methionine cycle in the liver. Methionine, together with lysine, forms L-carnitine in the liver and kidneys.

L-carnitine is critical for the transportation of long-chain fatty acids. NASH patients display abnormal parameters in liver function tests, such as elevated levels of aspartate transaminase (AST) and alanine transaminase (ALT);[5] however, L-carnitine has been shown in several studies to improve liver function in NASH patients and prevent the progression of the disease.[6–8]

This study investigated what effect L-carnitine supplementation could have on liver metabolites. In this study, the authors hypothesised that L-carnitine could elevate choline in the liver through a regulation of betaine. Furthermore, they aimed to investigate liver function to understand how L-carnitine affects key liver enzymes.

A total of 16 healthy male Wistar rats (approximately 200 g) were treated daily with either saline (n=8) or L-carnitine (n=8; intra-peritoneal injections, 3 g/kg/day) for 2 weeks. Following treatment, body weight was measured and blood obtained, before euthanasia and extraction of liver tissue for metabolomic analysis. Liver tissue was crushed and prepared with previously described methods to separate the aqueous, lipid, and protein layers,[9] and sent to the Department of Biochemistry at the University of Cambridge, Cambridge, UK, where liquid chromatography mass spectrometry was undertaken.

The L-carnitine-treated group had 15% lower body weight ($p<0.01$) and 55% reduced serum TAG levels compared to the saline-treated group. L-carnitine resulted in higher liver choline levels (47%; $p=0.05$) and reduced levels of betaine (24%; $p=0.04$). Alanine was elevated in the liver by a factor of 76.6, while oxaloacetate was elevated by a factor of 1.3 following L-carnitine treatment compared to saline treatment. Pyruvate, α-ketoglutarate, glutamate, and aspartate all stayed constant between the two groups (Figure 1).

Metabolism in the liver was modulated by L-carnitine. The liver is critical for choline metabolism, and studies have shown that NASH is associated with lower choline

concentrations. This study demonstrated that some of the beneficial effects of L-carnitine could be mediated through increased liver choline levels, possibly by an elevation of betaine, which was reduced in the liver when treated with L-carnitine. In addition, L-carnitine improved the AST/ALT ratio, which is commonly used to assess liver damage, where an elevation signifies advancement of fibrosis.[10] L-carnitine elevated alanine levels considerably more so than it elevated oxaloacetate, forcing the AST/ALT ratio to reduce, and thereby improving liver function. Future studies should investigate liver enzymes and metabolites in NASH patients treated with L-carnitine, which could help elucidate the mechanism through which L-carnitine can prove beneficial to liver function and provide insights into novel therapeutics.

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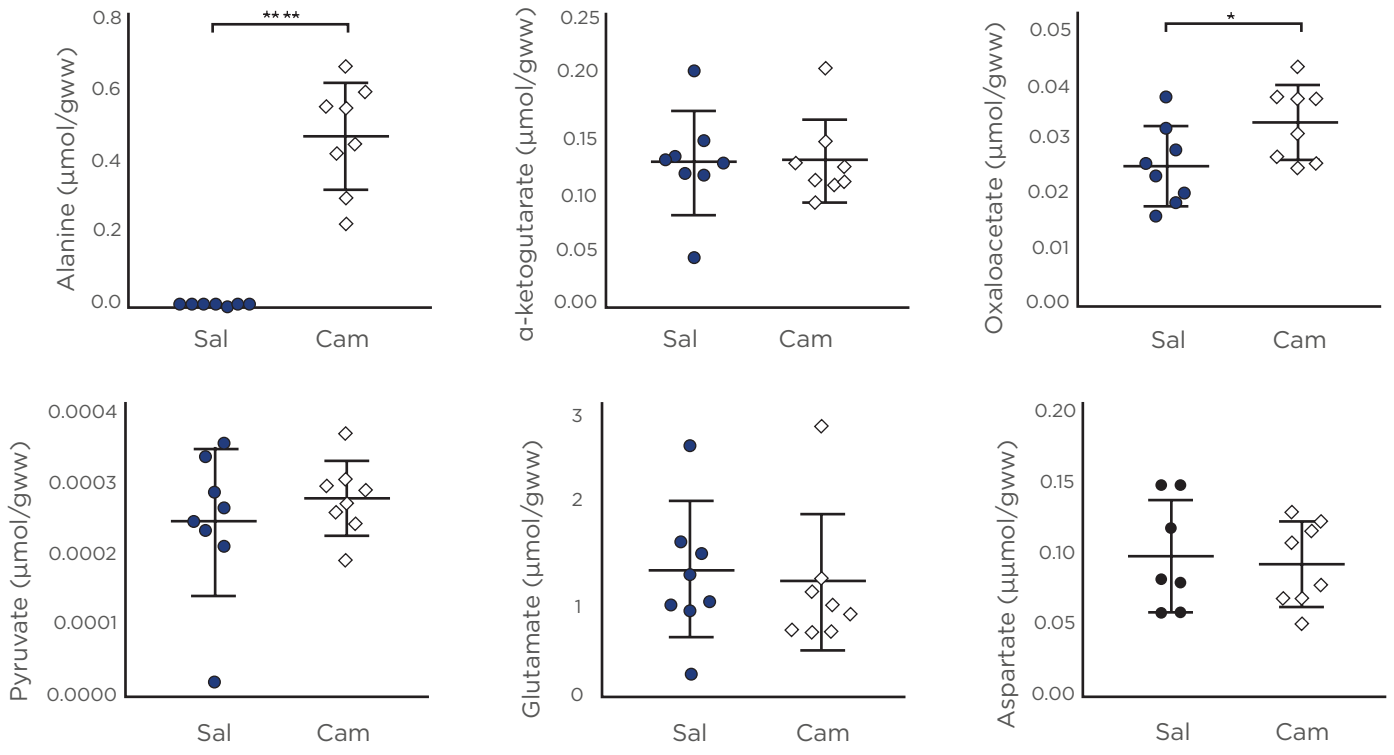

Figure 1: Liver function measured in the saline (Sal) treated group and L-carnitine (Carn) treated group.

All data presented as mean±standard deviation, significant values presented as *p<0.05 and ****p<0.0001 Metabolites relevant for alanine transaminase and aspartate transaminase measured with metabolomics (µmol/gww).

miRNA-21 Ablation in Mice Protects Against Gut Microbiota Dysbiosis During Cholestasis

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Keywords: Bile acid homeostasis, bile duct ligation, gut microbiota, *Lactobacillus*, miR-21.

Citation: EMJ Hepatol. 2019;7[1]:49-51. Abstract Review No. AR6.

Gut microbiota play an important role in gut–liver axis homeostasis. The understanding of the gut–liver relationship is crucial for the advances of research into the microbiome-based, diagnostic, prognostic, and therapeutic modalities to improve management of liver diseases. It is extremely difficult to establish a common set of 'healthy' gut microbiota because it may vary even between individuals of the same species. The onset and progression of liver diseases have been associated with gut microbiota dysbiosis through increasing lipopolysaccharides and gut barrier dysfunction.[1] In particular, several liver disorders, such as alcoholic liver disease and nonalcoholic fatty liver disease, have been associated with small intestine bacterial overgrowth.[2] Besides the impact of gut microbiota, other strategies to restore liver injury are based on the modulation of specific miRNA that might be involved in liver protection or injury.[3–5] miRNA participate in a variety of biological processes, such as cellular differentiation, metabolism, proliferation, immune response, and apoptosis.[6] New evidence suggests that endogenous miRNA, secreted into the intestinal lumen, may modulate gut microbiota function and abundance.[7] For instance, the loss of miR-21 alters gut microbiota composition in mice and protects against inflammatory bowel disease.[8] Of note, the authors have previously showed that miR-21 deletion ameliorates liver fibrosis in experimental cholestasis and improves adaptative response to bile acid dysregulation.[9] In this study, the authors aimed to characterise changes occurring in the gut microbiota of miR-21 knockout (miR-21KO) mice after bile duct ligation (BDL).

To test their hypothesis, 3-month-old C57BL/6 wild-type (WT) and whole body miR-21KO mice were subjected to sham or BDL surgeries. After 3 days, the small intestine was collected for qRT-PCR analysis of intestinal permeability-related genes. Serum was also collected for biochemical analyses. Gut microbiota composition was evaluated by sequencing the 16S rRNA gene V3 region of bacterial DNA from the small intestinal lumen. For the co-housing experiments, WT and miR-21KO animals were housed together for 1 month and then separated into different boxes for an additional month.

Results showed that miR-21KO mice were protected against small intestinal dysbiosis induced by BDL. In particular, depletion of miR-21 in mice positively correlated with increased *Lactobacillus sp*. and diminished Proteobacteria. This effect was independent from the BDL, as miR-21KO co-housed mice displayed increased relative abundance of *Lactobacillus sp*. in the small intestine, compared with WT mice. Of note, separated miR-21KO animals showed higher amounts of *Lactobacillus* sp. when compared with co-housed miR-21KO. Furthermore, mRNA expression of small intestinal tight junctions (ZO-1, JAM-A, and Occludin-1) and stem cell markers (Lgr5 and Olfm4) were decreased in

WT mice after BDL but remained unaltered in miR-21KO animals. Finally, miR-21 ablation also correlated with increased farnesoid X receptor mRNA expression in the small intestine, increased bile acid homeostasis, and reduced liver injury.

Genetic ablation of miR-21 modulated small intestinal permeability and farnesoid X receptor expression, impacting on bile acid production and contributing to improved gut microbiota and host homeostasis. These results reinforce the importance of the gut–liver axis in protecting the liver after acute cholestasis.

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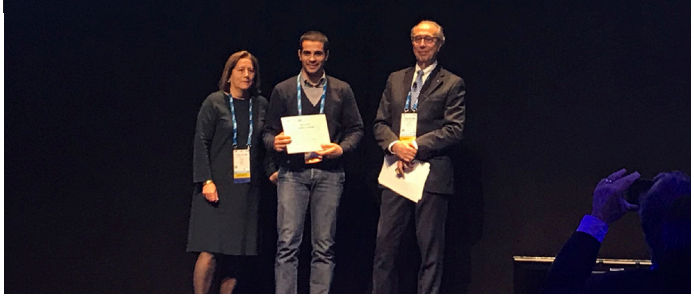
MEET THE PRESENTER



The EMJ team were privileged to meet with study author Dr André Fernando Anastácio dos Santos after his presentation to discuss his work and his experience as a first-time presenter at the EASL ILC.

Could you please summarise how you came to work in your current laboratory?

As a biologist, I am passionate about micro-organisms and their capability to adapt to a variety of environments. My curiosity about microbial adaptation led me to embrace the studies of the bacterial metabolic pathways of the sulfate/sulfite reducers. My PhD project was entitled 'Novel insights into dissimilatory sulfite reductase', where I studied the sulfide producers and realised their vital role in several anaerobic environments, such as the human gut. Knowing that these organisms are associated with several human diseases, such as inflammatory bowel disease, Crohn's disease, and autism, I became intrigued by their mechanisms of action. Now, I want to continue my personal project of understanding how micro-organisms strikingly influence human disorders, with a focus on the gut–liver axis. To fulfil my goal of connecting bacterial metabolism with human pathology, I have teamed up with Dr Cecília Rodrigues, head of the Cellular Function and Therapeutic Targeting laboratory at the iMed.ULisboa, who is a recognised expert on liver diseases with interest in the gut–liver axis.

What is it like to present research at an international conference like this one?

I must say that I was very nervous, but it was a wonderful sensation. To have the opportunity to present my work to the experts in the field is a huge pleasure.

The gut–liver axis has been an important topic for gastroenterologists for some years now and is of growing significance. What can we expect from research into this connection in the future?

I am new to this field, having started my work roughly 3 years ago. Nevertheless, to my knowledge, the gut–liver axis, and particularly bacteria and bacterial products, will be crucial to understanding liver and gut disorders, as they modulate gut homeostasis and contribute to energy availability. I think that in the future we will combine therapeutic strategies such as pro and prebiotics with targeted therapy to the specific diseased pathways or disease mechanisms, both in the liver and the gut.

Congratulations on winning an award at this year's congress! Could you please tell us about this EASL award and why you were selected?

Thank you. I am very honoured to have been awarded the Daniel Alagille Award by the EASL. The purpose of this award, which honours legendary Prof Daniel Alagille, is to encourage biomedical research in the field of paediatric and adult genetic cholestatic diseases sharing mutual disease-causing mechanisms. Our project aims to understand why 80% of patients with primary sclerosing cholangitis (PSC) also have inflammatory bowel disease (IBD). We propose investigating the role of miR-21, an oncogenic microRNA secreted in faeces, and correlate it with the PSC–IBD profile. Our final goal will be to assess if the expression levels of this marker in the blood plasma, faeces, and intestinal tissue can enlighten our understanding of the combined pathology.

What advice would you give to other young researchers presenting and attending an event like this for the first time?

My advice would be 'don't be nervous and enjoy the moment.' I could not follow my own advice, but I really think that this is a great opportunity to discuss our results and to show to peers our point of view. We normally look at the leaders in the field as 'scary monsters,' but I strongly think that we are all here with the same goal of finding new strategies to treat, or even cure, liver disease.

Neutrophil-to-Lymphocyte Ratio and Corticosteroid Response in Alcoholic Hepatitis

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Keywords: Alcoholic hepatitis, corticosteroids, lymphocytes, neutrophils.

Citation: EMJ Hepatol. 2019;7[1]:51-53. Abstract Review No. AR7.

INTRODUCTION

Treatment of alcoholic hepatitis with corticosteroids remains controversial, with recent studies indicating only a 28-day survival benefit.[1] The neutrophil-to-lymphocyte ratio

(NLR) may reflect the systemic inflammatory response, and the authors have previously shown that the NLR can affect the outcome of corticosteroid response in alcoholic hepatitis patients.[2] These abstracts aimed to determine the associations of baseline NLR with acute kidney injury (AKI), infection, and Lille response, as well as to externally validate the NLR as a means of identifying patients for corticosteroid therapy.

A) BASELINE NEUTROPHIL-TO-LYMPHOCYTE RATIO INDICATES BOTH PREVALENT AND INCIDENT INFECTION AND ACUTE KIDNEY INJURY, AND PREDICTS LILLE CORTICOSTEROID RESPONSE IN ALCOHOLIC HEPATITIS

The NLR was calculated from 789 patients who participated in the STOPAH trial, with patients randomised to receiving or not receiving prednisolone. Prevalent AKI was defined by an initial creatinine level ≥133.0 µmol/L and incident AKI was defined as either an increase of serum creatinine by 26.5 µmol/L or by 50% by Day 7 in those without baseline AKI. Patients presenting with infections (prevalent infection) were treated prior to randomisation; incident infections were determined after inclusion in the trial.

Those with prevalent AKI had a higher NLR than those without (11.1 versus 6.0; p=0.001; 95% confidence interval [CI]: 2.6–7.6), as did those with prevalent infection compared to those without (7.8 versus 6.3; p=0.02; 95% CI: 0.2–2.8).

A favourable Lille score with prednisolone treatment was more likely if NLR ≥5 versus <5 (56.5% versus 41.1%: p=0.01; overall response [OR]: 1.86; 95% CI: 1.16–2.99). The risk of developing infection after prednisolone treatment was greater if NLR >8 versus <8 after 7 days (17.3% versus 7.4%: p=0.006; OR: 2.60; 95% CI: 1.32–5.14) and 28 days (30.6% versus 20.0%: p=0.031; OR: 1.76; 95% CI: 1.05–2.96). The risk of incident AKI after prednisolone treatment was greater for those with NLR >8 versus <8 (20.8% versus 7.0%: p=0.008; OR: 3.46; 95% CI: 1.39–8.62).

The finding reaffirmed previous observations that prednisolone does not improve outcomes for those with NLR <5 or >8 but does improve outcome beyond 28 days for those with NLR 5–8 (Table 1). This may be explained by the greater likelihood of achieving a Lille response if NLR <5, but only achieving a reduction in AKI and infection if NLR ≤8.

B) A MODIFIED GLASGOW ALCOHOLIC HEPATITIS SCORE INCORPORATING THE NEUTROPHIL-TO-LYMPHOCYTE RATIO IS SUPERIOR TO OTHER BASELINE SCORES OF PROGNOSIS IN ALCOHOLIC HEPATITIS

In the same cohort of patients, there was a strong correlation between the total white blood cell count (WCC) and NLR: r=0.564; 95% CI: 0.52–0.60; p<0.0001. In view of this, the NLR was incorporated into the Glasgow Alcoholic Hepatitis Score (GAHS)[3] with an NLR threshold of 5 replacing the total WCC threshold of 15, creating a modified GAHS (mGAHS). The areas under the curve for mGAHS for 28-day and 90-day outcomes were 0.783 (95% CI: 0.752–0.812) and 0.739 (95% CI: 0.706–0.770), respectively. For 28-day outcome, the mGAHS area under the curve was superior to that of the Maddrey's discriminant function (0.684: p<0.0001; 95% CI: 0.05–0.14), the original GAHS (0.763: p=0.027; 95% CI: 0.002–0.040), and the model for end-stage liver disease (0.739: p=0.0014; 95% CI: 0.009–0.080).

C) VALIDATION OF THE PRE-TREATMENT NEUTROPHIL-TO-LYMPHOCYTE RATIO TO PREDICT RESPONSE TO CORTICOSTEROIDS IN SEVERE ALCOHOLIC HEPATITIS

The observations from the previous abstract were then tested in an independent validation group of 237 patients. Again, no improvement in outcome with corticosteroids was seen at either 28 days or 90 days for NLR <5 (28-day mortality 10.0% versus 5.1%; p=0.416; 90-day mortality 16.3% versus 18.4% p=0.843) or NLR >8 (28-day mortality 23.5% versus 28.0%; p=0.469; 90-day mortality 39.2% versus 48.0%; p=0.362). In total, 28-day mortality for NLR 5–8 was reduced by corticosteroid treatment (2.7% versus 28.6%; p=0.0023) with a trend to reduced mortality at Day 90: 21.6% versus 37.4%; p=0.097. Those with a mGAHS ≥9 and NLR 5–8 had a significant reduction in 90-day mortality with prednisolone

treatment: 23.3% versus 46.4%; p=0.036; hazard ratio: 0.268; 95% CI: 0.10–0.71.

CONCLUSIONS

These abstracts indicate the association of NLR with risk of infection and AKI in alcoholic hepatitis, as well as the chance of a Lille response to alcoholic hepatitis. Incorporation of the NLR in place of WCC improves prognostic accuracy. These results reaffirm and externally validate the observation that corticosteroid treatment for those with NLR <5 or >8 is ineffective but improves outcomes for those with NLR 5–8 beyond 28 days.

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Table 1: Mortality relative to neutrophil-to-lymphocyte ratio category and prednisolone treatment in all patients.

	NLR <5		NLR 5–8		NLR >8	
Prednisolone	Untreated n=182	Treated n=190	Untreated n=113	Treated n=105	Untreated n=101	Treated n=98
28-day mortality	8.2%	5.8%	25.7%	5.7%	29.7%	30.6%
	p=0.359		p=0.0001 HR 0.20 (0.10, 0.38)		p=0.923	
90-day mortality	13.2%	19.0%	34.5%	21.0%	37.6%	41.8%
	p=0.176		p=0.012 HR 0.52 (0.31, 0.86)		p=0.604	

HR: hazard ratio; NLR: neutrophil-to-lymphocyte ratio.

The Good Use of Paracetamol: Prospective Assessment of the Knowledge of Prescribers, Pharmacists, Care Providers, and Students in a University Medical Centre

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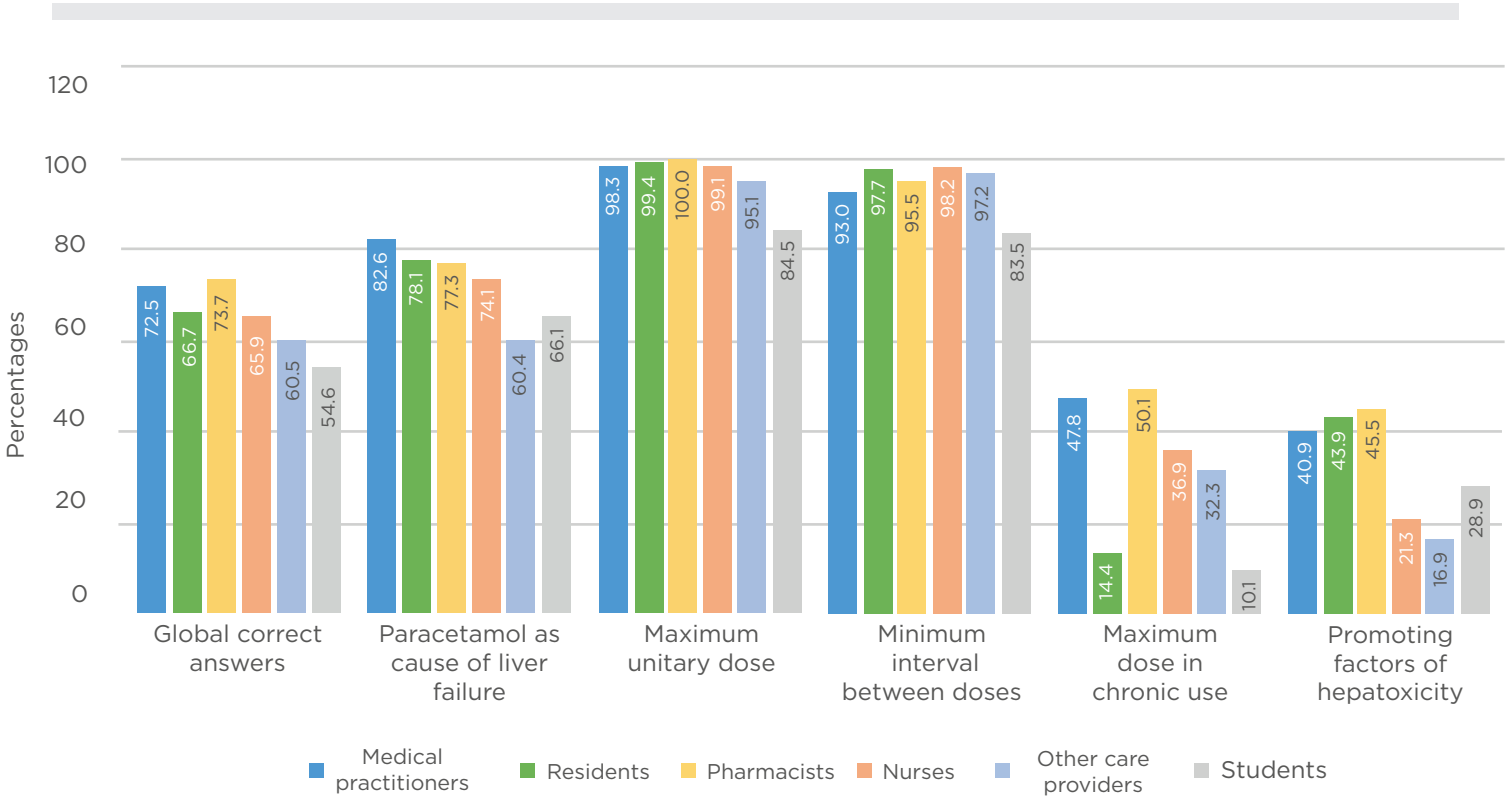

Figure 1: Distribution of answers by interviewed care providers.

Paracetamol, or acetaminophen, is the most commonly used drug in Western countries. Use of the drug is markedly commonplace because it can be obtained without a prescription. A recent nationwide study (SALT III) showed that paracetamol-induced acute liver failure is increasing and is the main cause of acute liver failure leading to registration for liver transplantation (42.1%, including 17.6% of paracetamol misuse).[1] The study aimed to assess the knowledge of prescribers (practitioners and residents), care and drug providers (including nurses and pharmacists), and students regarding paracetamol use in the University Medical Centre, Montpellier School of Medicine, Montpellier, France.

This prospective study was performed using a questionnaire comprising five questions regarding paracetamol prescription in adult patients:

- What is the primary cause of liver failure in France?
- What is the maximal daily dose of paracetamol for chronic usage?
- What is the maximal unitary dose of paracetamol?
- What is the minimal time interval between two doses?
- What are the promoting factors of paracetamol hepatotoxicity?

The questionnaire has been validated by the Committee for the Security of Medication Use (COSEMED) of the hospital and submitted from November 2017–October 2018 during professional meetings or via the hospital intranet website.

During this 1-year period, 1,118 questionnaires were completed by various care providers: medical practitioners (n=130; 12%), nurses (n=408; 36%), students (n=101; 9%), residents (n=201; 18%), and pharmacists (n=30; 3%). The overall rate of correct answers ranged from 54.6% for students to 73.7% for pharmacists. The answers were excellent regarding the maximum unit dose (95.1–99.4% for care workers and 84.5% for students) and the interval between doses (93.0–98.2% for care workers and 83.5% for students). Regarding the role of paracetamol in liver failure, the answers were good (60.4–82.6% for care workers and 66.1% for students). In contrast, the answers were poorer concerning the maximum daily chronic dose (14.4–50.1% for care workers and 10.1% for students), with some answers of 6 g per day. The identification of cofactors of

hepatotoxicity was variable: good for chronic alcoholism, but low for undernutrition or antalgic co-medications (Figure 1).

The knowledge regarding the proper use of paracetamol is insufficient for the maximum chronic dosage, the risk of paracetamol hepatotoxicity, and its contributing factors. These results encourage the promotion of a more intensive and specific teaching of all care providers involved in the use of this drug.

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Management of Hepatitis C in People Who Inject Drugs: Some Practical Lessons from the Frontline of the Elimination Battle

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INTRODUCTION

In 2016, the World Health Organization (WHO) set out its vision to combat the significant morbidity and mortality associated with viral hepatitis.[1] This strategy for viral hepatitis elimination has been adopted by many countries worldwide and, in January 2018, the National Health Service (NHS) in England committed to achieving this by 2025, a full 5 years ahead of the WHO plan. However, there are many cultural, economic, societal, strategic, and organisational barriers to overcome to see this ambition turned into reality. One of the critical populations in this battle to achieve elimination is people who inject drugs (PWID), who in the UK represent 85% of the hepatitis C virus (HCV)-infected prevalent population. PWID also represent majority prevalent populations in many other developed countries, such as the USA, Australia, and most Western European countries. Such individuals are often extremely marginalised and require tailored management to help overcome the significant barriers they experience in accessing HCV care.

In this opinion piece, I will share with the reader some of the lessons I have learnt over the last 5 years that I have been treating PWID in different settings. It is important to state that I do not claim that this is an exhaustive list and neither do I claim to have all the answers to the many difficulties I and others face in trying to ensure adequate penetration of services into this vulnerable population. Furthermore, where I make generalisations in the following text, I am by no means discounting that there are exceptions to the rule, and I hope the reader forgives me this indulgence. Finally, I will not be discussing management of PWID in the secure setting, as this is a topic worthy of separate consideration.

LOCATION IS KEY

It is abundantly clear that the attendance of PWID (especially active users) at secondary care institutes is extremely poor.[2] Through their life experiences, most of these people develop a deep mistrust of societal structures and institutions. Many suffer from extremely low self-esteem, which is often enforced by the way most of society treats them. Imagine for a second what it must feel like to be homeless in a major urban city and have thousands of people walk past you every day without even looking at you or giving you the time of day. It is unsurprising, therefore, that such individuals do not choose to follow secondary care institutions' rigid and regimental appointment rules. Even if they had the will to attend, transport costs are often prohibitive. This has led to many clinical teams locating services in areas where PWID already access services. Indeed, the majority of such outreach clinics in the community are co-located in drug and alcohol services where opiate substitution therapy (OST) is administered, in needle exchanges, or in homeless hostels. Setting up such clinics involves the engagement of local healthcare managers in the vision of

hepatitis elimination and a clear explanation of the benefits of effectively running a secondary care clinic in a different location.[3] Contracting with multiple stakeholders is often required and set-up times can be lengthy. Indeed, in my experience, 2 years is not an unusual time frame.

In addition, it is important to stress that co-location does not guarantee immediate success. Advertising the clinic, educating and motivating colleagues working in the aforementioned services, and changing the stigma associated with HCV, as well as countering some of the myths from the interferon era that are still pervasive, are all required to stimulate linkage to care. It is not unusual for a lag period of 6–12 months to be required prior to successful engagement in the service. Ongoing motivation of the staff in drug and alcohol services is required to change the culture surrounding HCV.

ENGAGEMENT WITH THE INDIVIDUAL PATIENT

As previously mentioned, PWID often have an inherent mistrust of healthcare professionals (HCP), as well as extensive experience of being talked down to or treated as inferior. The first consultation is, therefore, critical in establishing a degree of trust in the individual patient and reassuring them that the sole focus is on their health, with no hidden agendas. A casual, non-judgemental consultation style works best in my experience and, over time, I have learnt more of the lexicon of the street. In my experience, referring to 'pins' rather than needles, talking about 'snowballing' for the practice of using both heroin and cocaine at the same time, and enquiring about the background of the individual and how their mental health is at present, all help to break down some of the traditional barriers that HCP encounter in dealing with PWID. I often find myself agreeing with my patients when they recount stories of how badly they were treated when they were admitted to hospital or during other encounters with the healthcare system, and such empathy also helps to develop a useful bond. Simple as it sounds, treating the patient as a fellow human being who has been unfortunate in their life experience to date (regardless of whether this was through their own choices) is the single most important means of gaining trust. Each of these individuals has a life story and enquiring about it, even briefly, has helped me to start to understand some of the real challenges that these patients face and has helped me to frame the importance of HCV treatment within these.

As a hepatologist, I had always had a liver-centric view of HCV. It has become clear to me, however, that discussing the need to avoid development of liver failure in 10–20 years is insufficient to motivate many of the patients I see in outreach to engage with HCV treatment. This has been recently demonstrated in qualitative research from Australia.[4] Instead, focussing on side effects, such as tiredness, itching, or the ill-defined concept of 'brain fog,' and the potential to improve these with curative HCV therapy, is more helpful in gaining traction with this patient group.

Finally, linkage to care is always better when individual patients are engaged with OST services.[5] While this may indicate the patient is starting on the road of recovery, it also provides a structure for HCV therapy. Indeed, tying in HCV therapy with OST pickup has been demonstrated to be efficacious in community pharmacies as well as other settings.[6] This should be taken into consideration when setting up services.

THE CRITICAL ROLE OF CONTINUITY, FLEXIBILITY, AND THE CLINICAL NURSE SPECIALIST

PWID can be very distrustful of HCP, as already discussed. Building trust is crucial. Meeting and starting to engage with the person who will see them through their treatment at the first appointment is, therefore, important. I am extremely privileged (as are many of my colleagues) to work with a fantastic team of prescribing hepatitis clinical nurse specialists who treat our patients. They start to build up a rapport with the patients from the first clinical consultation with myself and continue this through to the sustained virologic response (SVR) visit and beyond. As such, it is important for the patient to see the same person (or a maximum of two people) throughout the treatment journey for this trust to be maintained. This continuity engenders better engagement, in my experience.

Furthermore, being flexible in the therapeutic relationship is also key to success. For example, arranging for the supplies of medicines to be couriered to the patient's home if they are unable to attend or giving 1 week's supply at a time for homeless patients who have nowhere to store a larger supply are both approaches that we have had to adopt. Similarly, flexibility in appointment times is critical. In this era of increasingly personalised medicine, I see no reason for this population of patients to be denied such an approach by insisting that they strictly adhere to rigid treatment protocols.

Finally, given the often difficult venous access in many PWID and given the high level of safety of modern direct-acting antiviral agents, on-treatment monitoring should be kept to a minimum, if not dispensed with all together. Indeed, in my practice we have very much moved towards a dry blood spot test (DBST) approach, with some patients only having a DBST at the start of treatment and a DBST at the SVR 12 weeks post-treatment timepoint to confirm cure.

PEER SUPPORT IN HCV OUTREACH SERVICES

There is increasing evidence that peer support in HCV clinics treating PWID significantly increases engagement and linkage to care.[7] This is indeed mirrored in my own experience. The Hepatitis C trust in the UK has expanded its peer co-ordinator pool over the last 1–2 years, and our services have been beneficiaries of this. While there is a lag period associated with training and integration of peers in HCV services, the benefits they bring are immense. Peers often relate to PWID in ways that most HCP are unable to. There is not the same inherent initial level of mistrust that exists towards HCP and the language that peers use is more relatable. Furthermore, peers can provide emotional support, reassurance through their own experience of HCV treatment, and logistical support through help with travel and reminders for appointments. They help engage individuals who we would have found impossible to reach using traditional methods.

Another avenue linked to peer programmes is providing PWID with voucher-based incentives to attend the first HCV clinic appointment. While there may be ethical objections to this among some HCP, in my experience such schemes help engage individuals and, when paired with strong peer support, can improve linkage to care in the even more marginalised PWID. The other important thing to state is that such individuals often require multiple points of contact and offers of appointments before they eventually engage with HCV care. Often this is because offers of support correspond with short windows of engagement in their often chaotic drug use. Successful engagement with HCV care can lengthen these windows of stability in their lives and with linkage into OST services can trigger the start of a full recovery journey. Once again, the lived experience of peers plays a crucial role in enabling this.

Finally, it is important to state that some PWID will never engage with HCV treatment, regardless of how many points of engagement they are offered or how many enablers are put into place. Although disappointing, HCP and peers should not feel disheartened by this. Instead, they should continue to try to engage with those individuals who show willingness, however small, to discuss their HCV status.

CONCLUSIONS

The management of PWID within the HCV continuum is associated with many challenges that are not traditionally faced by HCP in their normal working lives. However, the potential to provide truly transformative care to marginalised and vulnerable individuals, as well as the knowledge that managing such patients represents the frontline in the battle to achieve HCV elimination, more than makes up for some of the frustrations that can be felt. I have found it a truly rewarding experience.

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Transjugular Intrahepatic Portosystemic Stent-Shunt (TIPSS) for Acute Variceal Bleeding: Has it Come of Age?

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INTRODUCTION

The transjugular intrahepatic portosystemic stent-shunt (TIPSS) originated from imaging studies in the 1960s and 1970s, which led to the establishment of transjugular intrahepatic portal vein cannulation and the creation of a portosystemic shunt.[1,2] The first successful clinical application of TIPSS using expandable stents was in 1988 for variceal bleeding.[3] The main reasons for its implementation were salvage therapy and for the prevention of variceal rebleeding. Despite the introduction of covered TIPSS, studies have not consistently demonstrated a survival benefit in secondary prevention.[4] However, controlled trials in the 21st century have led to a paradigm shift in the utility of TIPSS in acute variceal bleeding as a result of careful patient selection and timing of the procedure.

PRE-EMPTIVE TIPSS FOR ACUTE VARICEAL BLEEDING (TABLE 1)

It is important to highlight that pre-emptive refers to TIPSS performed during the acute variceal bleeding episode in a stable patient, in contrast to secondary prevention where patients undergo TIPSS after the acute bleeding episode as an elective procedure. The aim is to select patients at high risk of rebleeding for a TIPSS procedure and at the earliest opportunity. The first of these trials was undertaken by Monescillo et al.[5] Patients were randomised to TIPSS or endoscopic therapy if they exhibited a hepatic venous pressure gradient (HVPG) >20 mmHg within 24 hours of acute variceal bleeding. The trial demonstrated better outcomes with improved survival in the pre-emptive TIPSS arm compared with standard of care.[5] Indeed, patients randomised to TIPSS fared better than those with initial HVPG ≤20 mmHg treated with endoscopic therapy. However, only bare stents were used, and the standard of care did not reflect current practice. Furthermore, the facility to perform HVPG measurement is only available in a few centres and does not reflect the standard of care in many countries. Therefore, this trial had minimal impact on clinical practice.

This study was followed by a randomised controlled trial (RCT) published nearly 10 years ago by Garcia-Pagan et al.,[6] who reported 12-month survival of 86% in the pre-emptive covered TIPSS group versus 61% with standard of care in Child–Pugh Class C (Child's C) cirrhosis patients or Child's B cirrhosis patients actively bleeding at the time of endoscopy. The standard of care was banding in combination with drug therapy. It is worth noting the high mortality rate of 33% at 6 weeks in the standard of care arm, which is higher than would normally be expected.[8]

Table 1: Pre-emptive transjugular intrahepatic portosystemic stent-shunt: Randomised controlled trials.

Trial	Type (number of recruiting institutions)	Randomisation/ entry criteria	Treatment arms (number of patients)	Follow up (months)	Early rebleeding (<5 days)	Encephalopathy	Treatment failure rate (failure to control bleed or prevent rebleed)	In -hospital mortality	1-year mortality	Comments
					TIPSS versus control					
Monescillo et al.,[5] 1997–2000 (Italy)	RCT (2)	HVPG >20 mmHg within 24 hours of admission.	Bare TIPSS (26) vs. Endoscopic and pharmacological therapy (26)	12 (all included patients)	4% vs. 12%; p=NS	31% vs. 35%; p=NS	12% vs. 50%; p=0.001	11% vs. 38%; p<0.05	31% vs. 65%; p<0.05	Standard of care does not reflect current management. Only bare stents used.
Garcia-Pagan et al.,[6] 2004–2007 (EU)	RCT (9)	Child's B with active bleeding or Child's C <14 points. Randomisation within 24 hours of admission.	Covered TIPSS within 72 hours (32) vs. Endoscopic and pharmacological therapy (33)	16 (median)	3% vs. 13%; p=NS	25% vs. 39%; p=NS	3% vs. 50%; p<0.001	N/A	14% vs. 39%; p<0.001	Patients with gastric varices GOV1 and GOV2 were included.
Lv et al.,[7] 2011–2017 (China) Abstract	RCT	Child's B or C.	Covered TIPSS within 72 hours (84) vs. Endoscopic and pharmacological therapy (45)	N/A	N/A	N/A	N/A	N/A	14% vs. 27%; p=0.039	Early TIPSS reduced early rebleeding and failure to control bleeding. Survival benefit seen regardless of severity of liver disease or active bleeding. MELD 12–18 had greatest survival benefit.

Child's B: Child–Pugh Class B; Child's C: Child–Pugh Class C; EU: European Union; MELD: Model for End-Stage Liver Disease; N/A: not applicable; NS: not significant; RCT: randomised controlled trial; TIPSS: transjugular intrahepatic portosystemic stent-shunt.

The definition of pre-emptive or 'early' TIPSS was within 72 hours of endoscopically controlling the bleed. This was followed by a retrospective post-RCT surveillance study by the same group screening 659 patients, of whom 584 were excluded.[9] Again, they found an 86% 12-month survival compared with 70% with endoscopy and drug therapy. However, this was only a trend to improvement compared with endoscopy and drug therapy, as opposed to reaching statistical significance (p=0.056).[9]

These two RCT were followed by a number of retrospective and prospective audits with variable results.[10-14] A French study reported better outcomes with pre-emptive TIPSS, but only 6.7% of those eligible for pre-emptive TIPSS underwent this procedure and this group tended to have less severe liver disease. Furthermore, it was liver disease severity that correlated with survival rather than pre-emptive TIPSS.[12] Recent data have led to some debate regarding the inclusion criteria for pre-emptive TIPSS.[12-16] While Child's C disease has been shown to consistently to correlate with improved survival following pre-emptive TIPSS, this has not been the case for Child's B patients with active bleeding.[12-16] A recent large observational study from China showed that only patients with Child's B disease and active bleeding obtain benefit from pre-emptive TIPSS regarding 1-year survival. However, the findings must be interpreted with caution in light of the intra-observer variability and heterogeneity of reporting active bleeding.[16] Moreover, patients with Child's A disease were also included. Thus, the evidence supporting bleeding as a high-risk criteria is not consistent and further controlled studies are necessary to confirm the utility of this criteria in selecting patients for pre-emptive TIPSS. A recent observational study also showed that patients with a Model for End-Stage Liver Disease (MELD) score ≥19 are likely to benefit from pre-emptive TIPSS,[15] a finding confirmed by Lv et al.[16] It is not clear from these studies if there is a ceiling of severity for liver disease beyond which there is no benefit from pre-emptive TIPSS, although a UK study reported that salvage TIPSS in patients with a Child-Pugh score >13 is likely to be ineffective.[17]

UPDATES FROM THE INTERNATIONAL LIVER CONGRESS 2019 (TABLE 1)

As mentioned previously, there are just two published RCT of pre-emptive TIPSS for acute variceal bleeding.[5,6] However, a relatively large RCT from China was presented at the 2019 International Liver Congress (ILC), Vienna, Austria, and published in abstract form.[7] The key difference between this trial and that of the previous trial of covered stents[6] is the inclusion of patients with Child's B and C cirrhosis without any requirement for active bleeding. Furthermore, active bleeding did not influence the risk of death or transplantation. The results confirmed that pre-emptive TIPSS resulted in improved transplant-free survival in all patient subgroups, with benefit seen particularly for those with a MELD score of 12–18.

A systematic review of individual data of 169 high risk patients undergoing pre-emptive TIPSS studied the benefit of pre-emptive TIPSS when risk was stratified according to age, Child-Pugh Class score, creatinine, and alcohol aetiology.[18] All groups obtained a survival benefit, in particular those stratified according to lower risk score.

CONCLUSION

The survival benefit of pre-emptive TIPSS is clear in high-risk patients. However, the high-risk criteria, in particular active bleeding in Child's B patients, is debatable due to conflicting data from RCT and observational studies.

One of the major barriers to implementation of pre-emptive TIPSS is the logistical issue of arranging a procedure as an 'emergency' in a stable patient where there is control of bleeding, even in centres with keen multidisciplinary teams. Clinicians may also be reluctant to accept the benefits of pre-emptive TIPSS, as shown in the study by Thabut et al.[12]

In conclusion, the data to support universal adoption of early or pre-emptive TIPSS in all high-risk groups are emerging at this time. The results of a UK RCT of pre-emptive TIPSS for variceal bleeding are eagerly awaited in view of the paucity of data from controlled trials.[19] A multicentre controlled trial collecting large numbers of patients is a research priority.

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Overview of Alpha-1 Antitrypsin Deficiency-Mediated Liver Disease

EDITOR'S PICK

Bouchecareilh et al. deliver a great overview on the molecular pathology and the clinical phenotypes of homozygous and heterozygous AATD-mediated liver disease. They go into great detail on the different isoforms and what they mean for chronic liver disease, including risk factors and differential diagnosis with various concurring conditions. For many years, hepatologists knew about the condition, but not much attention was given to it because of the lack of established treatment options beyond liver transplantation once the liver advanced to end-stage liver disease. It was not until recently that several new treatment options started to emerge: from promoting autophagy using old drugs licensed for very different indications (e.g., carbamazepine) to innovative gene silencing (siRNA) or even gene editing (CRISPR-Cas) methods currently under clinical or pre-clinical development. These are starting to become exciting times for a once neglected chronic liver disease.

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Abstract

Alpha-1 antitrypsin (AAT), encoded by the *SERPINA1* gene, is a protein mainly produced and secreted by hepatocytes. Some specific mutations affecting *SERPINA1* may cause accumulation of misfolded AAT in the endoplasmic reticulum of the hepatocytes leading to AAT deficiency (AATD). Z-AAT is the most severe and common deficient variant. This mutant is not only retained in the endoplasmic reticulum but accumulates as an aggregate that triggers a cascade of intracellular signalling pathways inducing hepatocyte injury and death. Nevertheless, among all the homozygous ZZ patients only 15% develop liver injury, with a wide-range of disease severities ranging from hepatic fibrosis to cirrhosis or even hepatocellular carcinoma. Due to the lack of knowledge surrounding modifiers associated with Z-AAT-mediated hepatocyte toxicity, it is impossible to screen for AATD patients at risk of liver damage and to develop accurate therapeutic strategies. This review aims to give an overview and update our knowledge of AATD associated with liver disease and discusses possible new therapeutic strategies.

INTRODUCTION

Alpha-1 antitrypsin deficiency (AATD), described in 1963 by Laurell and Eriksson,[1] is a rare inherited disorder with a prevalence of approximately 1 in 2,000–5,000 births in North American and European populations. AATD is associated with lung (emphysema) and/or liver damage (cirrhosis). Mutations in *SERPINA1* (chromosome 14), which encodes Alpha-1 antitrypsin (AAT) protein, cause AATD, which leads to a reduced level of AAT in serum.[2,3] The secretion rate functionality of AAT depends on the inherited mutation.[3]

Hepatocytes are the major source of AAT production in the liver. Around 1.5 g/L of this protein is secreted into the bloodstream in healthy adults. The main function of AAT is to protect the lung from non-specific protease-mediated degradation. AAT is carried by blood to the lung interstitium and the alveolar lining fluid, where it inhibits neutrophil serine proteases, such as neutrophil elastase (NE), cathepsin G, and proteinase-3. AAT is the main natural antagonist of NE. The latter is responsible for elastin degradation, the main constituent of the alveoli essential for the lung function.[4] NE also presides over a wide range of pro-inflammatory actions potentially leading to several types of lung alterations, including emphysema and bronchiectasis.[5] Thus, a reduced AAT serum concentration, as observed in AATD, results in lung tissue damage and major respiratory diseases, such as emphysema.[6]

In addition to the lung issues, some AATD patients may also develop liver diseases such as cirrhosis or hepatocellular carcinoma (HCC). AATD is the most common genetic cause of liver disease in children.[7] Over the past 20 years, there has been an increase in the prevalence of the adult form of AATD-mediated liver disease. More than 88% of patients who undergo liver transplantation for AATD are adults, with the peak age range being 50–64 years. The adult form of the disease seems to be an age-dependent degenerative disease. This is in contrast to the paediatric form of the disease, the progression and severity of which is associated with other genetic factors.[8]

This wide variation in the severity and form of liver disease among patients is still not well understood, but genetic predisposition could play a role.[9,10] However, the genetic underpinnings of AATD remain unclear, and new mutations, including severe deficient mutations, are yet to be detected. A better understanding of the genetic factors involved in AATD-mediated liver disease could help in the development of new therapeutic strategies. This review aims to provide an overview of our understanding of AATD-mediated liver disease, from the clinical features to the molecular mechanisms. Additionally, it opens a discussion about the therapeutic prospects.

GENERALITY: ALPHA-1 ANTITRYPSIN PRODUCTION, SECRETION, AND ACTION

SERPINA1 is highly polymorphic, with more than 100 alleles identified.[11] The gene is composed of 7 exons and 6 introns located on the q arm of the human chromosome 14 (14q32.1). Transcription

of *SERPINA1* results in 11 mRNA divided into 5 mRNA products: 1.8, 1.9, 1.95, and 2.0 kb-sized transcripts expressed by monocytes, and a 1.6 kb-sized transcript expressed exclusively by hepatocytes. They are generated by variability in transcription start sites and alternative splicing of untranslated exons according to tissues. A translation site beginning at exon 2 results in the production of 418 residues with a 24-residue peptide signal.[12] This peptide signal promotes the targeting of the AAT protein towards the endoplasmic reticulum (ER). In this organelle, AAT is 3-N glycosylated and then acquires native form in the Golgi. AAT is composed of two ß sheets, nine α helices, and a reactive loop, and is secreted into the bloodstream.

AAT is the most abundant serine proteinase inhibitor in human plasma and is mainly produced and secreted by hepatocytes. However, other cells such as neutrophils, macrophages, monocytes, and epithelial cells also produce AAT in smaller quantities. AAT plasma concentration varies from 0.9–2.0 g/L and according to the body's inflammatory regulation.[13] During inflammation (acute phase), AAT levels increase rapidly by 3–4-fold. The protein inhibits a range of pro-inflammatory proteases, such as proteinase 3, cathepsin G, and NE.[14] The mechanism of inhibition has been determined by crystallographic studies. Targets, such as NE, bind to the reactive loop of AAT, which constitutes a trap for the target protease, and cleave at a precise site causing a conformational transition. The amino terminal half of the reactive loop is inserted into the main ß-sheet, which acquires an extra strand. The reactive loop residue, located upstream of the cleavage, flips from the upper to the lower pole of the protein, carrying with it the protease trapped within a covalent complex. The resulting inactive AAT/protease complex is highly stable.[15]

AAT is crucial in inhibiting these molecules and maintaining the balance of protease/anti-protease that are important for lung integrity. Consequently, AATD predisposes patients to lung injury.

ALPHA-1 ANTITRYPSIN DEFICIENCY

As previously mentioned, AATD is caused by a mutation in the *SERPINA1* gene that predisposes not only to lung injury but also liver damage. Over 100 mutations have been described to date and are classified as deficient or null.[16] Null variants are characterised by undetectable levels of AAT in the serum due to nonsense mutations or frameshifts leading to a premature stop codon. However, deficient variants, such as the well-known Z-variant, are characterised by low levels of circulating AAT, generally due to a point mutation or small deletions.[16]

The two variants named 'Z' and 'S' are the most common. These mutants are so named because of their isoelectrofocussing pattern in which they migrate slowly compared to the wild type (WT) isoform referred to as M-AAT. These two variants are caused by point mutations (Glu342Lys and Glu264Val, respectively), which result in aberrantly folded protein.[1,17] The Z variant is the most severe form and its mutation results in the aggregation of Z-AAT, resulting in greater plasma deficiency and potentially hepatocyte toxicity and liver damage.

Z-AAT Mutant

As previously mentioned, the Z-variant is the result of a lysine substitution by glutamate at position 342. This mutation leads to the retention of the Z-variant in the ER,[18] where it interacts with chaperones such as Grp78/BiP, Grp94, Grp170, and calnexin, all of which have been shown to contribute to its ER retention (Figure 1).[19] The Z-variant is retained within the ER in both soluble and aggregate forms. The latter may be bound into the hepatocytes in inclusion bodies (IB) and may predispose to liver injury.[20]

This histopathological hallmark of the disease stains positively in hepatocytes with periodic acid–Schiff after treatment with diastase. The mechanism of IB formation is not clearly understood. It was proposed that these IB are a part of the ER components and could have a protective effect on hepatocytes.[21] Moreover, there is heterogeneity in the distribution and size of IB within hepatocytes, many of which are free or resemble small clouds of 'dust', while others are huge and well-marked. This difference and its association with the disease outcome is yet to be explained.

These structures are formed by the opening of ß-sheet, which can accept the loop of another molecule to form a dimer that extends into

aggregates.[18] Due to the retention of the Z mutant in the ER of hepatocytes, homozygous ZZ patients have approximately 85% lower circulating levels of AAT. The retention and accumulation of this mutant in the ER could have a gain-of-function toxic effect leading to hepatocyte toxicity and death, thus predisposing homozygotes to liver diseases.[22]

Other Mutant-Mediated Liver Disease

The Z-variant is not the only AAT mutant involved in liver diseases. Rare variants, such as Mmalton (Phe51/52 del) and Siiyama (Ser53Phe), may cause liver disease (Table 1).[23,24] An Italian study has reported that among subjects with severe AATD, 11% carried a genotype other than the common Z or S/Z, and 13% of this population had developed a chronic liver disease.[27] According to the Spanish Register of AATD patients, Mmalton is the most prevalent deficient rare variant.[28] Unfortunately, given the extreme rarity of such variants, their detection seems to be underestimated.

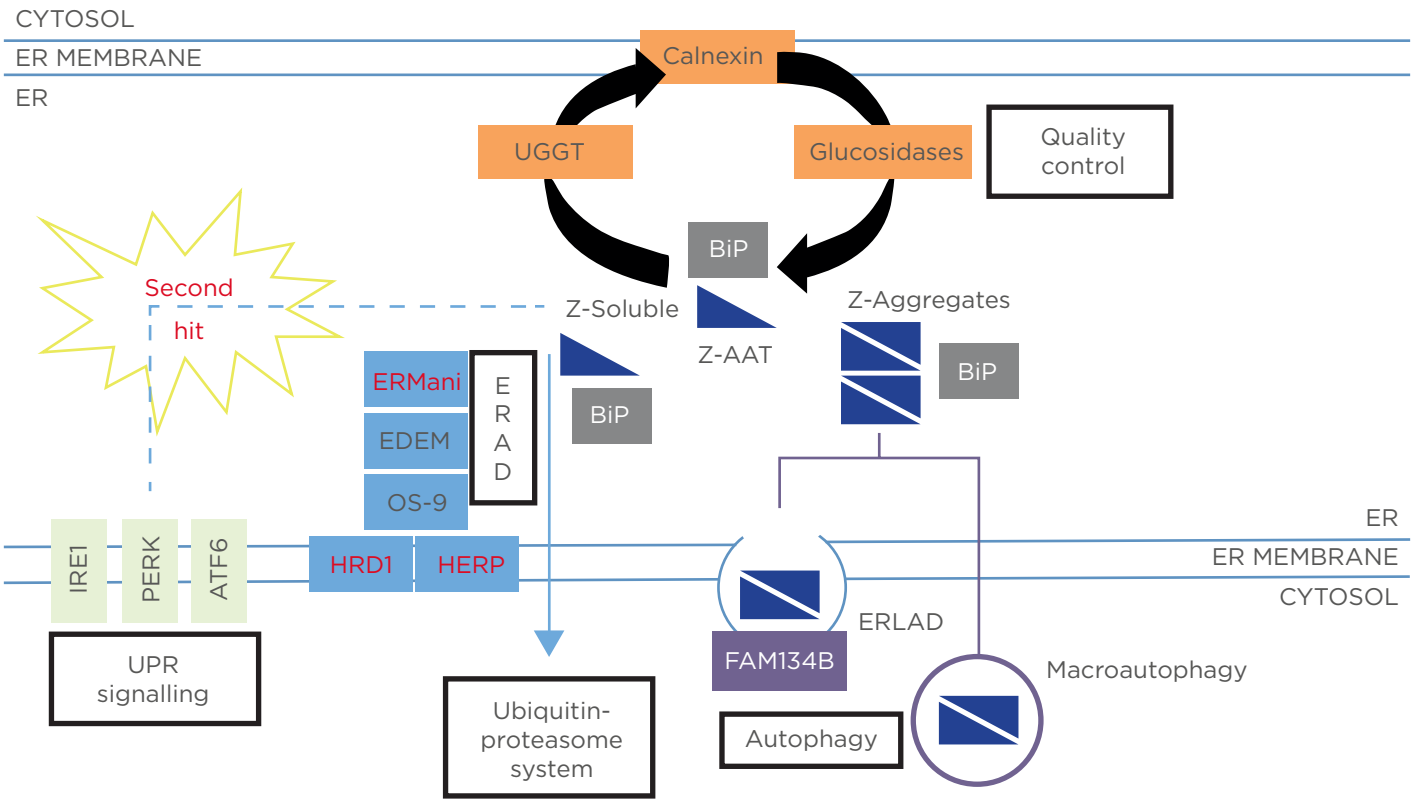

Figure 1: Fate of Z-Alpha-1 antitrypsin within the endoplasmic reticulum.

The nascent Z-AAT undergoes N-glycosylation and enters the quality control/calnexin cycle. A prolonged time of association of Z-AAT with members of the calnexin cycle coincides with its targeting to the degradation pathway. Two major pathways are involved in Z-AAT degradation: ERAD/ubiquitin-proteasome system for the soluble forms (left), and autophagy for the aggregate forms (right). It was shown recently that Z-aggregates undergo a novel clearance pathway named ERLAD. This event is under the control of the ER-phagy receptor FAM134B. The expression and accumulation of intracellular Z-aggregates is not associated with the induction of the UPR. Nevertheless, those aggregates seem to sensitise the cell to a 'second hit', such as *HDR1* or *HERP* mutations (in red) that can induce a UPR more efficiently than observed in wild type cells. Mutations in ERAD members (in red) have been associated with liver disease.

AAT: alpha-1 antitrypsin; ATF6: activating transcription factor 6; BiP: binding immunoglobulin protein; EDEM: ER degradation-enhancing α-mannosidase-like protein; ER: endoplasmic reticulum; ERAD: ER-associated degradation; ERLAD: ER-to-lysosome-associated degradation pathway; ERManI: ER α-mannosidase I; HRD1: HMG-CoA reductase degradation 1; IRE1: inositol-requiring enzyme 1; PERK: protein kinase RNA-like ER kinase; UDP: uridine diphosphate; UGGT1: UDP-glucose glycoprotein glucosyltransferase 1; UPR: unfolded protein response.

Table 1: Alpha-1 antitrypsin variant-mediated liver disease.

Variant	Mutation site	Liver disease	Detection	References
Z	Glu342Lys	Liver damage: fibrosis, cirrhosis, hepatocellular carcinoma	PAS-Diastase positive; polyclonal and monoclonal antibodies	18, 25
Mmalton	Phe51/52 Δ	Risk of severe liver disease	PAS-Diastase positive; calcium precipitates detected by Von Kossa staining	23, 26
Siiyama	Ser53Phe	Risk of severe liver disease	PAS-Diastase positive	24

PAS: periodic acid–Schiff.

Similar to the Z mutant, Mmalton and Siiyama mutants form polymers and IB in the hepatocytes. However, the formative processes and nature of these aggregates are different depending on the variant. First, only the Z aggregates are recognised by the non-commercial monoclonal antibody ATZ11.[25] In addition, IB resulting from the Mmalton variant contain calcium precipitates.[26] A better diagnostic, molecular characterisation, and identification of the factors and stress pathways induced by these rare variants must be undertaken.

ALPHA-1 ANTITRYPSIN DEFICIENCY AND LIVER DISEASE

In 1969, Sharp et al.[29] were the first to establish a link between AATD paediatric homozygous ZZ patients and liver disease. It is now well-known that AATD may be associated with liver damage in infancy, childhood, and adolescence, as well as later in adult life.

However, not all patients develop liver disease, and the symptoms and outcomes of the disease are highly variable: these include the age or stage of the first manifestation, as well as the rate of progression of the damage. Both an acute or chronic form of liver injury may be encountered. A recent review of the Organ Procurement and Transplantation Network database for liver transplantation in the USA has shown that there are two peak ages for severe AATD-associated liver disease: 0–5 and 50–65 years of age.[8]

In brief, a small subset of affected children will develop severe liver disease in the first decade of their life, others may develop and grow up normally, because most adolescents display normal liver enzyme levels[30] even in the presence of advanced liver disease.[31]

In this chapter, the authors discuss the clinical characteristics of the disease, such as the different forms and symptoms associated with AATD during childhood and adulthood. We also discuss the incidence of genetic and environmental factors and the heterozygous status in the development of liver diseases associated with AATD.

Liver Disease During Childhood

The main clinical characteristic of childhood liver disease is usually persistent jaundice, which is a general hallmark of neonatal cholestasis observed during the first 1–2 months after birth. In addition to this, infants may present with itching, bleeding, trouble eating, and elevated liver enzyme levels. Although neonatal cholestasis disappears in 3–6 months, in severe cases, this cholestasis may progress to cirrhosis with early development of portal hypertension and liver failure.[32] In toddlers, the liver damage associated with AATD may present without chronic liver disease or with isolated signs such as an accumulation of fluid in the abdominal cavity (ascites), splenomegaly, or hepatomegaly.

Much of what is known about AATD-associated liver disease during neonatal period and during childhood comes from a prospective study by Sveger,[33] who has determined the frequency of liver disease. In his study, Sveger[33] screened 200,000 newborns in Sweden and identified 127 homozygous ZZ patients who were monitored until 12 and 18 years of age. From this group of homozygotes, 14 had prolonged obstructive jaundice and 9 of the 14 had severe liver disease. The authors concluded that only 15% of homozygous ZZ patients had developed clinically significant liver disease in the first 4 decades of life, and <3% of those had progressed to life-threatening end-stage disease as infants.[33,34] Recently, a study from the French paediatric cohort named DEFI-ALPHA has observed that in France, 18.3% paediatric AATD patients had a severe liver disease and almost half of them ended up with a liver transplantation.[35] The lower rate of liver disease reported in the Swedish study might be due to the mode of patient recruitment. Indeed, the patients in the study by Sveger[33] were recruited through neonatal screening, whereas only patients with known AATD were included in the French study.

As previously mentioned, the majority of AATD patients are healthy or free of chronic disease by 18 years of age. However, for some of them, this period of few or no signs of liver damage will end and severe, progressive liver disease will onset during their adulthood.

Liver Disease During Adulthood

AATD adults may develop chronic hepatitis, cirrhosis, and HCC, and the incidence of liver disease increases with age.[36] Liver disease is more common in adults than in children. Chu et al.[8] have shown that currently more than 88% of patients who undergo a liver transplantation for AATD are adults, with the peak age range being 50–64 years. This condition is under-recognised and undiagnosed during adulthood because biochemical and histopathologic analyses in homozygous ZZ adults may produce results similar to those for alcoholic liver disease.

In adult AATD, advanced liver fibrosis is present without clinical liver disease, and fibrosis on biopsy is detected in approximately one-third of AATD adults.[37-39] In addition, several studies have reported obesity as a risk factor for liver disease in AATD adults.[39,40] The prevalence of hepatic steatosis in adult AATD is higher than 20–30% expected for the general USA population and represents a secondary cause of hepatic steatosis similar to other genetic/metabolic diseases. Valenti et al.[41] evaluated the impact of the two most relevant AAT variants in two large cohorts of nonalcoholic fatty liver disease and chronic alcohol misuse. The authors found that the Z-variant is a major risk factor for cirrhosis in the context of chronic metabolic injury such as nonalcoholic fatty liver disease and chronic alcohol misuse.[41]

To summarise, risk factors for adults with liver disease in AATD are age >50 years, male sex, repeated elevated liver enzymes, hepatitis virus infection, obesity, diabetes, and metabolic syndromes.[39]

Liver Disease and Genetic and Environmental Influences

As previously mentioned, only about 15% of homozygous ZZ patients develop clinically significant liver disease in their childhood. Other co-factors, such as hepatitis, obesity, metabolic syndromes, or alcohol intake, could augment the risk of hepatic disease during adulthood but obviously not during childhood. Consequently, the modifiers in the infant form of severe and progressive liver disease remain unidentified. Since not all patients with the homozygous ZZ genotype develop end-stage disease, it has been suggested that genetic and environmental factors may be implicated in variability of onset and disease severity. From a clinical point of view, AATD patients with neonatal cholestasis are likely to develop severe liver disease.[35] Other risk factors, such as male sex or the absence of breastfeeding, were found to not be associated with severe liver damage.[35] From a cellular/genetic perspective, in 2009, based on a candidate gene-sequencing strategy, Pan et al.[10] demonstrated that differences in ER mannosidase I expression were associated with an earlier age-of-onset for end-stage liver disease. However, the significance of this association has been challenged, as a replicate study in another cohort failed to reproduce the results.[42] Recently, a study that focussed on the *MAN1B1* gene (that encodes the ER mannosidase I protein) and *SERPINA1*

gene showed that no genetic polymorphisms in these two genes influence the onset and severity of liver disease in AATD during childhood.[43] Finally, the authors' group had identified two mutations, *HERPUD1* R50H and *HFE* H63D, that were associated with the advanced liver disease component of AATD.[9] Based on this work, they have also observed that specific pathways, including ER-associated degradation pathway (ERAD) and Unfolded Protein Response (UPR), could be novel risk factors for AATD-caused liver disease (Figure 1).[9] However, these results are in line with the findings of a large-scale screening study for AATD.[9]

The AATD-associated liver disease during adulthood could be predominantly an age-dependent degenerative disease. This hypothesis is consistent with different studies using an AATD mice model that showed differences in the activation and expression of proteins and molecular pathways between livers from young mice and older mice.[44,45] These results suggest that different modifiers (co-factors, stress pathways) are involved in the infant and the adult form of the disease, and that their identification is crucial for accurate treatment of patients.

Alpha-1 Antitrypsin Deficiency and Hepatocellular Carcinoma

Although the incidence of AATD on HCC development is still controversial, some studies have pinpointed AATD as a risk factor for the development of the cancer.[46] Based on the AATD mouse model,[47] the livers of 79 Z-AATD and 18 WT mice were analysed. Liver pathology was seen more frequently in Z-AATD livers (47/79) than in WT (5/18), a development that was also age-related. In older Z-AATD mice (18–24 months), livers showed malignant tumours (HCC and angiosarcoma) (17/50), hyperplastic nodules (28/50), and non-specific changes (33/50), whereas only 9/50 were normal.[47] Similarly, a Swedish autopsy study based on 31 autopsied adults with severe AATD has shown that in the homozygous ZZ group, there were 13 cases of cirrhosis, 5 cases of HCC, and 8 cases of gallstone disease.[48] Consequently, AATD patients are at greater risk of cirrhosis and HCC, with the risk of HCC being higher in males.

From a molecular perspective, a comparative 'omics' approach between diseased human-induced pluripotent stem cell-derived and WT hepatocytes has pinpointed specific proteins associated with predisposition to malignancy that are highly upregulated in the former.[49] More recently, another study has examined the contribution of DNA methylation to AATD adult liver disease heterogeneity, where the global analysis revealed significant genomic hypomethylation in AATD liver impacting genes related to liver cancer.[50]

Heterozygotes

Retention of Z-AAT within hepatocytes is responsible for liver disease. Recently, a clear relationship between Z-AAT accumulation and fibrosis was demonstrated.[39] According to that study, only patients carrying aggregate-forming AAT variants are susceptible to developing liver disease. Thus, it is not surprising that heterozygous patients, such as those with SZ or MZ variants, can also develop severe liver disease in childhood and adulthood.[35,51] Hence, it has been suggested that heterozygosis increases the risk of developing liver disease. Interestingly, the incidence of liver disease could be higher in heterozygotes with the deficiency than in the general population, especially if the affected individuals have other liver comorbidities.[5] Many patients who undergo liver transplantation with a diagnosis of AATD are actually heterozygotes who also have other risk factors (e.g., alcohol usage or steatosis).[8,52] Recently, two cohort studies have shown that that Z allele is a risk factor for cirrhosis in alcoholic and nonalcoholic fatty liver diseases.[53] Among patients with cirrhosis, decompensation of cirrhosis with ascites or encephalopathy was significantly more frequent in patients with MZ than in homozygous MM or WT allele patients.[54] The MZ genotype is a genetic risk factor for more advanced cirrhosis and decompensation, especially if the affected individuals have other liver comorbidities. Z heterozygous variants seem to be strong risk factors for the severity of chronic liver diseases.

Given that the MZ genotype represents 2% of the USA and European populations, and the fact that the Z allele represents a strong disease modifier, Z allele carriers are at higher risk. Hence, genotyping for the Z allele should be considered as the first-line test in all patients with cirrhosis.

Finally, further research is needed to define the clinical features of heterozygous Z and S carriers and determine whether those AAT variants present a clinical phenotype.[53,54]

In conclusion, there is a wide variation in the forms and severity of liver injury among AATD patients and, as mentioned above, very little is known about predispositions. This variability may be related to how the affected individual responds to the intracellular accumulation of Z-AAT in hepatocytes.

Z MUTANT AND MOLECULAR MECHANISM: FROM DEGRADATION TO HEPATOCYTE TOXICITY

Z-Variant Disposal

In the ER, Z-AAT mutant accumulates both in soluble and aggregate forms (Figure 1). Several lines of evidence suggest a dose–response relationship between Z-AAT intracellular protein accumulation and liver injury. Thus, the accumulation of these aggregates is the first event of liver injury cascade. To prevent this dramatic issue, hepatocytes use different degradation pathways to protect themselves from proteotoxicity. Depending on the form of the mutant, soluble or aggregated, different degradation pathways have been identified (Figure 1). The soluble form, which represents most of the intracellular forms of the Z-variant (>80%), interacts with several proteins involved in important pathways, such as quality control or ERAD, before finally being degraded by the proteasome (Figure 1). Previous work has shown that among all the molecules involved in those pathways, one molecule present in the ER, calnexin, is a critical point of control. Calnexin is a transmembrane ER chaperone that binds Z-AAT and targets it for degradation. For instance, it has been shown that inhibitors indirectly affecting the interaction of the Z mutant with calnexin, such as kifunensin (mannosidase inhibitor I and II) or castanospermine (glucosidase inhibitor), restore the secretion of this variant.[55] In addition to calnexin, studies in human fibroblast cell lines from homozygous ZZ patients have shown that patients with liver disease have less efficient Z-AAT degradation than homozygous ZZ patients without liver disease.[56] These results suggest that many other proteins involved in the disposal of the Z mutant could be critical in the liver damage linked to AATD. Among all these potential proteins, different studies have pinpointed the ERAD pathway, with genetic modification in genes encoding the proteins Man1B1 or Herpud1 perhaps altering susceptibility to liver injury by changing the efficiency of Z-AAT degradation.[9,35]

The autophagy pathway is another degradation pathway activated by the cell to prevent liver toxicity. This is a highly conserved and important degradation system specialised in disposal of protein aggregates and large structures via the formation of autophagosomes. Consequently, autophagy could rather be involved in the degradation of Z mutant aggregates that are not capable of being eliminated by the protea some. It has been shown that autophagosomes were abundant in the hepatocytes of homozygous ZZ patients and that Z-AAT retention in the ER is associated with a marked autophagic response.[50] Consequently, some studies have observed that enhancing autophagy may reduce hepatotoxic effect. For instance, weekly administration of rapamycin, a mTOR pathway inhibitor, in a mouse model increased autophagy and diminished Z-AAT aggregates accumulation, subsequently leading to reduction of liver injury.[57] This is also observed using another autophagic drug, carbamazepine (CBZ), a drug that reduces the hepatic fibrosis and inflammatory response associated with the Z-AAT aggregates in a mouse model.[58]

Nevertheless, several questions concerning autophagy and AATD are still unclear and controversial. What activates this pathway? Which type of autophagy (e.g., ER-phagy, macroautophagy) is activated by the aggregates? What is the mechanism of vesicle/autophagosome formation? Some studies suggest an intervention of macroautophagy in the clearance of luminal aggregates; this would imply their dislocation across the ER membrane, or the capture of ER portions containing them by autophagosomes.[59] Conversely, other studies based on the transcription factor EB master gene (*TFEB*) that regulates the autophagy pathway have speculated that mechanisms independent of mTOR or other classical macroautophagy were involved in the disposal of Z-AAT aggregates.[58,60] In this case, autophagy is induced due to reduced intracellular calcium and inositol levels.

Finally, it has been recently described that an ER-to-lysosome-associated degradation pathway was implicated in the degradation of Z-AAT aggregates.[61] This pathway involves calnexin and the engagement of the LC3 lipidation machinery by the ER-resident ER-phagy receptor, FAM134B (Figure 1). Consequently, Z-AAT aggregate delivery from the ER lumen to endolysosomes for clearance does not require ER capture within autophagosomes. Rather, it relies on vesicular transport, where single-membrane, ER-derived, Z-AAT-containing vesicles release their luminal content within endolysosomes upon membrane-membrane fusion events.[61]

Among these two degradation pathways, ERAD/proteasome and autophagy, it remains unclear whether autophagy is a specific response to the accumulation of Z-AAT or rather a secondary process that becomes more important when ERAD and/or the proteasome are overwhelmed. Based on a yeast Z-AAT expression system, it seems that the trigger for induction of autophagy by Z-AAT might be the formation of Z-AAT aggregates.[62] Indeed, at low levels, the Z mutant remains soluble and is disposed by the ERAD/proteasome pathway. Conversely, higher levels of Z-AAT expression induce aggregate formation, thereby activating the autophagy pathway required for their degradation. Another study on Z mouse liver extracts showed that polyubiquitin conjugates were accumulating, despite normal recruitment to catalytically active 26S proteasomes. This suggests that a defect at the 26S proteasome, other than the compromised binding to polyubiquitin chain or peptidase activity, plays a role in this accumulation.[63] It is still unknown whether this event reflects a lack of response to an increased demand for the proteasome or a response counteract by rapid elimination of damaged subunits and/or complexes.

In addition to these two major degradation pathways, the cell can also protect itself from Z-AAT aggregates by activating other degradation pathways. For example, in a murine hepatoma cell line, Z-AAT is degraded by a non-proteasomal mechanism, which is sensitive to tyrosine phosphatase inhibitors.[64]

Even if hepatocytes use different systems to protect themselves from the Z-AAT proteotoxicity consequences, in 15% of the homozygous ZZ patients those degradation pathways are not sufficient and Z-AAT aggregates will trigger multiple signalling events, finally leading to cellular toxicity and death.

Z Mutant and Proteotoxicity

Although several aspects of the disease pathogenesis are still unclear, accumulation of Z-AAT aggregates can affect various intracellular signalling pathways leading to injury cascade, including apoptosis. Hence, mitochondria have been closely associated with the Z-AAT aggregates toxicity via their role in apoptosis induction. In a Z-AAT mouse model, both mitochondrial and liver injuries were reduced with the administration of cyclosporin A, an inhibitor of mitochondrial permeability transition.[65] Recently, transcriptome and proteome analyses using human induced pluripotent stem cells derived from patients with Z-AAT differentiated into hepatocyte-like cells in comparison to patient-specific genetically corrected hepatocyte-like cells, have confirmed that Z-AAT aggregates were associated with disrupted mitochondrial structure.[49]

Other pathways have been linked to the Z-variant and cell toxicity. For instance, the accumulation of Z-AAT aggregates triggers NF-κB signalling through a pathway named 'ER overload response'.[66] Although this response remains unclear, it seems that a leakage of calcium from the ER might have a role in its activation. Moreover, it has been shown that the kinases c-Jun N-terminal kinase 1 (JNK1), 2 (JNK2), and c-Jun play important roles in the pathogenesis of the liver disease by controlling the degree of Z-AAT accumulation.[67] Finally, oxidative stress was shown to contribute to liver damage in a murine model of Z-AAT. Higher levels of reactive oxygen species and a more oxidised cellular redox state are observed in liver tissue from Z-AAT mice compared to WT mice.[67]

Interestingly, the accumulation of intracellular Z aggregates is associated with multiple pathways, but in the absence of the induction of the UPR. This pathway is generally activated when misfolded proteins accumulate within the ER. Thus, UPR activation reduces the entry of nascent proteins into the ER and improves the folding and degradation of misfolded proteins. One hypothesis is that the

absence of UPR activation might be caused by the structure of the Z-AAT aggregates. Indeed, these structures are relatively well-folded, and they might not contain hydrophobic areas/misfolded parts. Nevertheless, the accumulation of Z-AAT aggregates seems to sensitise the cell to a 'second hit' that can induce an UPR more efficient than that observed in WT cells (Figure 1).[9,68]

THERAPEUTIC STRATEGIES

Currently, there is no specific treatment for AAT-associated liver disease, other than standard liver supportive care, such as ursodeoxycholic acid.[69] Generally, management of the disease focusses on preventing the complications of chronic liver disease.[69] In severe cases, when life-threatening liver disease does develop, liver transplantation is performed with excellent success rates.[69] Following liver transplantation, the serum AAT levels return to normal.[69] To overcome the absence of specific treatments, several therapeutic strategies have been employed. They consist of interfering in the different pathways in which the Z-variant is involved (Table 2).

Reducing the Amount of Z-AAT Aggregates

Since the accumulation of the Z-variant aggregates into the ER can trigger liver damage, one of the first strategies implemented is to reduce this accumulation in the ER.

For this purpose, small interfering RNA (siRNA), targeting mRNA encoding human AAT, have been developed by two biotechnology companies: Arrowhead Research Corporation and Alnylam Pharmaceuticals, Inc. In transgenic mice expressing the Z-variant, siRNA treatment reduced the accumulation of Z-AAT in hepatocytes, the formation of inclusion bodies, and liver fibrosis.[70] Moreover, in non-human primates, 80% of AAT in the bloodstream is reduced following siRNA treatment. This strategy has been extended to humans. In June 2015, Phase I/II clinical trials using the siRNA named ALN-AAT was conducted in healthy patients and homozygous ZZ patients who developed liver damage. Although circulating AAT levels decreased significantly (about 75% on average) up to 6 months at a single dose of 6 mg/kg, liver enzymes increased in three patients, indicating liver.[83] The other candidate is the Arrowhead siRNA AAT, named ARC-AAT. A Phase I study, in healthy volunteers and AATD patients, had been launched and was terminated in January 2017. ARC-AAT was well-tolerated and induced deep and durable reduction of AAT. Thus, a Phase II study was recently initiated to determine the safety and effect on circulating and intrahepatic AAT levels of ARC-AAT.[84,85] Further details about and the conclusions of this programme are pending.

Rather than targeting mRNA, another way to prevent the accumulation of Z-AAT in the ER would be to directly correct the mutated gene by using a gene editing approach, such as CRISPR/Cas9. The CRISPR/Cas9 approach uses a guide RNA, which is homologous to that of the DNA to be excised/targeted, and a Cas9 enzyme, which is an endonuclease, the co-operation of which leads to a DNA double strand break at a specific location. This approach has already been used in a transgenic mouse model expressing human Z-AAT.[71] A guide RNA specific for *hSERPINA1* expressed in the liver has been used with the aim of disrupting the gene and reversing the disease phenotype. Thus, with this treatment, the author has shown a reduction in protein aggregation, hepatocellular proliferation, and liver fibrosis without off-target DNA editing.[72]

Increasing the Degradation Pathway

Autophagy and the ERAD/proteasome pathway mainly remove the aggregate and the soluble Z-AAT forms, respectively. Therefore, one of the strategies would be to increase these degradation pathways. The most promising advances have been with autophagy enhancer treatments. One interesting avenue involves CBZ, a drug largely used for epileptic treatment, which has been shown to enhance both autophagy and proteasome pathways in AATD cellular and mouse models. CBZ increases intracellular degradation of the Z-variant and, in a mouse model, administration of large doses (>10-times the recommended dose for humans) reduced intrahepatic Z-AAT inclusion and reversed hepatic fibrosis associated with AATD.[58] Following these encouraging results in 2012, a Phase II clinical trial has been conducted

on 30 AATD patients with severe liver disease. This clinical trial will be finished in 2020 and will provide us more details on CBZ efficacy in humans.[86]

Table 2: Therapeutic strategies for alpha-1 antitrypsin deficiency-associated liver disease.

	Name	Mechanism	Disease models	Stage of development	Reference(s)
Genetic approaches	siRNA	Targeting mRNA encoding human AAT; reducing the accumulation of AAT in hepatocytes and liver fibrosis; reducing the formation of inclusion bodies	Mouse model; non-human primates	Clinical trials	70
	CRISPR/Cas9	Gene editing approach; reducing protein aggregation, hepatocellular proliferation; and liver fibrosis	Mouse model	Clinical trials	71, 72
Autophagic enhancers	Drugs: carbamazepine, rapamycin, fluphenazine, pimozide Gene therapy: transcription factor EB	Autophagy-inducing agents; induce autophagic disposal of Z-AAT aggregates, decrease liver aggregates; inflammation, and fibrosis	*Caenorhabditis elegans;* mammalian cell lines; mouse model	Clinical trials for carbamazepine compounds	57, 60, 73-76
Chaperones	4-phenylbutyric acid; suberoylanilide hydroxamic acid	Histone deacetylase inhibitors; increase Z-AAT secretion	Mammalian cell lines; mouse model; human	Human randomised trial failed	55, 77, 78
Aggregation blockers	Structure-based drugs; monoclonal antibody: mAb4B12	Structure-based drugs that target the hydrophobic pocket and the reactive centre loop of Z-AAT. The antibody interacts with the helix-rich region spanning helices A, C, G, H, and I. These agents reduce the aggregation of Z-AAT	*In silico* screening; *in vitro*; mammalian cell lines	These agents have not been tested in animal models	79-82

AAT: alpha-1 antitrypsin; PAS: periodic acid–Schiff.

As observed with CBZ, another pro-autophagic agent, rapamycin, has been shown to be effective in clearing Z-AAT aggregates in cell lines and in mice that express the Z-variant. This was associated with a reduction in markers of hepatocellular injury, such as hepatic fibrosis.[57] Given these findings, and as these two pro-autophagic agents do not act through the same molecular pathway, a combined treatment of CBZ (mTOR-independent) and rapamycin (mTOR-dependent), would be interesting to test. In the light of pro-autophagy and AATD, a drug screen based on an AATD *Caenorhabditis elegans* model has identified several other drugs that enhance the autophagic disposal of Z-AAT.[73] Finally, an alternative approach to enhance autophagy is to use a viral vector that expresses the transcription factor TFEB, which is a master regulator of autophagy. TFEB localises to the lysosomal membrane, and, upon dephosphorylation, the factor is activated and transported into the nucleus where it acts as a transcription factor. TFEB has been shown to modulate lysosomal clearance[74] and induce autophagy.[75] The effect of TFEB gene transfer on the induction of autophagy and lysosomal biogenesis has already shown efficacy in lysosomal storage diseases.[76] Concerning AATD, it has been shown in a mouse model that gene transfer of TFEB reduced the accumulation of Z-AAT, liver apoptosis, and fibrosis.[60]

Improve the Folding/Inhibit Polymerisation

Another strategy to reduce the accumulation of Z-AAT is to improve its folding and/or increase its secretion by, for instance, using chemical chaperones that can facilitate mutant protein folding and trafficking. Among these chemical chaperones, suberoylanilide hydroxamic and 4-phenylbutyrate, two histone deacetylase inhibitors, have been shown to increase the secretion of Z-AAT in cellular and *in vivo* models.[55,77] However, a human randomised trial failed to demonstrate the efficacy of 4-phenylbutyrate.[78]

Another avenue of treatment could be a 'structure-based' drug development.[79] A better knowledge of polymer formation has permitted drug discovery. Polymers have a hydrophobic pocket, which accepts an exogenous reactive loop peptide. Mutation in this hydrophobic pocket has been initially shown to reduce Z-AAT aggregate formation and increase the secretion of Z-AAT using a *Xenopus* oocyte expression system.[80] Furthermore, small-molecule drugs that target the hydrophobic pocket and peptides that target the reactive centre loop of AAT have been tested and were found to prevent Z-AAT aggregation.[81] Nevertheless, some of these drugs and peptides increase intracellular degradation rather than Z-AAT secretion, or improve the rate of secretion but lead to more Z-AAT intracellular accumulation. Moreover, these peptides have not been tested in animal models.

Recently, monoclonal antibodies such as mAb4B12 were shown to reduce the accumulation of Z-AAT polymers *in vitro* but failed to prevent Z aggregation and increased its secretion *in cellulo.*[82]

Other Treatments

As mentioned previously, oxidative stress has been associated with AATD liver damage. Antioxidant therapy, such as with Vitamins A, C, and E, and N-acetylcysteine could be interesting to test and evaluate.

With the recent advances in our knowledge of gene editing, a cell transplantation therapy for AATD could be also considered. Ding et al.[87] demonstrated that WT donor hepatocytes could almost completely repopulate the liver of the AATD mouse model. In addition, a biallelic correction of the Z-AAT point mutation (Glu342Lys) in human iPSC (from a homozygous ZZ patient) had been achieved by a combination of zinc finger nucleases and piggyBac technology.[51] Subsequently, these cells were engrafted into the liver of a transgenic mouse model, which were able to colonise the liver *in vivo* and display functional activities. This exciting strategy could protect against COPD and liver damage associated with AATD.

CONCLUSION

AATD is a genetic disorder associated with an increased risk of liver disease in children and adults. AATD is caused by mutations in the *SERPINA1* gene encoding the AAT protein. Among mutations responsible for AATD, Z mutant is the most severe and common deficient variant.

Homozygous ZZ patients have low circulating level of AAT. This is caused by the retention and accumulation of soluble and aggregate Z-variants in the ER of hepatocytes where it is mainly synthesised and secreted. The accumulation of Z-variant aggregate triggers intracellular signalling pathways, such as apoptosis, NF-κB, and JNK signalling, and causes liver damage. Some homozygous ZZ patients may develop liver damage, with variable severity, such as cirrhosis or HCC. The cause of this variability in severity of liver disease remains unclear. The development of new therapies, for example RNA technology, gene repair (CRISPR/Cas9), stimulating degradation pathways, and inhibiting aggregation via chemical chaperones and drugs, would negate the need for liver transplantation, which is currently the only curative treatment.

In the future, one of the major challenges will be to identify modifier-mediated Z-AAT toxicity and understand how these modifiers act. This will open a new personalised approach to treatment and could be used as biomarkers of the hepatic disease outcome. Moreover, further studies are needed on liver disease among adults with AATD and on the potential risk to subjects carrying rare variants of the disease.

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Non-Invasive Imaging Modalities in Nonalcoholic Fatty Liver Disease: Where Do We Stand?

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. Liver biopsy is the gold standard for diagnosis and staging of fibrosis in patients with NAFLD; however, it is invasive, costly, and may be associated with morbidity and even mortality, so is not suitable for screening the large number of individuals who are at risk of, or have, NAFLD. Therefore, there has been tremendous focus on finding non-invasive diagnostic modalities, including imaging. New imaging modalities are emerging and may potentially replace biopsy. This review discusses the different non-invasive imaging modalities for the assessment of NAFLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in developed countries.[1] It is defined as the presence of at least 5% of hepatic steatosis on histology or imaging in absence of significant alcohol use and other secondary causes of steatosis.[2] NAFLD has been clinically associated with metabolic disorders such as obesity, diabetes, and dyslipidaemia. It consists of a wide spectrum of clinico-pathologic presentations ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC).[3-6] The top three leading causes of death in patients with NAFLD, in descending order, are cardiovascular disease, cancer, and liver disease.[3] Therefore, early identification of this disease is paramount.

The gold standard for diagnosis of NASH is liver biopsy; however, this is invasive, costly, and risks complications.[7] Thus, biopsy is not practical for the screening or monitoring of NAFLD.[8,9] Non-invasive diagnostic techniques, such as serum biomarkers and imaging studies, have emerged. Imaging, in particular, has gained importance in the non-invasive diagnosis of hepatic steatosis.

IMAGING IN NAFLD/NASH

Ultrasonography

Ultrasonography is the most commonly used imaging modality for evaluating hepatic steatosis.

Ultrasound (US) is accepted as an initial screening for fatty liver because it is safe, widely available, well tolerated, and inexpensive.[10-13] It also plays

a key role in ruling out focal liver lesions and characterising them.[14] There are numerous sonographic features of steatosis, such as the 'echogenicity' of the liver relative to the adjacent right kidney, hepatomegaly, and blunting of liver structures. Recent studies suggest that fatty infiltration of the liver can change the Doppler waveform of the hepatic veins.[15,16] The degree of steatosis can be subjectively scored as mild, moderate, and severe, or, as reported in some studies, by using ordinal US scores.[17,18]

In a large meta-analysis of patients with suspected or known liver diseases, the reported sensitivity and specificity of US in distinguishing moderate-to-severe fatty liver from the absence of steatosis, was 85% (80–89%) and 93% (87–97%), respectively. Nevertheless, US lacks the sensitivity for detection of liver fat and is considered inaccurate in differentiating fibrosis from steatosis or quantifying the fat accumulation. US can only detect steatosis if the liver fat content is above 12.5–20.0%.[9] Another major weakness of US is its operator dependency. Numerous factors can affect the sonographic features besides hepatic steatosis, such as obesity, renal disease, equipment-related factors, operator dependency, and the qualitative interpretation. Consequently, US has limited accuracy, repeatability, and reproducibility for diagnosis and evaluation of the degree of hepatic steatosis.[20-23] Such limitations may be at least partially overcome by semi-quantitative indices, which are correlated with metabolic derangements and histological features in various liver diseases, notably including NAFLD both in adults and in children.[24,25] Despite its undisputed limitations, US remains a first-line option technique in the investigation of NAFLD.[26]

Computed Tomography

X-ray CT uses the density of liver to spleen ratio to detect hepatic steatosis. NAFLD is typically an incidental finding on CT that is being performed for another indication. CT has fallen out of favour for diagnosis of hepatic steatosis for multiple reasons, including exposure to ionising radiation and lack of accuracy and reliability, especially for the detection of small fractions of fatty infiltration.[27] Moreover, it has been demonstrated that CT attenuation values vary significantly between different manufacturers' scanners and image processing techniques.[28]

Box 1: Relative cost of current available non-invasive techniques for liver steatosis assessment.

Technique	Procedure cost
US	Low
CT	Fair
MRI	High
MRS	High

CT: computed tomography; MRI: magnetic resonance imaging; MRS: magnetic resonance spectroscopy; US: ultrasonography.

Magnetic Resonance Imaging

Magnetic resonance (MR) spectroscopy (MRS) is reportedly the most accurate method for the quantification of steatosis,[29,30] but its use is currently limited to research. MRS may be better than histology in assessing longitudinal changes in liver fat content, and is also safe; however, it is expensive and not widely available (Box 1).[31]

Magnetic Resonance Elastography

Magnetic resonance elastography (MRE) is the MR equivalent of transient elastography that is considered among the final options to assess hepatic fibrosis in patients with NAFLD. It uses a modified phase-contrast method to image the propagation of the shear wave in the liver parenchyma. MRE has demonstrated excellent diagnostic accuracy and ability to exclude significant fibrosis. Studies have shown that MRE has a sensitivity and specificity of 98% and 99%, respectively, for detecting all grades of fibrosis.[32,33] When coupled with MRI, MRE can be helpful for the screening of HCC. Another advantage is that MRE accuracy is not affected by obesity or cirrhosis. Since the measured liver area is large on MRE, it can avoid potential sampling errors. On the other hand, MRE may be inaccurate in inflammatory conditions and iron overload. MRE may not be practical for routine screening of NAFLD patients because it is costly, time-consuming, and not readily available. The best indication for MRE may be in morbidly obese patients who fail US-based elastography or need detailed liver imaging.

Magnetic Resonance Spectroscopy

(MRS) is the gold standard for quantification of fat in the liver,[34] therefore it can accurately diagnose NAFLD.[35] MRS measures the chemical composition of tissue based on proton signals frequency. Most of the identifiable peaks are derived from water and fat, and the fat signal fraction, also known as proton density fat fraction (PDFF) can be calculated.[34,36] Therefore, MRS is considered the most sensitive and accurate non-invasive method of quantifying liver fat.[30,31,36] MRS has important limitations that preclude its widespread use.[37] MRS is time-consuming, not readily available, and requires additional equipment and special expertise.

Vibration-Controlled Transient Elastography

Vibration-controlled transient elastography (VCTE), also known as Fibroscan® (Echosens, Paris, France), is the most commonly used elastography method.[38] VCTE is a non-invasive point-of-care method of assessing liver fibrosis by using an US-based technology for estimation of liver stiffness measurement (LSM).[39,40] VCTE was originally validated for use mainly in the setting of viral hepatitis.[41,42] Studies have shown robust VCTE quality criteria in patients with NAFLD, which include a minimum of 10 measurements that are used to obtain the median LSM and the interquartile range. Two probes are now available: the M-probe and the XL-probe. The latter probe has been introduced due to the high failure rate of VCTE in obese patients.[43,44] XL-probes possess a deeper focal length, increased amplitude, and lower shear wave frequency; therefore, they are more reliable in obese patients.[45] A multicentre prospective study by Siddiqui et al.[46] on NAFLD patients who underwent VCTE found that the diagnostic accuracy of VCTE in differentiating fibrosis stages was lower than previously reported by Tapper et al.[47]

Controlled Attenuation Parameter

The controlled attenuation parameter (CAP) is a novel tool for the assessment of hepatic steatosis available as an adjunct to VCTE.[48] Based on studies, CAP relies on an M-probe of Fibroscan; therefore, it shares the same limitations as VCTE.[43] The first study that assessed its performance in patients with chronic liver diseases has reported that CAP was able to accurately detect steatosis ≥11%, ≥33%, and ≥66% with an area under the curve of the receiver operating characteristic (AUROC) of 0.91, 0.95, and 0.89, respectively.[49] Nevertheless, a meta-analysis by Karlas et al.[50] suggested that CAP does not provide accurate reliable quantification of liver fat. Another meta-analysis of studies using the M-probe has suggested optimal cut-offs of 248 (237–261) dB/m, 268 (257–284) dB/m, and 280 (268–294) dB/m, respectively, for detection of steatosis.[51] Others have proposed an optimal cut-off of 288 dB/m.[52] The differences in proposed cut-offs can be explained by the variation in BMI and diabetes prevalence in heterogeneous populations, the use of M-probe, and the small sample size in most studies. A multicentre study in NAFLD patients using the XL-probe reported that CAP had an AUROC of 0.76 for detecting steatosis >5% and a 96% positive predictive value.[53] Only two studies have performed a head-to-head comparison of CAP with US, showing that the performance of CAP for detecting and grading liver steatosis was higher than that of US; however, the rate of overestimation was significantly higher for CAP than for US (30.5% versus 12.4%; $p<0.05$).[54] Overall, CAP is a useful technique for the rapid quantification of steatosis, but it still needs to be better validated with the XL-probe in patients with NAFLD.

Acoustic Resonance Forced Impulse Imaging and Shear Wave Elastography

Acoustic resonance forced impulse imaging (ARFI) is integrated into a conventional US device and relies on elastography to estimate the LSM in shear wave speed. Shear wave elastography (SWE) adapts US imaging to evaluate liver stiffness. SWE can perform measurements over a wide range of frequencies and regions and thereby reduce sampling errors. SWE may be considered a screening test for patients with mild fibrosis stages according to Cassinotto et al.[55] and Leung et al.;[56] however, further studies are needed to confirm its applicability to patients with NAFLD. In general, SWE and ARFI are more reliable compared to VCTE in the assessment of liver fibrosis, but the utility of their use in NAFLD is yet to be confirmed as data are currently limited. The quality criteria for the application of ARFI or

SWE are limited; thus, further studies are needed to establish those criteria and to define the role of ARFI and SWE in NAFLD so their readings can be standardised.

Discussion

US is not sensitive but is highly specific for detection of moderate-to-severe hepatic steatosis. MRI–PDFF/MRE is considered the gold standard to quantify liver fat due to its high diagnostic accuracy; however, it may not be routinely available and is expensive. It may be used when other tests fail and can otherwise be reserved for clinical studies. CAP readings can be highly reliable if the interquartile range is <30 db/m.[57] It becomes less accurate with a dynamic range of liver fat; therefore, it is not reliable in differentiating closely related steatosis stages.[42] CAP, when combined with VCTE, may be helpful in screening obese patients for NAFLD. Elastography has gained wide acceptance. The most validated imaging modality in NAFLD is VCTE, which can be performed as a point-of-care test. It is best used to exclude significant fibrosis; however, VCTE is less accurate for low stages of fibrosis. SWE or ARFI may be useful for risk stratification of patients with NAFLD. Imaging in NAFLD is an area of increasing research focus. Further studies are needed to evaluate and quantify the relationship between imaging modalities and clinical status in NAFLD.

Non-invasive imaging methods, together with serum-based biomarkers, can be used as part of targeted screening strategies for NAFLD in primary care settings to improve specialist referral. There is a need for an integrated management plan for NAFLD between primary and secondary care, with robust pathways for subsequent referrals. The absence of well-defined referral strategies can potentially result in missing a substantial proportion of the population at risk.[58]

CONCLUSION

The non-invasive assessment of NAFLD has progressed significantly. It is important to tailor the choice of non-invasive tests to the setting (primary care, tertiary referral centre, or clinical trial) and clinical needs (screening, staging of fibrosis, or follow-up). Although various imaging techniques are available, US remains the first line technique to be adopted in the evaluation of NAFLD. MRI–PDFF is the most accurate method for detection and grading of steatosis, but it is neither routinely available nor affordable, making it strictly used in research. Until now, there is no imaging modality that can reliably discriminate NASH from simple steatosis. Imaging can help with the identification of advanced fibrosis and, therefore, the appropriate referral for a liver biopsy. The combination of serum markers and liver stiffness, measured using transient elastography, can identify NAFLD patients at a high risk of liver-related complications.

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Predictors of Fibrosis Progression in Chronic Active Hepatitis C Related Nephropathy

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Abstract

Background: Hepatitis C virus (HCV) patients have a higher risk of developing renal impairment than health-matched controls. Fibrosis progression in HCV-related nephropathy could be accelerated. The role of angiopoietin 2 (Ang-2) in HCV-related nephropathy and its relationship with platelet parameters and thrombopoietin (TPO) is evaluated in this article.

Methods: Three patient groups were selected: HCV without nephropathy (n=90), HCV-related nephropathy (n=90), and controls (n=60). Laboratory analysis included complete blood count to reveal mean platelet volume and platelet distribution width (PDW), albumin creatinine excretion ratio, estimated glomerular filtration rate, and cryoglobulins. Quantitative real-time PCR, serum Ang-2, and TPO by ELISA, abdominal ultrasonography, and liver stiffness measurement by fibroscan were all conducted.

Results: Ang-2 was significantly higher in HCV-related nephropathy patients (43.0±36.9 pg/mL) when compared to healthy controls (16.6±4.3 pg/mL) (p=0.001). However, when compared to HCV without nephropathy (30.3±22.9 pg/mL), a statistically insignificant difference was noted (p=0.45). Logistic regression analysis revealed that significant fibrosis in HCV-related nephropathy is independently associated with platelet count (β: 0.98; p=0.000; odds ratio [OR]: 2.7), PDW (β: 0.722; p=0.000; OR: 2.1), serum TPO (β = 1.180; p=0.000; OR: 3.25), liver stiffness measurement by fibroscan (β: 1.29; p=0.000; OR: 3.63), and FIB4 (β: 1.07; p=0.000; OR: 2.9).

Conclusion: Ang-2, TPO, PDW, FIB4, and liver stiffness measurement are markers of liver fibrosis and portal hypertension in HCV-related nephropathy.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection represents a great health burden placing significant strain on healthcare resources. More than 180 million patients are chronically infected with HCV globally.[1] Egypt has the highest seroprevalence of HCV, approaching

15% in 15–59-year-old patients in 2008; however, this has shown a promising decline after treatment was revolutionised with the introduction of direct-acting antiviral drugs.[2]

Patients with chronic active HCV have a greater possibility of developing renal impairment than their health-matched controls.[3,4] The pathogenesis of hepatitis C-related nephropathy remains incompletely explained, but is mostly attributed to the deposition of HCV-associated immune complexes in the renal glomeruli and mesangial matrix causing renal injury.[5]

HCV-mediated renal injury could be explained by the presence of CD81 and SR-B1, which act as HCV receptors in the renal parenchyma facilitating viral entrance by endocytosis. HCV-induced upregulation of TLR3 induces mesangial proliferation and continuous immune pressure on B lymphocytes. Chronic HCV enhances the production of cryoglobulins, which are deposited in the mesangial matrix and glomerular capillaries, causing renal injury, glomerular fibrinoid necrosis, and thrombosis due to enhanced platelet aggregation.[6] Insulin resistance induced by HCV enhances the production of intrarenal IGF-1 and TGF-β, which induces endothelin-1 expression, with reduced endogenous nitric oxide production perpetuating renal ischaemia.[7]

Angiogenesis can be stimulated under physiological conditions to allow hepatic regeneration, but when it exceeds these compensatory limits, such as in ischaemic hepatic conditions, it creates a hypoxic microenvironment, and thus angiogenesis had been previously labelled as one of the principle causes of disease progression in viral hepatitis.[7,8] It is well known that angiogenesis is regulated and maintained by angiogenic cytokines, such as vascular permeability factor and angiopoietins.[9]

The angiopoietin/Tie2 signalling system represents an important regulator of angiogenesis. Angiopoietins, including angiopoietin 1 (Ang-1), bind to endothelial Tie2 and trigger autophosphorylation, which enhances endothelial cell survival, stabilisation, and vascular maturation, resulting in anti-inflammatory effects by blocking the effect of TNF-α on leukocyte migration. Tie2 activation induces trafficking of perivascular cells (vascular myocytes and pericytes) through paracrine endothelial substances. Ang-2 competes for Tie2 binding with Ang-1 and, as such, is considered an Ang-1 antagonist. The balance of Ang-1 and 2 is disrupted in chronic inflammatory states and neoplastic conditions, which enhances the vascular instability and leakage.[10-12] Vascular permeability factor mediates the cellular effects of angiopoietins.[13] Integrins, mainly integrin α5β1, have a role in upregulating Ang-1/Tie2 crosstalk.[14]

HCV structural proteins, mainly NS3, may have an important role in stimulation of proangiogenic factors and cytokines but the mechanisms underlying hepatic angiogenesis in chronic HCV-infected patients remain unclear and need to be evaluated.[15]

Thrombopoietin (TPO) is the principal regulator of megakaryocytopoiesis and thrombopoiesis and is mainly produced in the liver. Binding of TPO to its receptors expressed on the surface of the effector cells stimulates thrombopoiesis through enhancing the JAK/STAT signalling pathways. TPO levels are directly proportional to platelet count and are reduced in liver cirrhosis due to decreased production or due to a direct effect by hepatotropic viruses such as HCV.[16] The current study aimed to assess potential markers of fibrosis progression in HCV-related nephropathy.

STUDY DESIGN

Patient Selection

This prospective case-control study was conducted in the outpatient clinic of the Internal Medicine and Hepatology Departments, Zagazig University Hospital, Zagazig University, Zagazig, Egypt, in the period from January 2014–March 2017.

Out of 1,435 patients with chronic active HCV who were evaluated for treatment with direct antiviral agents, 180 patients (12.5%) were enrolled in this study. Questionnaires regarding medical history, drug history, and family history of all participants were obtained. The inclusion criteria the study used were patients aged 18–60 years, with seropositivity for HCV antibodies, positive HCV RNA, and HCV-related nephropathy. The patients were classified into two groups: Group 1 included 90 patients

with chronic active hepatitis C virus without nephropathy with a mean age of 45.2±6.2 years and Group 2 included 90 patients with chronic hepatitis C virus-related nephropathy with a mean age of 44.8±6.6 years. A control group included 60 healthy subjects matched for age and sex after exclusion of HCV, HBV, diabetes, hypertension, and nephropathy with a mean age of 43.9±4.5 years.

Exclusion Criteria

Exclusion criteria used to select patients for the study included causes of nephropathy other than HCV or any condition which may alter ANG-2 levels, such as patients with hepatorenal syndrome; hepatic decompensation; hepatitis B virus; diabetes; autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis; obesity or essential hypertension; current treatments that may induce nephropathy, such as non-steroidal anti-inflammatory drugs, D-penicillamine, gold, alcohol, and contrast dye; previous treatment with antiviral or immunosuppressive drugs; current urinary tract infections; malignancy; and pregnancy.

METHODS

All patients had a clinical examination to assess clinical signs of portal hypertension, such as dilated abdominal veins, splenomegaly, and the condition of the liver, whether shrunken or enlarged; exclusion of features of liver cell failure, such as jaundice, ascites, lower limb oedema, fetor hepaticus, flapping tremors, spider angiomas, palmer erythema; and clinical signs of nephropathy, such as oedema of lower limbs, haematuria, oliguria, hypertension, and signs of volume overload.

All procedures were performed in accordance with the ethical standards of the Zagazig University's Faculty of Medicine research committee and with the 1975 Declaration of Helsinki and its later amendments. Written informed consent was obtained from patients for interview, anthropometric measurements, and blood sampling.

Laboratory Analysis

Complete blood count assessments were performed, including mean platelet volume (MPV) and platelet distribution width (PDW). Liver function tests were also executed, including assessment of total and direct serum bilirubin, serum albumin, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time, prothrombin concentration (%), and international normalised ratio. Kidney function tests included blood urea and serum creatinine.

Albumin-creatinine excretion ratio was classed as normal up to 20 mg/g creatinine, and urine microalbuminuria was classed as normal up to 30 mg/24 hours. Estimated glomerular filtration rate (GFR) was calculated as 140–age x weight x 0.85 if female/72 x serum creatinine.[17] The stages of GFR were classed as:

- Stage 1: 90 mL/min
- Stage 2: 60–89 mL/min
- Stage 3a and 3b: 30–59 mL/min
- Stage 4: 15–29 mL/min
- Stage 5: <15 mL/min or on dialysis

Estimation of cryoglobulins, rheumatoid factor, and complement levels in blood and fasting blood sugar were classified according to the American Diabetes Association (ADA) criteria 2010.

The HBsAg surface antigen, serum α fetoprotein, and HCV antibodies were detected using Cobas® e 411 (Roche Diagnostics GmbH, Mannheim, Germany), a third-generation, commercially available enzyme-linked immunosorbent assay kit.

Quantitative measurement of HCV load in patient sera was carried out by real-time, quantitative PCR (COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0, Roche Diagnostic Systems Inc., Welwyn Garden City, UK), with a detection limit of 15 IU/mL.

Serum level of Ang-2 was measured by ELISA, following the manufacturer's recommendations (Quantikine, R&D Systems, Minneapolis, Minnesota, USA). All measurements were made in duplicate. Absorbance was read at 450 nm and corrected at 570 nm. Assay range: 1.6–140.0 pg/mL.

Serum TPO level was measured using a commercial quantitative sandwich enzyme immunoassay (Quantikine Immunoassay Control Set 934 Human TPO, R&D Systems) with a reference range of 31–2,000 pg/mL.

Abdominal Ultrasonography

All patients were examined using a real-time grey-scale device by a transducer with a frequency of 2.5–5.0 MHz. The patients were examined after a 6-hour fast. Criteria for cirrhosis diagnosis were determined by a coarse, nodular appearance and shrunken size with prominent caudate lobe. Criteria for portal hypertension diagnosis included portal vein diameter >13 mm measured at point of crossing inferior vena cava, splenic bipolar diameter >130 mm, and splenic vein diameter 10 mm. Features of chronic renal disease were excluded.

Liver Stiffness Measurement

Liver stiffness measurement (LSM) was performed by a fibroscan. The number of shots was 10 and the interquartile range ≤25%. Generally, a liver stiffness of 2.5–7.0 kPa denotes F0–1, 7–9.5 kPa indicates F2, 9.5–12.5 kPa indicates F3, and >12.5 kPa denotes cirrhosis.[18]

Statistical Analysis

All data were collected, tabulated, and statistically analysed using SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as the mean±standard deviation and median (range), and qualitative data were expressed as absolute frequencies, both numbers and percentages. For independent samples a t-test was used to compare between two groups of normally distributed variables while the Mann–Whitney U test was used for non-normally distributed variables. The F-test was used to compare more than two groups of normally distributed variables. The Kruskal–Wallis test was used to compare between more than two groups of non-normally distributed variables. Percent of categorical variables were compared using the chi-squared test or Fisher's exact test when appropriate. The Pearson correlation coefficient was calculated to assess the relationships between various study variables. All tests were two-sided. A p value <0.05 was considered statistically significant. Logistic regression analysis was used to elucidate the independent relationships associated with significant fibrosis in HCV-related nephropathy and odds ratios were calculated from exponential beta.

RESULTS

The baseline laboratory, metabolic, LSM by fibroscan values of patient subgroups are shown in Table 1. HCV genotyping revealed that genotype 4a (n=94, 52%) and 4c (n=67, 37%) were predominant, mixed 4.1b (n=19, 11%). The mean values of serum TPO, platelet count, and PDW were significantly lower and aspartate aminotransferase/alanine aminotransferase and FIB4 were significantly higher in patients with nephropathy.

The mean value of LSM by fibroscan was significantly higher in HCV-related nephropathy patients (9.9±3.2 kPa) compared with HCV patients without nephropathy (7.0±1.6 kPa) (p=0.04). F4 or cirrhosis by fibroscan was diagnosed in 10 patients in the HCV-related nephropathy group (11%) versus no patients in the HCV without nephropathy group, which was statistically highly significant (p=0.007), as shown in Table 1.

In the HCV nephropathy group, 48 patients (53.3%) (F4=10, F3=2, F2=4, F0–1=32) had higher values of Ang-2 (74.9±52.6 pg/mL) when compared to 16 patients (17.8%) in HCV without nephropathy (62.3±32.5 pg/mL) (F4=0, F3=4, F2=8, F0–1=4) and no patient in the healthy group showed a high value of Ang-2 (p=0.001). The mean value of Ang-2 was significantly higher in HCV related nephropathy group when compared to the healthy controls (43.0±36.9 pg/mL, 16.6±4.3 pg/mL, respectively; p=0.001). However, when compared to the HCV without nephropathy group (30.3±22.9 pg/mL), a statistically insignificant difference was noted (p=0.45).

In patients with nephropathy and thrombocytopenia, Ang-2 showed a significant negative correlation with platelet count (r=-0.780; p=0.001), PDW (r=-0.540; p=0.001), serum TPO (r=-0.802; p=0.000) (Figure 1), and GFR (r=-0.770; p=0.000), and a significant positive correlation with LSM by fibroscan (r=0.910; p=0.000) (Figure 1), FIB4 (r=0.823; p=0.000), creatinine (r=0.495; p=0.001), and microalbuminuria (r=0.418; p=0.004).

Logistic regression was performed to determine variables independently associated with significant fibrosis in HCV-related nephropathy.

Table 1: Baseline laboratory, metabolic, and fibroscan values of patient subgroups.

Variable	HCV with nephropathy (n=90)	HCV without nephropathy(n=90)	Healthy control (n=60)	p value
Sex (M/F)	54/36	56/34	36/24	0.500
Age	44.8±6.6	45.2±6.2	43.9±4.5	0.700
BMI (K/m^2)	23.0±1.4	23.5±1.2	22.9±1.1	0.300
AST (IU/L)	68.7±4.6	44.7±16.0	16.0±3.0	0.003
ALT (IU/L)	60.0±14.5	50.0±19.0	21.0±7.0	0.001
AST/ALT	1.14±0.21	0.88±0.23	0.78±0.19	0.001
Albumin (gm/dL)	3.7±0.5	4.4±0.2	4.5±0.4	0.030
Total bilirubin (mg/dL)	1.0±0.1	1.12±0.1	0.9±0.1	0.200
Hb (gm/dL)	12.0±1.9	12.3±1.5	13.0±0.9	0.100
Platelet (10^3/µL)	145.0±51.0	206.0±31.0	275.0±29.0	0.014
FIB-4	2.79±0.65	1.42±0.25	0.66±0.18	0.001
MPV (fL)	10.6±1.8	11.3±2.1	11.7±1.6	0.120
PDW (%)	11.9±1.9	14.9±2.4	14.2±3.1	0.035
TPO (pg/mL)	52.4±6.9	89.0±10.9	180.0±17.0	0.001
Ang-2 (pg/mL)	43.0±36.9	30.3±22.9	16.6±4.3	0.001
FBS (mg/dL)	93.5±5.3	85.0±20.0	82.0±10.0	0.340
HbAIc (%)	5.60±0.37	5.2±0.9	4.40±0.37	0.090
Urea (mg/dL)	89.0±14.6	22.0±3.4	19.0±4.0	0.002
Creatinine (mg/dL)	2.60±0.47	0.90±0.08	0.88±0.05	0.001
GFR (mL/min/1.73m^2)	56.0±6.7	91.0±10.6	105.0±1.0	0.010
Albumin/creatinine ratio	618.0±208.0	32.7±11.0	19.4±3.5	0.001
Microalbuminuria	40.0±8.3	20.6±8.9	13.2±7.8	0.001
Cryoglobulins	3.0	0.0	0.0	0.160
LSM (kPa)	9.9±3.2	7.0±1.6	*	0.040
Fibrosis stage n (%)				
F0–1	74 (82)	64 (71)	*	0.120
F2	4 (5)	22 (24)	*	0.001
F3	2 (2)	4 (5)	*	0.400
F4	10 (11)	0	*	0.007

*These tests were not performed for healthy controls.

ALT: alanine aminotransferase; Ang-2: angiopoeitin; AST: aspartate aminotransferase; BMI: body mass index; FBS: fetal bovine serum; GFR: glomerular filtration rate; Hb: haemoglobin; HCV: hepatitis C virus; LSM: liver stiffness measurement; MPV: mean platelet volume; PDW: platelet distribution width; TPO: thrombopoietin.

The variables identified were platelet count (β: 0.98; p=0.000; odds ratio [OR]: 2.70), PDW (β: 0.722; p=0.000; OR: 2.10), serum TPO (β: 1.180; p=0.000; OR: 3.25), LSM by fibroscan (β: 1.29; p=0.000; OR: 3.63), and FIB4 (β: 1.07; p=0.000; OR: 2.90).

When the patients were stratified according to stages of fibrosis by fibroscan, Ang-2 showed a highly significant increase with progression of fibrosis stage compared to serum TPO, platelet count, MPV, and PDW, which showed a significant decrease (Table 2).

DISCUSSION

Renal affection due to chronic hepatitis C is mainly due to immune-complexes or cryoglobulins deposition, vasculitic affection of the renal blood vessels, and a direct viral cytopathic injury.[19] Subclinical renal involvement

should also be highlighted due to the possibility of an underlying occult HCV infection.[20] Still, these mechanisms of renal injury cannot explain all the documented lesions. In addition, the microscopic characteristics of HCV nephropathy have unique features which prove that the virus may induce renal injury via specific mechanisms, including induction of apoptosis, modulating caspases, and NS3 binding to TLR3, which induces mesangial proliferation.[21]

Vascular permeability factor, Ang-2, and matrix metalloproteinase 9 are concomitantly increased in chronic HCV and play an important role in vascular remodelling and fibrosis progression through perpetuation of inflammation, release of fibrogenic molecules from the stimulated endothelial cells, and directly affect hepatic stellate cells. This effect was supported by the increased expression of Ang-2 mRNA in liver biopsies taken from the study patients.[22]

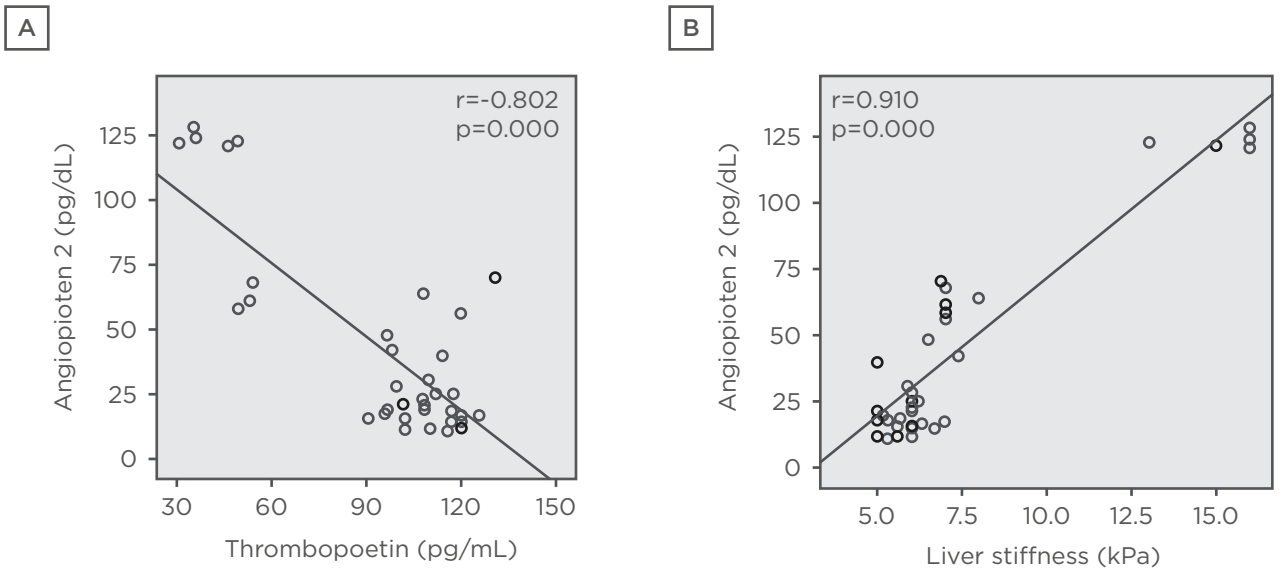

Figure 1: A) Negative correlation between thrombopoietin and angiopoietin 2 levels in HCV positive patients with nephropathy. B) Positive correlation between liver stiffness by fibroscan value and angiopoietin 2 levels in HCV positive patients with nephropathy.

HCV: hepatitis C virus.

Table 2: Relation between fibrosis stage progression by fibroscan, angiopoietin 2, thrombopoietin, and platelet parameters in the studied patients.

	F0–1 (2.5–7.0 kPa) (n=138)	F2 (7.0–9.5 kPa) (n=26)	F3 (9.5–12.5 kPa) (n=6)	F4 (>12.5 kPa) (n=10)	p value
Ang-2 (pg/mL)	36.6±16.7	48.0±20.3	130.0±19.2	143.4±12.8	0.001
TPO (pg/mL)	185.5±13.8	130.9±20.1	72.8±17.5	51.4±8.7	0.001
Platelet count	271.6±22.5	178.3±17.5	134.4±12.5	78.3±3.5	0.001
MPV (fL)	11.2±2.1	10.8±1.8	10.2±1.4	8.9±1.1	0.043
PDW (%)	13.4±1.8	12.6±2.1	11.4±1.9	10.3±1.8	0.036
FIB-4	0.73±0.17	1.16±0.20	2.83±0.19	3.22±0.50	0.001

Ang-2: angiopoietin; MPV: mean platelet volume; PDW: platelet distribution width; TPO: thrombopoietin.

A positive, significant correlation between Ang-2 and stage of hepatic fibrosis in chronic HCV patients would help in the diagnosis and monitoring for disease progression. A non-invasive model has been suggested that includes evaluation of platelets count, transaminases, and Ang-2.[23] Serum values of Ang-2 and Ang-1 correlated with fibrosis progression in HCV patients. The ratio of Ang-2 and Ang-1 may prove to be a useful index for monitoring the progression of chronic liver disease.[24]

Previous research could not define a direct relation between portal pressure and platelet count; however, thrombocytopenia that persists after splenectomy may be corrected after successful liver transplantation.[25]

The level of TPO as the main factor affecting the level of circulating platelets remains variable in cirrhosis due to increased levels as a result of conditions associated with platelet destruction due to enlarged spleen or reduced levels in patients with advanced liver diseases.[26] MPV is directly proportional to the rate of TPO production, therefore it is increased in cases of increased destruction. PDW can indicate the degree of difference in platelet size; however, their variability and use in liver cirrhosis is rarely discussed or studied. Increased MPV and PDW in cirrhosis denotes an increased destruction of platelets along with increased levels of young platelets in the blood if serum TPO is increased.

The current study showed a significant negative correlation between Ang-2 and serum level TPO, and platelet count, MPV, and PDW, as well as a positive correlation between FIB-4 and LSM by fibroscan. Platelet parameters and TPO were decreased in patients with advanced fibrosis or cirrhosis due to the occurrence of portal hypertension. This observation is supported by a study that revealed Ang-2 levels were elevated in patients with cirrhosis and hepatocellular carcinoma compared to the healthy controls (p=0.001) and correlated inversely with markers of synthetic liver function, such as serum albumin and prothrombin concentration, and correlated positively with markers of excretory function, such as serum bilirubin.[27,28]

There was a significant elevation of serum level of Ang-2 in patients with HCV infection when compared to the healthy controls, but insignificant difference was detected among HCV patients when stratified by the presence of nephropathy (p=0.45).

A significant positive correlation was found between Ang-2 and markers of renal injury in HCV as microalbuminuria (p=0.004). These findings were also supported by a study that showed that hepatitis C infection was independently associated with microalbuminuria in subjects without diabetes (OR: 1.99; 95% confidence interval: 1.38–2.85; p=0.008).[29]

This study did not investigate the impact of HCV eradication by direct-acting antiviral agents on the levels of Ang-2 or TPO. Patients with HCV related nephropathy were treated with ritonavir, ombitasvir, and paritaprevir, which was a limitation of the current study.

CONCLUSION

In conclusion, in HCV-related nephropathy, histological progression is anticipated. This can be predicted by platelet count, PDW, FIB-4, serum TPO, and serum angiopoietin 2, which may justify their use as markers of liver fibrosis and portal hypertension in patients with chronic HCV-related nephropathy.

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Overlap Syndrome with Autoimmune Hepatitis and Primary Sclerosing Cholangitis

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Abstract

Patients with autoimmune liver disease frequently fit diagnostic criteria for more than one condition. Up to 12.5% of autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) cohorts have a label of AIH/PSC overlap. There can be an interval of many years between the diagnoses of the two conditions, and the sequence in which they are made is unpredictable. Issues exist with the use of diagnostic criteria validated for AIH in patients with AIH/PSC overlap. There are no agreed criteria for the diagnosis of AIH/PSC overlap, it is based on a combination of biochemistry, autoantibody profile, cholangiogram, and liver histology. A positive diagnosis of AIH/PSC overlap impacts therapeutic options and prognosis. There is a beneficial role for immunosuppression, albeit with a higher relapse rate and evidence of progressive liver disease despite immunosuppression in some cases. Liver related outcomes sit somewhere between the constituent diseases, with better outcomes than PSC but poorer outcomes than AIH. There is an increasing body of data for patients with AIH/PSC overlap undergoing liver transplantation for end-stage disease.

Nearly half of patients with autoantibody positive liver disease in childhood have autoimmune sclerosing cholangitis (ASC). ASC patients are differentiated from those with AIH by having abnormal cholangiograms. Histological analysis shows chronic hepatitis in <50% of ASC cases. The biochemical response to immunosuppression in ASC patients is less than that seen in AIH patients, and cholangiograms commonly show progressive disease. Transplant-free survival of the ASC population is poorer than in AIH.

INTRODUCTION

The autoimmune liver diseases (AILD) have been categorised classically as either autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), or primary biliary cholangitis (PBC). The AILD are a heterogeneous group of conditions with differing pathogenesis, patterns of hepatic injury, and clinical outcomes.[1] Despite this, AIH, PSC, and PBC are frequently grouped together as the AILD because of similarities in clinical presentation, immunological markers, and treatment options.

A subgroup of patients with AILD share common features relating to the different subtypes of AILD; these have been termed the 'overlap syndromes' or 'variant syndromes.'

The significance of these overlap syndromes in AILD remains controversial.[2,3] Do patients simultaneously have two diseases? Are they the product of inaccurate diagnostic criteria that results in the diagnosis of two conditions when patients only have one? Is there, in fact, a continuum between the two disorders? (Figure 1)

This review will summarise the overlap syndromes that share features of AIH and PSC, namely AIH/PSC overlap and autoimmune sclerosing cholangitis (ASC). The authors will review the different presentations between children and adults, which are important as patients transition to adult services.

ADULTS

Demographics

Adults diagnosed with AIH/PSC overlap are significantly younger at the time of diagnosis than those with AIH (AIH/PSC diagnosis age: 24–27 years, compared to 39–46 years for PSC patients).[4-6] Analysis of the United Network for Organ Sharing (UNOS) database of patients who underwent liver transplantation for PSC categorised by age group, identified a higher prevalence of AIH/PSC in the 18–39-year-old age group (2.1%) than in older patients (1.0% of patients aged 40–59 and 0.5% in those aged >60 years).[7] The proportion of adult males with AIH/PSC overlap is 69–81%, which is higher than seen in AIH, which is more prevalent in females.[5,6,8]

Frequency

The frequency of AIH/PSC overlap varies based on the diagnostic criteria used[9] and whether the prevalence has been taken as a proportion of AIH or PSC patients. Biliary changes have been identified in 24% of patients with AIH on liver histology[10] and magnetic resonance cholangiopancreatography (MRCP);[11] however, these cases were not related to a cholestatic syndrome and were thought to be secondary to fibrosis. The diagnosis of AIH/PSC within cohorts of AIH patients ranges from 1.7–12.5%.[4,6,11,12]

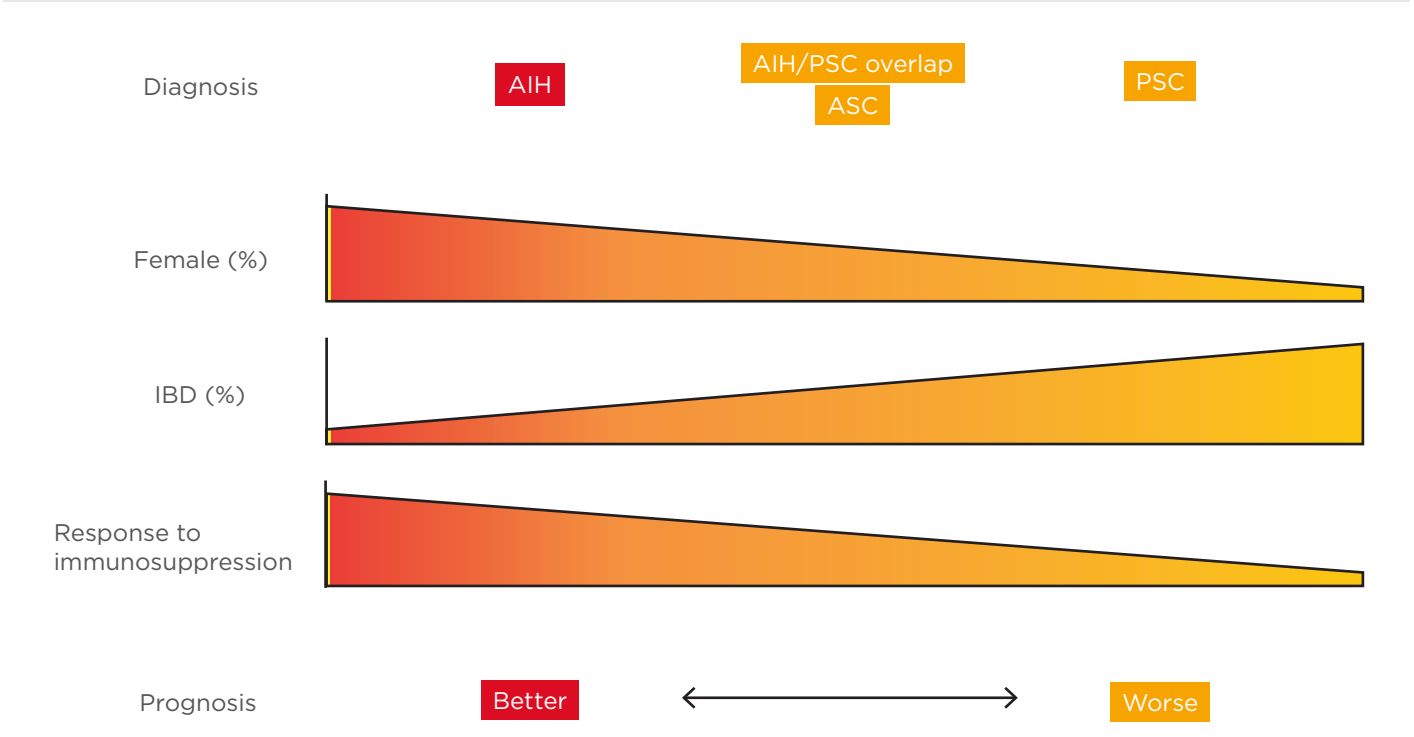

Figure 1: A number of demographics and clinical characteristics differ between autoimmune liver diseases, indicating a better or worse prognosis.

There is a spectrum between AIH and PSC with overlap found in the middle.

AIH: autoimmune hepatitis; ASC: autoimmune sclerosing cholangitis; IBD: inflammatory bowel disease; PSC: primary sclerosing cholangitis.

Larger studies of PSC patients have a definite diagnosis of AIH/PSC in 1.4–9.0%, with up to a further 33.0% with probable AIH/PSC.[13-15]

Inflammatory Bowel Disease

The rates of concurrent inflammatory bowel disease (IBD) diagnosis in patients with AIH/PSC varies significantly between series (ranges between 13–89%) but appears to be less prevalent than in PSC.[4,15-17] Cholangiography was performed to further investigate a subgroup of patients with AIH who were undergoing annual surveillance endoscopy and diagnosed with IBD.[18] At least 29% of those with AIH and IBD had features of PSC which were previously unrecognised. If a patient with AIH develops IBD then further evaluation for PSC is likely warranted.

Timing of Diagnoses

In patients who presented with AIH, the subsequent diagnosis of AIH/PSC overlap was made at a mean interval of 5–9 years later, with cases of up to 15 years from presentation.[16,17] When PSC was the initial diagnosis the mean interval to AIH/PSC overlap was 3.3 years.[15] No cases of small duct PSC progressing to AIH/PSC overlap have been reported.[19] AIH appears to be the more common primary diagnosis (in 31–63% of cases) compared with PSC (19–44% of cases), and in a significant proportion of patients the diagnosis is contemporaneous (19–42%).[8,15,19] Despite the interval between presentation and a formal diagnosis of AIH/PSC often being a number of years, closer analysis of the initial histology frequently identifies features of both disease processes at presentation.[8] A diagnosis of AIH/PSC overlap should be considered at all stages in a clinical course, but particularly early in the diagnosis and if atypical features develop.

Diagnosis

AIH/PSC overlap is mainly seen in young adults, in whom there is a characteristic clinical, biochemical, immunological, and histological picture of AIH with a classical cholangiogram of PSC.[20,21] Up to 94% of AIH/PSC overlap patients have antinuclear antibodies, antismooth muscle antibodies, or anti-liver-kidney antibodies at titres of ≥1:40, which is comparable to AIH and higher than in PSC.[14,22] Aspartate aminotransferase (AST) level at presentation in AIH/PSC is lower than in AIH,[12] while serum globulins and IgG levels are higher in AIH/PSC than in PSC.[14] However, there is a significant challenge in making a definitive diagnosis of AIH/PSC because the diagnostic scoring systems used may have only been validated in AIH and there is no dedicated diagnostic criteria for AIH/PSC overlap.[22,23]

There have been three iterations of the International Autoimmune Hepatitis Group (IAIHG) scoring system for AIH (published in 1993, 1999, and 2008).[24-26] All three IAIHG scoring systems are dedicated to AIH and only 2 of 250 patients in the 2008 derivation cohort had AIH/PSC overlap. Although there are similarities between the IAIHG scores there are also key differences. These may reflect the changes in prevalence of AIH/PSC reported in studies that have used different scores. The original 1993 IAIHG scoring system deducts points for elevated alkaline phosphatase (ALP) to aminotransferase ratio and for biliary features on histology.[24] Out of a group of 114 patients with PSC who were scored using the 1993 IAIHG score, 2% were found to have definite AIH and a further 33% were probable for AIH.[13] This is likely to be a high false positive rate, reflecting the similarities between patients with AIH and PSC and inaccuracies of the scoring system, as opposed to the true prevalence of AIH/PSC. Some modifications were made to the 1999 IAIHG scoring system, and 28 of the 40 patients with PSC who were rescored were reclassified from probable AIH to not AIH.[25] In a larger series of 113 PSC patients who had their 1993 and 1999 IAIHG scores calculated, the 1999 score was a lower numerical score.[15] Further analysis of 89 patients with AIH using both scores reclassified 15% of cases, a demotion using the 1999 IAIHG score was mainly related to the presence of biochemical and serological characteristics of biliary disease.[27] The score was simplified in 2008, with removal of the liver enzyme profile, response to therapy, and deductions for biliary features on histology.[26] The 2008 IAIHG score has excellent specificity for PSC: among 147 PSC patients 0.0% were definite and 1.4% probable for AIH.[28]

The performance of these scoring systems in AIH/PSC overlap have only been reported in a few studies involving small patient numbers. In nine patients with AIH/PSC overlap there was no difference in the 1993 and 1999 scores, with

eight definite AIH and one probable AIH in both.[15] The 1999 score was applied to three patients with AIH/PSC; of the three patients, one was graded as probable AIH and two were not AIH.[29] The 16 patients with AIH/PSC had a lower 1999 score compared to those of AIH patients.[5] The 2008 score was probable or definite AIH in 65% of 17 patients with AIH/PSC,[22] although this was 0% when the score was applied to an additional cohort of three patients.[30]

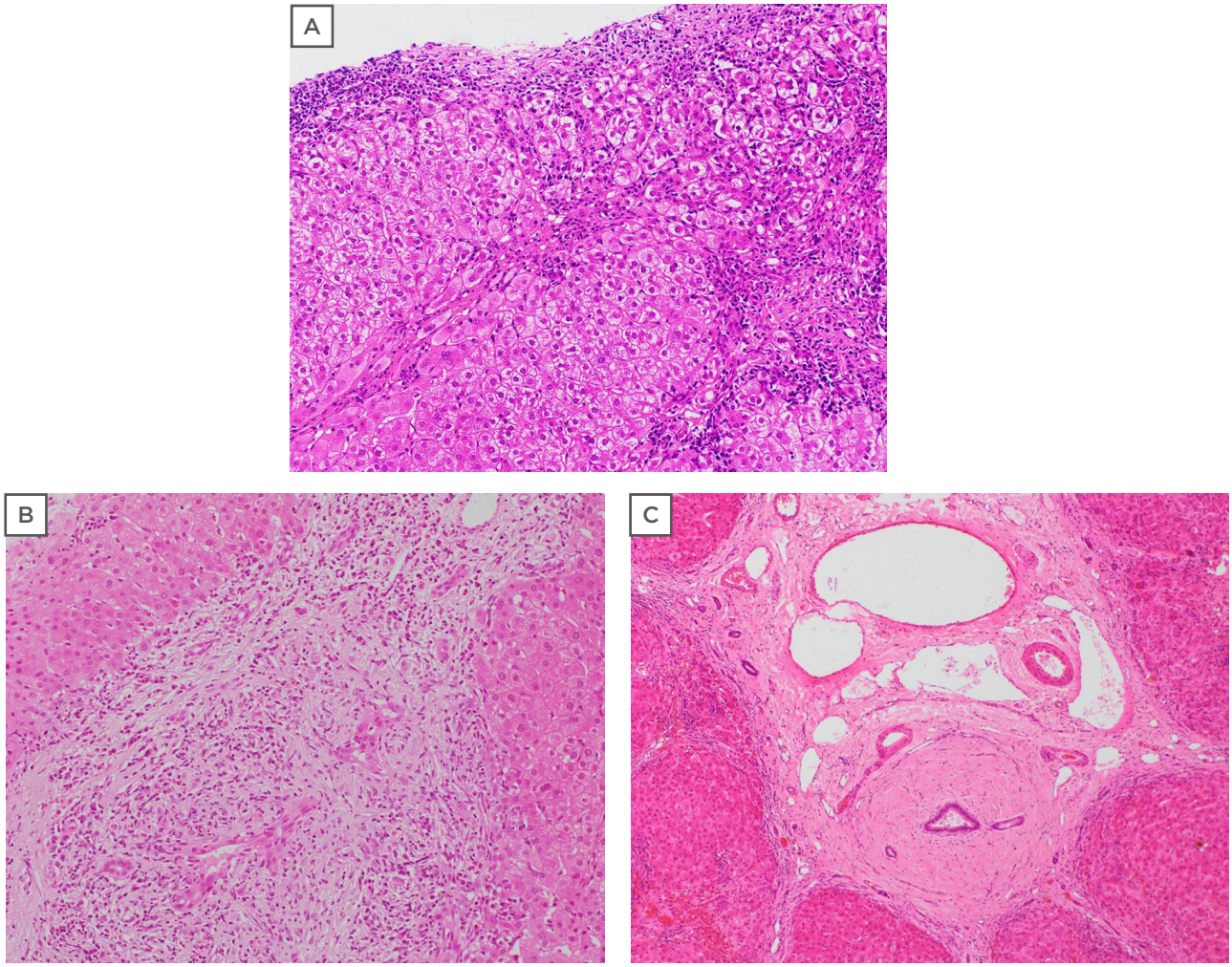

Figure 2: Sequential liver histology from a patient with an initial diagnosis of autoimmune hepatitis and subsequently autoimmune/primary sclerosing cholangitis overlap.

The patient was a 14-year-old girl who presented with ALT 320 UI/L, raised IgG, and positive autoantibodies (ANA 1/160 and ASM 1/160).

A) Autoimmune liver disease with features of autoimmune hepatitis only. Liver biopsy at age 14 years. Sections show advanced-stage chronic hepatitis, with marked lymphoplasmacytic portoseptal inflammation with plasma cell enrichment and moderate interface activity. Hepatocyte rosettes and emperipolesis activity were also observed at higher magnification. Bile duct lesions were not identified (H/E, 100X).

B) Autoimmune liver disease with chronic cholangiopathy. Liver biopsy of the same patient at age 19 years, with radiological features of a cholangiopathy. Obvious bile duct lesions and persistent interface activity, lymphoplasmacytic. The main indication at this time was a cholangiopathy (H/E, 100X).

C) End-stage autoimmune liver disease with sclerosing cholangitis features. Explant of the same patient at age 21 years, with radiological features of an established cholangiopathy. Diffuse periductal concentric fibrosis (sclerosing cholangitis type lesions) was observed. The hepatic parenchyma shows end-stage cirrhosis (H/E, 40X).

AIH: autoimmune hepatitis; ALT: alanine aminotransferase; ANA: antinuclear antibody; ASM: antismooth muscle antibody; H/E: haematoxylin and eosin; PSC: primary sclerosing cholangitis.

When assigning a diagnosis of AIH/PSC, experts advise caution in using the IAIHG scoring system in clinical practice and advocate the importance of clinical judgement.[31] When a diagnosis of AIH/PSC overlap is suspected, key diagnostic tools are MRCP and liver histology, and a final decision can be made as a composite of these results irrespective of IAIHG score (Figure 2). Caution should be used with regards to the biliary changes that can be seen in AIH, which do not represent a cholangiopathy of PSC.[10,11]

Treatment

Treatment in AIH/PSC overlap is a significant challenge due to the small number of studies and lack of randomised controlled trials. Therapeutic options are extrapolated from the management of the constituent syndromes.[2]

Data on the benefits of immunosuppression in AIH/PSC overlap are variable. There is some data suggesting a benefit from corticosteroids in PSC patients with histological features of AIH, higher bilirubin and alanine aminotransferase (ALT),[32] and in patients with AIH/PSC and large duct cholangiopathy.[19] Normalisation of ALT was achieved in 88% of patients with AIH/PSC overlap; however, this took a mean of 26 months, much longer than for AIH.[8] Remission is achieved less frequently with immunosuppression in AIH/PSC than in classical AIH (22% versus 64%;[21] 73% [when combined with AIH/PBC] versus 95%).[22] Univariate analysis of overlap syndromes (AIH/PSC and AIH/PBC analysed together) detected overlap syndromes were associated with a suboptimal response to immunosuppression.[6] Relapse upon reduction of immunosuppression (combination of corticosteroids and azathioprine) was seen in 44% of patients with AIH/PSC overlap; however, the patients responded to increased levels of immunosuppression.[8] There is a particularly poor response to immunosuppression in those with small duct AIH/PSC.[19] Response to immunosuppression in AIH patients with histological biliary changes is not suggestive of PSC; however, they have similar outcomes to those without.[10]

In small case series of patients with initially immunosuppression-responsive AIH and subsequent diagnosis of PSC, there is biochemical relapse upon reduction of immunosuppression or progressive cholestasis.[17,33] These suggest that in some cases the cholangiopathy has developed despite adequate immunosuppression and may not respond to immunosuppression.

A group of four patients with AIH/PSC overlap, who were either azathioprine non-responders or azathioprine intolerant, were treated with mycophenolate mofetil as a second-line steroid-sparing agent.[34] There was a biochemical response in all patients, and three of the four patients achieved remission. Other second-line immunosuppressive agents that have been used in AIH, such as tacrolimus, have not been reported in AIH/PSC overlap.

Combination therapy using immunosuppression (maintenance 10–15 mg per day prednisolone and 50–75 mg per day azathioprine) and 15–20 mg/kg per day ursodeoxycholic acid (UDCA) was associated with a reduction in AST over 5 years in seven patients with AIH/PSC overlap.[35] However, there was no change in their cholestatic enzymes (ALP and gamma glutamyl transferase [GGT]). Among the 16 patients with AIH/PSC overlap, a reduction in ALT within 6 months was seen with immunosuppression, irrespective of combination with UDCA.[8] These studies raise the question as to whether UDCA provides an additional benefit to immunosuppression in AIH/PSC overlap. The effect of UDCA monotherapy was assessed in seven patients, only 29% achieved remission or a good response in aminotransferase reduction.[19]

Given the paucity of evidence in the area, guidelines currently recommend empirical combination therapy with immunosuppression and UDCA in AIH/PSC overlap, and liver transplantation in end-stage disease.[36] On a practical level, the care of patients should be individualised to address their own balance of hepatitis and cholestasis. Immunosuppression should be targeted at the hepatic component and UDCA at the cholestatic component. The response to therapy can be assessed through a combination of clinical, biochemical, histological, and radiological parameters. As evidenced from the literature, the phenotype of patients can switch during the course of their disease, and modifications in therapy should be made accordingly (Figure 3).

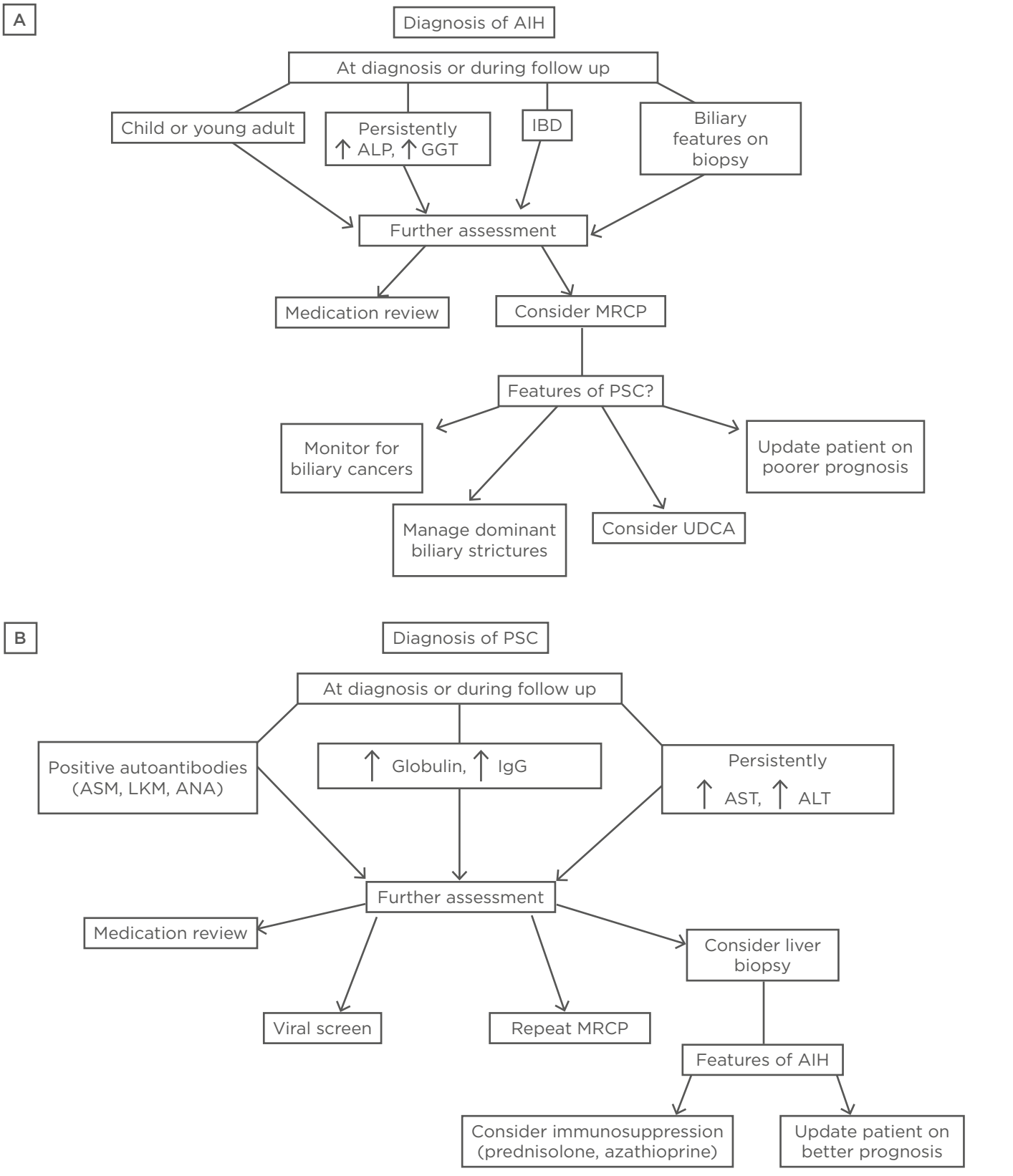

Figure 3: A schematic for the proposed management, either at first diagnosis or during follow up, of patients with AIH (A) or PSC (B) or with features of AIH/PSC overlap.

AIH: autoimmune hepatitis; ALP: alkaline phosphatase; ALT: alanine aminotransferase; ANA: antinuclear antibody; ASM: antismooth muscle antibody; AST: aspartate aminotransferase; GGT: gamma-glutamyltransferase; IBD: inflammatory bowel disease; LKM: liver-kidney microsomal antibody; MRCP: magnetic resonance cholangiopancreatography; PSC: primary sclerosing cholangitis; UDCA: ursodeoxycholic acid.

Outcomes

Despite treatment with currently recommended therapies, progression is common (Figure 2). Over a mean of 12 years follow-up in patients with AIH/PSC overlap on treatment, the proportion with cirrhosis increased from 19% at presentation to 56% at the end of observation.[8] There is a wide range (14–80%) reported in the literature for the requirement of liver transplantation for end-stage AIH/PSC overlap and it is limited to small case series with variable duration of follow up.[5,16,35,37,38]

Long-term patient survival is poorer in AIH/PSC compared with AIH (odds ratio: 2.08; p=0.039),[5] and liver-related death and liver transplantation is more common (33% versus 8%; p=0.05).[21] The outcomes from AIH/PSC overlap are better than PSC, with no increase in Mayo score prognostic risk index during follow up, no cases of cholangiocarcinoma (15% in PSC), and no deaths (26% in PSC).[35]

Following liver transplantation, patients with overlap AILD (AIH/PSC and AIH/PBC) had a higher rate of recurrence of disease in their transplanted liver at 5 years compared to single AILD (overlap 53%, AIH 16%, and PSC 18%), but comparable graft and patient survival.[39] Recurrent disease was diagnosed in two patients with AIH/PSC overlap, one of whom had features of AIH/PSC overlap and the other had only those of PSC.

PAEDIATRIC AND YOUNG ADULTS

From early reports of children with PSC it was clear that there were many clinical similarities between PSC and AIH, and in fact these patients had often initially been managed as having AIH.[40,41] In approximately a third of these PSC cases, the diagnosis was only made after subsequent investigations, including cholangiography, which revealed features of PSC.

A study that systematically evaluated consecutive children with liver disease and positive antibodies consistent with AIH, with screening cholangiogram and liver biopsy, found ~50% had abnormal cholangiograms.[42] The term ASC was used for those with abnormal cholangiograms and positive autoantibodies, who had different characteristics to those with normal cholangiograms. The condition has only been described in paediatric populations.

Demographics

The age at diagnosis in paediatric AILD does not appear to differ between subtypes. No difference was seen between AIH/PSC overlap and PSC (11.3 versus 11.5 years),[43] or ASC and AIH (11.8 versus 10.5 years).[42] However, PSC exhibits a slight male predominance compared with AIH/PSC overlap, with 64% of males presenting with PSC compared to 55% with AIH/PSC overlap.[43] A sex difference was not seen between AIH and ASC subgroups (79% versus 55% female).[42]

Frequency

A diagnosis of AIH/PSC is much more common in paediatric populations than in adult cohorts; in a large multicentre cohort of 781 children with PSC, 33% had AIH/PSC overlap.[43] When consecutive children who presented to a single centre with suspected AILD and positive autoantibodies underwent liver biopsy and cholangiogram, a diagnosis of ASC was made in 49% and the remainder were diagnosed with AIH.[42]

Inflammatory Bowel Disease

Coexistent IBD is present in 63% of patients with AIH/PSC overlap, which is fewer than the 82% seen in PSC (p<0.001).[43] ASC patients, on the other hand, more frequently had IBD than AIH (44% versus 18%; p=0.03), and had fewer cases of autoimmune disease in first degree relatives (37% versus 71%).[42]

Diagnosis

The diagnostic criteria used for AIH/PSC overlap in children is similar to that used in adults. It involves an abnormal cholangiogram or liver histological features of PSC, in combination with a probable or definite classification on simplified AIH criteria.[43] The biochemical profile in AIH/PSC overlap was of higher AST and higher ALT, with comparable ALP and GGT to PSC. They also had higher globulin fraction and IgG, and more were antinuclear antibody positive (62%) and antismooth muscle antibody positive (61%).

Although lacking formal diagnostic criteria, ASC was first described in patients with suspected AILD, a positive autoantibody test, and an abnormal cholangiogram.[42] This differs from a diagnosis of AIH/PSC overlap because the only feature of AIH required is a positive autoantibody test. The patients had lower bilirubin levels, lower AST levels, and a lower ALP:AST ratio than AIH. All had positive autoantibodies, and 74% had positive antismooth muscle antibodies. Histological analysis in ASC identified less lobular activity, less portal tract inflammation, lower histological inflammatory activity index, and more acute or chronic cholangitis than in the AIH patients. Only 23% had a histological diagnosis of chronic hepatitis on their index liver biopsy, 42% had sclerosing cholangitis, and 19% chronic hepatitis with biliary features.

Treatment

Most ASC patients (85%) were treated with 2 mg/kg per day prednisolone (maximum dose 60 mg);[42] however, of those with abnormal baseline AST, 61% did not normalise, compared to all those with AIH treated with prednisolone. In total, 59% of ASC patients required 1–2 mg/kg per day of azathioprine, either due to increasing AST on tapering prednisolone or prednisolone side effects. Further escalation of therapy to penicillamine, cyclosporin, or colchicine was initiated in 22% of patients due to persistent AST elevation. Subsequent data from the same centre have shown 89% response to second-line mycophenolate mofetil in azathioprine non-responders in AIH, compared to only 25% in ASC.[44] Outcomes from specific treatment regimens have not been reported for children with AIH/PSC, although 81% have been treated with UDCA.[43]

Outcomes

Patients with AIH/PSC overlap have similar outcomes in terms of event-free survival and transplant-free survival to PSC patients.[43,45] No cases of cholangiocarcinoma have been reported in the paediatric literature for AIH/PSC and are limited to PSC only.[43]

Follow up assessment in ASC patients was undertaken with biopsies in 17 patients and endoscopic retrograde cholangiopancreatography in 17 patients.[42] Histology revealed a significant decrease in histological inflammatory activity index score from baseline biopsy, which was comparable to the improvement seen in the AIH patients. However, the cholangiograms showed progressive intra and extra-hepatic cholangiopathy in 47% of patients. This may, in part, explain the significantly poorer transplant-free survival for ASC compared to AIH, with 15% of the ASC patients requiring liver transplantation during the study period.[42] The 10-year transplant-free survival from other series is 89% in ASC, which is comparable to paediatric PSC patients.[46] When PSC, ASC, and AIH are compared, the chances of a complication of liver disease within 5 years of diagnosis are 37% PSC, 25% ASC, and 15% AIH, respectively.[47] The limited data from these studies suggest that the outcomes for patients with ASC sits between AIH and PSC.

CONCLUSION

There is limited literature on AIH/PSC overlap and ASC. Not only is the number of studies small, but the data is heterogeneous, in part related to the lack of consensus on diagnostic criteria and the variety of scoring systems used across different studies. Despite this, there appears to be sufficient evidence that patients with AIH/PSC overlap and ASC have a different clinical course and different response to therapy than patients with AIH and PSC alone.

In children, and possibly young adults, with AIH, it is reasonable to routinely perform a MRCP at diagnosis to assess for a cholangiopathy. Otherwise an evaluation of the biliary tree should be limited to those with AIH and an incomplete response to immunosuppression or ensuing cholestasis (Figure 3A). Diagnosing a cholangiopathy may allow for therapeutic biliary intervention, heightened awareness of poorer prognosis, lower threshold for investigating for IBD, and cholangiocarcinoma surveillance.

Performing a liver biopsy in patients with PSC to look for evidence of AIH should be restricted to those with a strong autoantibody profile or persistently elevated transaminases (Figure 3B). If an AIH component is detected, then the patient may derive benefit from immunosuppression. However, there should be an awareness of the poor response rates to immunosuppression in these patients, in terms

of progressive cholangiopathy and chronic liver disease despite adequate immunosuppression, prior to embarking on therapy.

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Liver Fibrosis: A Clinical Update

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Abstract

Liver fibrosis is a disease that affects patients with hepatitis B virus or hepatitis C virus, harmful alcohol consumption levels, and nonalcoholic fatty liver disease. It is important to assess the cause, disease severity, and prognosis at the time of presentation to determine suitable treatment. The aim of this review article is to outline the recent advances in the diagnosis, management, and treatment of liver fibrosis. A PubMed review was performed encompassing the years 1982–2019 using the following search terms: 'liver fibrosis', 'hepatitis C virus', 'hepatitis B virus', 'non-alcoholic fatty liver disease', and 'alcoholic liver disease'. Results showed that the cornerstone therapy for liver fibrosis is to remove the offending agent and treat the underlying disease. The gold standard method of diagnosis is liver biopsy; however, this procedure is invasive and thus multiple laboratory and radiologic tests are used to help determine the degree of fibrosis. There are few pharmacological agents known to treat fibrosis and they are disease specific. For example, the only proven therapy for fibrosis improvement in alcoholic liver disease is abstinence. The authors concluded that liver fibrosis carries a high morbidity and mortality risk with few therapeutic options depending on the cause and degree of fibrosis. Larger multicentre prospective studies are needed to examine effective agents to prevent, stop, or reduce fibrosis.

INTRODUCTION

Liver fibrosis is the common sequelae of chronic insult to the liver from any aetiology. The most common causes are alcohol-related, fatty liver disease, chronic hepatitis B or C viral infections, autoimmune hepatitis, and metabolic or genetic liver diseases. The disease spectrum of liver fibrosis ranges from non-cirrhotic (stages F0–F3) to cirrhotic (stage F4). Fibrosis is the replacement of tissue with a collagenous scar as a result of repetitive liver insults. Cirrhosis is the end stage of liver fibrosis resulting in regenerative nodular hepatic echotexture surrounded by fibrotic bands and distortion of hepatic vasculature.[1,2] Liver fibrosis is a major cause of morbidity and mortality.[3] A survey by the Centers for Disease Control and Prevention in 2016 found that there were 4.9 million people living with liver disease.[4] Chronic liver disease and cirrhosis is the sixth leading cause of all-cause mortality in people aged 25–64 years.[5] Patients may be asymptomatic or present with a wide range of symptoms, including decompensation and liver failure. Liver biopsy is the gold standard for

diagnosis; however, many recent advances in biomarkers and imaging are being used as non-invasive methods of diagnosis.[6] Rates of fibrosis differ depending on the type of insult, age, and sex.[7] Liver fibrosis was previously thought to be a unidirectional process, but many clinical studies have shown that it is a dynamic process with potential for reversibility. The goal of current and future therapies for any chronic liver disease is to prevent, reduce, and reverse the progression of fibrosis to cirrhosis with its complications and the need for liver transplantation.[8,9] This review will discuss the current and future advances in the diagnosis, management, and treatment of liver fibrosis.

CLINICAL PRESENTATION

Liver fibrosis often goes unrecognised unless the patient manifests symptoms from complications of cirrhosis. When a patient presents with liver disease, it is important to exclude or confirm cirrhosis, especially when the presentation is with incidental findings of elevated serum aminotransferases, unexplained thrombocytopenia, or abnormal liver imaging. Risk factors for developing liver fibrosis include metabolic syndrome, heavy alcohol consumption, exposure to hepatotoxic substances, and the use of hepatotoxic medications.[10] Thus, a careful clinical history and index of suspicion is important to identify the disease early. Physical exam findings that assist with diagnosis include jaundice, spider angioma,[11] a nodular liver on palpation,[10] splenomegaly, ascites,[12] caput medusae, palmar erythema, gynecomastia,[13] asterixis,[2] and Type 2 diabetes.[14] However, many patients are without physical findings and advanced fibrosis is diagnosed by abnormalities on haematological, biochemical, endoscopic, or radiologic evaluation.[2,15]

PATHOPHYSIOLOGY

Fibrosis is a wound-healing process that becomes dysregulated when repeated insults result in pathologic, chronic fibrinogenesis.[16] The common aetiologic agents for chronic repetitive liver damage are harmful alcohol consumption, metabolic syndrome and diabetes, viral infections with hepatitis C virus (HCV) or hepatitis B virus (HBV), toxins and drugs, and autoimmune or metabolic diseases.[17,18] All liver cell lines undergo alterations in phenotype due to changes in the microenvironment in the space of Disse.[19] The hepatic stellate cell (HSC) is the major driver of hepatic fibrosis followed by portal fibroblasts and bone-marrow derived fibrocytes. Extracellular signals from the innate and adaptive immune systems, such as Kupffer cells, macrophages, natural killer cells, T cells, and B cells, modulate HSC activation, also known as the initiation phase.[18,20] In early liver injury, endothelial cells produce a variant of fibronectin that also stimulates HSC activation.[19] Hepatocytes stimulate activation through lipid peroxidases leading to oxidative stress, and Kupffer cells stimulate matrix synthesis, cell proliferation, and the release of retinoids by stellate cells.[19] HSC release chemokines and cytokines that recruit and activate inflammatory immune cells, contributing to the perpetuation phase of fibrogenesis. In this phase, the HSC proliferate and lead to contractility, fibrogenesis, chemotaxis, matrix degradation, retinoid loss, and cytokine release.[18,19] Stellate cell mitogens, such as platelet derived growth factor, endothelin-1, thrombin, fibroblast growth factor, and insulin-like growth factor lead to proliferation. Endothelin-1, along with arginine vasopressin, adrenomedullin, and eicosanoids, activate HSC to increase portal pressures and resistance by constricting sinusoids and contracting the liver. Transforming growth factor B1 is the primary fibrinogenic factor and is upregulated by the transcription factors Sp1 and Zf9. Other factors involved in fibrinogenesis include TNF, lipid peroxides, and acetaldehyde.[19] The extracellular matrix, which is made up of molecules such as collagens, glycoproteins, proteoglycans, and glycosaminoglycans, further promotes HSC activation. When the liver becomes fibrotic, the interstitial collagen increases 3–8-fold, a concept known as 'capillarisation' that causes destruction of hepatocyte microvilli and endothelial fenestrations.[19] As a result, the transport of important solutes to hepatocytes is impaired, leading to hepatic dysfunction.[21] Stellate cells are a known source of matrix metalloproteinase-2. Matrix metalloproteinases have been identified as responsive for extracellular matrix remodelling; however, their regulators have not been identified.[19] HSC activation and proliferation can be inhibited and even reversed. Mechanisms of reversal involve apoptosis, immune elimination,

senescence, and reversion to an inactivated state.[20,22] These pathways are promising targets for novel therapeutic agents.

DIAGNOSIS

Diagnosing and assessing the degree of liver fibrosis is important in predicting liver-related morbidity and mortality and the emergence of complications of portal hypertension.[23] Histologic scoring systems have been developed to grade (degree of inflammation that reflects ongoing liver disease injury) and stage (amount of current fibrosis) the extent of hepatic disease. The major determinants of inflammatory activity are lymphocytic piecemeal necrosis, lobular necroinflammation, and portal inflammation, which are graded 0–4 in most classification systems. The degree of fibrosis is based on the expansion of fibrotic areas between portal tracts. Stages of fibrosis can range from 0–4 or 0–6 depending on which staging system is used. There are multiple validated scoring systems, including Scheuer/Batts–Ludwig/Tsui which grades on a scale of 0–4; METAVIR, on a scale of 0–4; and Ishak et al.,[24] on a scale of 0–6 (Table 1). There are invasive and non-invasive methods of staging for liver fibrosis (Table 2). Combination testing may be a more effective prognostic tool when compared to any individual non-invasive method. In one study, the aspartate aminotransferase (AST)-to-platelet ratio index (APRI), in combination with ultrasound, had a positive predictive value of 80%.[27,28]

Liver Biopsy

Liver biopsy for diagnosis of cirrhosis is needed when the diagnosis is uncertain based on clinical or biochemical and radiological assessment.[10,27]

Table 1: Fibroscan evidence-based cut-off references.

	*Bonder-Afdahl[25]	*Tapper-Castera-Afdahl[26]
HBV	F0–F1 ≤6.0	Significant fibrosis ≥9.0
	F2 >6.0	
	F3 ≥9.0	Cirrhosis ≥11.7
	F4 ≥12.0	
HCV (HCV and HIV)	F0–F1 ≤7.0 (≤7.0)	Significant fibrosis ≥7.3
	F2 >7.0 (≤10.0)	
	F3 ≥9.5 (≥11.0)	Cirrhosis ≥12.5
	F4 ≥12.0 (≥14.0)	
NAFLD/NASH	F0–F1 ≤7.0	Cirrhosis ≥10.3
	F2 ≥7.5	
	F3 ≤10.0	
	F4 ≥14.0	
Alcoholic liver disease	Abstinent	Cirrhosis ≥12.5
	Drinking	Cirrhosis ≥22.7
Cholestatic	F0–F1 ≤7.0	Cirrhosis ≥17.9
	F2 ≥7.5	
	F3 ≥10.0	
	F4 ≥17.0	

*Bonder–Afdahl and Tapper–Castera–Afdahl are names of studies validating different techniques for measuring degree of fibrosis when using Fibroscan.

HBV: hepatitis B virus; HCV: hepatitis C virus; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic fatty steatohepatitis.

Table 2: Assessing the degree of liver fibrosis.

	Pros	Cons
Liver biopsy	Most accurate diagnosis.	Invasive; intra-observer variability.
Scoring Systems		
ARR, APRI, Fib-4, UIC	Uses standard laboratory tests.	Cannot discriminate the intermediate stages of fibrosis.
FibroTest	Uses software algorithm and adjusts for age and sex with applicability of 99%.	Does not detect significant fibrosis or cirrhosis; need to combine with other methods.
Hepascore	Computer-based score adjusted for sex and age.	Not useful in NAFLD or those co-infected with HIV.
Fibrospect-II	Computer generated.	Overestimates in African Americans and HCV.
Imaging		
Fibroscan	Accurate and reproducible.	Limited in obesity and acute inflammatory flares.
ARIF	Only requires standard equipment.	Operator dependent.
SWE	Higher accuracy than TE or ARIF.	Operator dependent.
MRE/MRI	More sensitive and specific than most other non-invasive tests, less operator dependent.	Time-consuming and costly.

APRI: aspartate aminotransferase-to-platelet ratio index; ARIF: acoustic radiation force impulse; ARR: alanine aminotransferase ratio; Fib-4: fibrosis-4; HCV: hepatitis C virus; MRE: magnetic resonance enterography; NAFLD: nonalcoholic fatty liver disease; SWE: shear wave elastography; TE: transient elastography; UIC: Universal Index for Cirrhosis.

In patients diagnosed with cirrhosis, the liver biopsy is sometimes performed for underlying aetiology of the disease, especially to rule out treatable diseases, such as autoimmune hepatitis. Biopsy can be obtained through a transthoracic, subcostal, or transvenous approach and can assist with diagnosis, prognosis, and management, particularly in those with atypical features or co-existing disorders.[23] In a recent prospective study of 176 patients, liver biopsy changed the diagnosis in 55 (31.2%) patients.[29] However, there are risks associated with liver biopsy. In a retrospective study by Chi et al.[30] there was a 6.00% rate of overall complications, most frequently pain followed by excessive bleeding with an overall risk of death of 0.03%. Absolute contraindications include an uncooperative patient, severe coagulopathy, infection of the hepatic bed, and extrahepatic biliary obstruction. Relative contraindications include ascites, morbid obesity, possible vascular lesion, amyloidosis, and hydatid disease.[29]

Non-Invasive Measures of Fibrosis

Given the invasiveness and potential for complications with liver biopsy, non-invasive methods to assess the stage of hepatic fibrosis are increasingly being used in clinical practice. The most common modalities include elastography using ultrasound and magnetic resonance technology, as well as measurement of serum biomarkers. Transient elastography (TE), or Fibroscan (Table 1), is an accurate and reproducible method to detect liver fibrosis using ultrasound that can be performed in the outpatient setting. It is also a successful predictor of fibrosis complications such as portal hypertension and hepatocellular carcinoma (HCC).[31] The transducer propagates vibrations of low amplitude (50 Hz) to the liver, and the velocity of this propagation is used to determine tissue stiffness. However, the accuracy of TE is limited in obese patients.[32] The sensitivity and specificity for the diagnosis of significant fibrosis, advanced

fibrosis, and cirrhosis in chronic hepatitis B is 71.6% and 81.6%, 79% and 84.6%, and 80% and 86.6%, respectively, with an overall sensitivity and specificity of 83% and 89%[33,34] (Table 1). TE in HCV has an area under the curve of receiver operating characteristic (AUROC) from 0.77–0.90, with a cut-off value of 6.20–8.70 kPa for assessment of significant fibrosis (F≥2); HBV, AUROC, 0.81–0.95; cut off value, 6.30–7.90 kPa; primary biliary cirrhosis (PBC), primary sclerosing cholangitis, and Wilson's disease, AUROC range is 0.81–0.95 for significant fibrosis.[35] TE has also been validated in nonalcoholic fatty liver disease (NAFLD)[36] and alcoholic liver disease (ALD).[37] However, TE overestimates the degree of fibrosis in the setting of inflammatory activity. Thus, if a patient is in an acute flare, it is recommended to wait until alanine aminotransferase (ALT) levels have stabilised.[35]

Acoustic radiation force impulse (ARIF) of the liver is an additional ultrasonographic method to measure liver fibrosis, with a sensitivity and specificity of 84% and 92%, respectively, and only requires standard ultrasound equipment. However, it is operator dependent.[34,38]

2D-shear wave elastography (SWE) is a real-time technique that produces a colour-coded image from radiation generated by an amplitude modulated beam of focussed ultrasound.[39] A recent meta-analysis proposed that SWE may be an equally helpful method for detecting liver fibrosis and may have higher accuracy than TE and ARIF at detecting fibrosis severity.[39] The pooled sensitivity and specificity for the varying stages of fibrosis are 85% and 81% for F2 or greater, 90% and 81% for F3 or greater, and 87% and 88% for F4 or greater.[40] However, this method is also operator dependent. As with ARIF, the operator has the potential to influence the findings based on where they place the region of interest, as opposed to TE where this variability in operator technique is not present.[41]

Magnetic resonance elastography is a contrast phase study that uses mechanical wave propagation to assess tissue stiffness and can also be used to assess portal hypertension and spleen stiffness simultaneously. The sensitivity in chronic hepatitis B in significant fibrosis, advance fibrosis, and cirrhosis were 92.8% and 93.7%, 89.6% and 93.2%, and 89.5% and 92%, respectively.[33] Overall sensitivity and specificity is 100% and 96%, respectively.[34] Although more sensitive and specific than the other non-invasive tests with less operator-variability, this method is more time-consuming and costly than the other imaging modalities.[42]

Serological Markers

These biomarkers for assessing fibrosis stage can be based on tests specifically used for this purpose or tests needed for standard of care.

Markers Based on Standard of Care Laboratory Parameters

Markers based on standard of care include many scoring systems, such as aminotransferase-to-ALT ratio (ARR),[43] APRI,[44] and fibrosis-4 (FIB-4).[45] One of the most commonly used formulas, APRI, is calculated using the patient's AST level, corrected for the upper limit of normal, and platelet count. When combining serum ferritin (SF) with the AAR, APRI, FIB-4, and Fibro-Q, SF plus APRI was the most reliable to predict cirrhosis.[43] On the other hand, the Universal Index for Cirrhosis (UIC) had the highest AUROC when compared to Fibro-Q, FIB4, APRI, and ARR, and can be used in all types of fibrosis.[46] Fibro-mark was found to be a superior predictor of fibrosis over existing scores in those with chronic HCV.[47] The NAFLD fibrosis score uses routine demographic and laboratory variables, such as age, glucose level, BMI, platelet count, albumin, and AST/ALT to differentiate those with advanced fibrosis with an AUROC of 0.88 and 0.82.[48] In addition, the BARD score is able to determine advanced fibrosis at stages F3 and F4, with a negative predictive value of 97%.[49] While accurate in excluding or confirming significant fibrosis, these formulas often fail to discriminate the intermediate stages of fibrosis necessitating the use of other non-invasive methods.

Markers Requiring Special Tests Outside Standard of Care Laboratory Parameters

FibroTest is a clinically validated measure of fibrosis that analyses serum biomarkers (α2-macroglobulin, apolipoprotein A1, haptoglobin, gamma-glutamyl-transpeptidase, and total bilirubin) and uses a software algorithm to determine an individual score while adjusting for age and sex at a mean applicability rate of 99.03%.[50] However, it is limited in detecting

significant fibrosis and cirrhosis and thus it is recommended to combine with other methods of diagnosis to improve accuracy.[51]

Hepascore is another computed-based fibrosis score adjusted for sex and age that analyses serum levels of total bilirubin, gamma-glutamyl transferase, α2-macroglobulin, and serum hyaluronic acid (HA). This test has been used as a primary screening method to determine the need for liver biopsy due to its ability to predict the level of fibrosis, particularly cirrhosis.[52] Hepascore has shown better diagnostic predictability in HCV, HBV, and ALD than for NAFLD and those co-infected with HIV.[53]

FIBROspect-II (FS-II) uses α-2 macroglobulin, HA, and tissue inhibitor metalloproteinase type 1 to estimate liver fibrosis.[54] However, FS-II may overestimate degree of fibrosis in African Americans with HCV.[55] In one study, HA was equally as effective at determining the stage of fibrosis in HCV as compared with the FS-II score and, thus, may be a more cost-effective alternative for screening.[56]

Enhanced liver fibrosis score uses procollagen III amino terminal peptide, HA, and tissue inhibitor of metalloproteinase I and can allow for the avoidance of liver biopsy in approximately 60% of patients.[57]

MANAGEMENT (TABLE 3)

Table 3 summarises effective therapies for the reduction of liver fibrosis in patients with various diseases.

General Management

Preventive hepatology focusses on nutrition; promoting a healthy lifestyle, including exercise and abstinence from alcohol consumption; vaccinations; and screening for HCC. Malnutrition is a frequent complication in chronic liver disease and, along with obesity and sarcopenia, can lead to a worse prognosis.[69] Dietary interventions should be individualised and may focus on nutritional micronutrient replacement, adequate protein calorie intake of 1.2–1.5 g/kg daily, and low sodium consumption. Common micronutrient deficiencies include thiamine, B12, folic acid, retinol, vitamin K, vitamin D, zinc, selenium, and magnesium. Patients should consume 5–7 small meals per day to prevent consumption of too much protein in a single meal. Some may benefit from a late-night snack due to the evidence supporting improvement in sarcopenia and quality of life.[70] In patients that are overweight, weight loss has been proven to not only improve ALT/AST and insulin resistance but also quality of life in ALD and HCV liver disease patients.[71]

Table 3: Therapy proven to aid in fibrosis regression.

HBV	Tenofovir alafenamide, tenofovir disoproxil fumarate with adefovir dipivoxil[58,59]
	Entecavir[60]
	Lamivudine[61]
HCV	Interferon-derived therapy[62]
	Direct-acting anti-virals[63]
NAFLD	Obeticholic acid[64]
	Selonsertib[65]
Alcoholic liver disease	Abstinence from alcohol[66]
Autoimmune hepatitis	Corticosteroids and azathioprine[67]
Primary biliary cirrhosis	Ursodiol[68]

HBV: hepatitis B virus; HCV: hepatitis C virus; NAFLD: nonalcoholic fatty liver disease.

Recommended vaccinations, other than those recommended for the general population, include hepatitis A and B and pneumococcal vaccinations, regardless of age.[72] Screening for oesophageal varices begins once the diagnosis of cirrhosis is made.[73] HCC screening is also typically performed once a patient has developed cirrhosis; however, there is increasing evidence to support the need for screening in those with earlier stages of fibrosis. In recent studies, the incidence of HCC in those without cirrhosis was found to be elevated in those with HBV, NAFLD, and metabolic syndrome.[74,75] However, those with F3 fibrosis have much lower cost-effectiveness for screening, as well as a decreased risk for development of liver disease complications and better survival than patients with cirrhosis (F4 fibrosis).[76] Portal hypertension is a complication of advanced liver fibrosis that can result in variceal bleeding and ascites. Once a patient develops cirrhosis, a variceal screening oesophagogastroduodenoscopy should be performed and repeated after 1–3 years, depending on findings.[77]

The most effective way to manage hepatic fibrosis is to eliminate the stimulus or harmful cause of hepatic damage, but this is not always feasible. No anti-fibrotic agents have been approved for human use that work effectively at eliminating or reducing fibrosis in the clinical setting. Due to the disease complexity, it is suspected that combination therapy may be needed to target two pathways to effectively treat fibrosis and cirrhosis.[78] The current mainstay of treatment for liver fibrosis is to treat the underlying disease.[8]

Hepatitis B Virus Infection

Long term suppression of chronic HBV can lead to regression of fibrosis and cirrhosis. Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir and, when compared to adefovir dipivoxil as single therapy for chronic HBV, demonstrated a significantly greater number of complete responders at 48 weeks, defined as HBV DNA <400 copies/mL and histological improvement (reduction of ≥2 points in Knodell necroinflammatory score).[79] In a study of patients randomised to TDF with adefovir dipivoxil, 87% had histological improvement and 51% had regression of fibrosis at Week 240 (p<0.0001). In addition, of the 96 patients with cirrhosis, 74% no longer had cirrhosis and only 3 of 252 patients progressed to cirrhosis at 5 years (p<0.0001).[58] Both nucleoside-naïve patients who were treated with entecavir[60] and patients treated with lamivudine therapy[61] had significant histological improvement and regression of fibrosis or cirrhosis. Furthermore, anti-viral therapy significantly improves decompensated cirrhosis, as well as liver function and mortality rates.[80] Tenofovir alafenamide is a prodrug that was developed to allow more efficient delivery of the active metabolite than TDF and had greater reductions in FibroTest scores at 48 weeks (mean change 0.07 versus 0.04; p=0.007).[59] It is important to continue HCC screening because HCC in serologically cured HBV can occur in those with pre-cirrhosis or cirrhosis.[81] However, those with F3 fibrosis have much lower cost-effectiveness for screening as well as decreased risk for development of liver disease complications and better survival than patients with cirrhosis (F4 fibrosis).[76]

Hepatitis C Virus Infection

Historically, HCV infection was treated with interferon and ribavirin. Interferon-derived therapy resulted in a 50% regression in cirrhosis in the 30% who achieved a sustained virologic response (SVR). However, in those with advanced cirrhosis, only 5% saw regression of their liver disease over a 10 year period.[62] Lower baseline stage of fibrosis, sustained viral response, age <40 years, BMI <27, and viral load <3.5 million copies per mL were independently associated with regression of fibrosis after treatment.[82]

The newer direct-acting antivirals (DAA) may eradicate HCV, but have not yet been proven to improve survival and complications.[83] In a prospective study of 70 patients, 48.6% had a >30% improvement in vibration-controlled TE.[74] In another prospective study of 304 patients, TE was used to assess the degree of fibrosis after DAA therapy and showed that 65.1% achieved at least a 20% reduction in liver stiffness.[84] Another study of 260 patients on DAA showed a significant fibrosis regression in 40% with baseline advanced fibrosis versus mild fibrosis (52.3 versus 22.5%; p<0.001).[85] Larger prospective trials are needed to further confirm these results. Furthermore, it is difficult to determine their effect on regression of fibrosis and cirrhosis because liver biopsy is not commonly used. TE may overestimate the degree of regression of

disease and is thought to falsely show lower fibrosis staging due to decreased inflammation once the virus is cleared.[63,86] Data matching TE and liver biopsy after SVR is lacking and it is unclear how long a patient will need monitoring after SVR. As with HBV clearance, those with HCV clearance will also need follow-up for HCC.[87] Some patients with HCV were even seen to have an unexpected high recurrence rate of HCC at 27–29% after treatment with ablation or radiation. This study suggests this population receiving DAA may need closer screening.[88] However, in the age of DAA, a reduction of 30-50% was seen in those with HCV requiring wait-listing and subsequently liver transplant, indicating a tremendous success with these medications. Furthermore, there are approximately 600 donor livers each year now being allocated to other forms of chronic liver disease.[89,90]

Nonalcoholic Fatty Liver Disease

The only proven treatment for NAFLD is lifestyle modification, including control of the components of metabolic syndrome. Thus, therapy is directed at controlling risk factors such as insulin resistance, decreasing delivery of fatty acids to the liver, and the use of hepatoprotective medications.[91] Weight loss improves histologic features of NAFLD, particularly nonalcoholic fatty steatohepatitis (NASH). The highest rate of their reduction is seen in those who lose >10% of bodyweight, with 90% resolution of NASH and 45% regression of fibrosis.[92] In a recent study, a text messaging approach encouraging a healthy lifestyle improved weight loss and hepatic function tests in patients with NAFLD.[92,93] In another study, some patients with only 3.0–4.9% weight loss achieved remission of NAFLD at 12 months.[94] Previously, NASH was thought to increase the risk of adverse outcomes, but, in a randomised retrospective study of 646 biopsy-proven patients with NAFLD, the stage of fibrosis rather than NASH was determined to predict adverse related events.[95] Furthermore, it is suggested that fibrosis stage should be part of predicting all-cause mortality secondary to cardiovascular disease and development of chronic kidney disease.[96,97]

There is currently no U.S. Food and Drug Administration (FDA) approved medication for the treatment of NAFLD or NASH, but multiple trials are underway. A meta-analysis of thiazolidinediones in the treatment of NASH showed significant histological improvement in ballooning degeneration, lobular inflammation, and steatosis, although this is at the expense of significant weight gain.[98] A Bayesian network meta-analysis found that thiazolidinediones, vitamin E, pentoxifylline, and obeticholic acid (OCA) improve ballooning degeneration, lobular inflammation, and steatosis, while only pentoxifylline and OCA improve fibrosis.[64] A Phase III, randomised, double-blind, placebo-controlled trial (REGENERATE)[99] is being conducted to assess the benefits of OCA in patients with NASH and advanced fibrosis. In the Phase IIb FLINT trial,[100] OCA demonstrated superiority over placebo based on an intention-to-treat (p=0.0002) in addition to improving liver fibrosis (p=0.004) in NASH and was well tolerated.[65] AURORA is a Phase III, randomised, double-blind, placebo-controlled study on cenicriviroc for the treatment of liver fibrosis for those with NASH.[101] RESOLVE-IT is a Phase III multicentre study looking at the effects of elafibranor in patients with NASH and fibrosis.[102] Selonsertib was studied in a Phase II trial[103] with NASH patients and was determined to be superior to placebo in improvement of one stage of fibrosis or greater, and improved fibrosis without worsening NASH.[65] STELLAR 3[104] and STELLAR 4[105] are Phase III studies examining selonsertib in those with NASH F3 and compensated F4 fibrosis, respectively. ATLAS[106] is a Phase II study examining selonsertib, firsocostat, and cilofexor, both individually and in combinations, in patients with bridging fibrosis or NASH; the results of this study have thus far been promising, with minimal side effects and a reduction of 30% measured hepatic fat based upon MRI.

Alcoholic Liver Disease

The mainstay of treatment for ALD is a reduction in alcohol use.[66] Abstinence can lead to total resolution of hepatic steatosis with the most benefits seen in patients with jaundice or ascites. Abstainers' probability of survival was found to be 87% compared to 55% in persistent drinkers.[107] A recent study suggested in those with NAFLD, even moderate alcohol consumption (10.0–29.9 g per day for men and 10.0–19.9 g per day for women) can result in worsening fibrosis.[108] Corticosteroids have been studied in both ALD

and alcoholic hepatitis (AH), although results are variable. Prednisolone studied in the STOPAH trial did not show a mortality benefit at 90 days or at 1 year in patients with severe AH.[109] However, the American Association for the Study of Liver Diseases (AASLD) and the American College of Gastroenterology (ACG) recommend a trial of steroids in patients with severe AH because of the trend for 28-day mortality benefit among STOPAH participants in the prednisolone therapy arm.[66,110] Oxidative stress is an important component in the pathology of ALD; however, antioxidants like s-adenosyl-L-methionine, vitamin E, and silymarin have failed to show efficacy in the treatment of ALD.[111]

A Phase II study of livercellgram,[112] a stem cell therapy, is being conducted in patients with ALD. Many ongoing clinical trials for ALD are focussed on targeting the gut–liver axis (probiotics, antibiotics, zinc), inflammation and oxidative stress (anakinra, extracorporeal cellular therapy, ASK-1 inhibitor selonsertib, and metadoxine), and regenerative agents (G-CSF and IL-22).[110,113-120] There is a growing interest in investigating the use of probiotics in ALD due to its close association with gut microbial alterations; however, the precise mechanism needs further investigation.[121] A current Phase II trial[122] is investigating rifaximin in ALD. The remainder of clinical trials focus on AH. Selonsertib (ASK-1 inhibitor) has completed a Phase II trial[123] with prednisolone versus selonsertib alone in those with AH and data is currently pending. Metadoxine showed improvement in 3 and 6-month mortality in those with severe AH, aided with alcohol abstinence.[124] A Phase IV trial[125] is underway investigating the efficacy of G-CSF in patients with severe AH. IL-22 is overexpressed in liver regeneration and repair, and a current Phase II trial[126] is underway evaluating the use of IL-22 in AH.

Autoimmune Hepatitis

Corticosteroids and anti-inflammatory agents are the mainstay of treatment for autoimmune hepatitis. In those with mild disease, a low dose of prednisone may be used, such as 30 mg daily. In those with more severe hepatitis, the recommendation is to begin with a higher dose of 60 mg prednisone daily. If patients are at increased risk of side effects (brittle diabetes, post-menopausal women, hypertension, emotional liability, obesity, or osteoporosis), a low dose of prednisone (30 mg daily) combined with azathioprine (50 mg daily) is used for initial treatment. If a patient has significant cytopenia, is pregnant, or has a malignancy, azathioprine should be avoided. Corticosteroids have been shown to improve or stabilise fibrosis in about two-thirds of patients.[67] If continued on steroids, patients should be monitored closely with annual bone densitometry and should also receive hepatitis A and B virus vaccinations, regardless of age.[127]

Primary Biliary Cirrhosis

Although PBC is thought to be a form of autoimmune disease, immunosuppressive therapy has not proven beneficial in this population. Ursodeoxycholic acid (UDCA) is the mainstay of treatment and has been shown to delay the time of the liver transplant and death in patients with PBC.[128] Although UDCA effectively decreases AST and ALT, it did not appear to improve existing cholestasis or fibrosis compared to placebo in one study.[129] However, in another study, UDCA was found to significantly delay the progression of fibrosis in PBC with 76% on UDCA remaining in early stage disease versus 29% in the placebo group.[68] Furthermore, the decreased need for ALT among all aetiologies for PBC treated with UDCA support its use.[130]

OCA showed benefits as monotherapy or in conjunction with UDCA over placebo in patients with PBC in single centre studies.[131] COBALT, a Phase IV, double-blinded, randomised, placebo-controlled multicentre trial study is being conducted to further assess OCA in PBC.[132] As HSC are the main drivers of liver fibrosis, they remain an important potential target for therapy. Although many drugs used in mouse models show improvement in liver fibrosis, medications targeting HSC have yet to be approved for the treatment of liver fibrosis.[133]

SUMMARY

Common causes of hepatic fibrosis are chronic viral infection with HBV or HCV, harmful alcohol consumption, and NAFLD. With accelerating obesity rates worldwide and effective cure of HCV with DAA, alcohol and NAFLD are emerging as the leading causes of hepatic fibrosis and cirrhosis, with its related complications. Apart from treatment of the underlying aetiology and

risk factors, several new therapeutic approaches are being studied with potential to prevent, stop, or reverse the progression of liver fibrosis. The field is advancing rapidly, especially in NAFLD, as many studies have consistently shown that fibrosis stage, and not fatty liver or inflammation severity, determines the long-term outcomes including hepatic and extra-hepatic outcomes.

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