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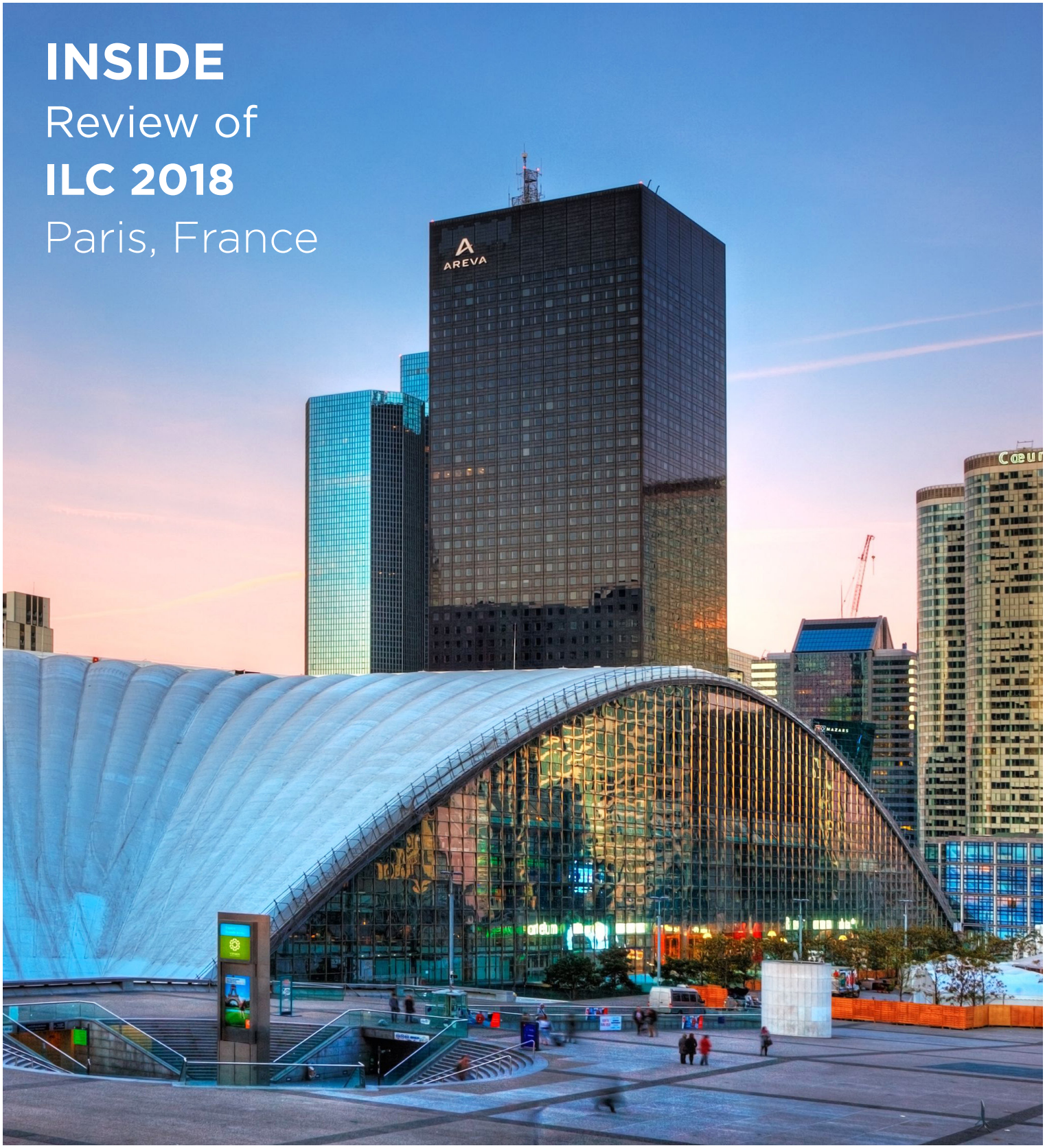
INSIDE

Review of
ILC 2018
Paris, France



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"We hope that the variety held within its pages will encourage great discussion among colleagues and encourage even greater attendance at the ILC next year."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

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Front cover and contents photograph: Paris, France, home of the ILC 2018. © Radu Razvan Gheorghe / 123rf.com

Masthead

EMJ Urology 6.1

The European Medical Journal would like to welcome readers to *EMJ Urology 6.1*, our first eJournal to review one of the major European medical conferences of 2018.

VIEW ALL JOURNALS ←

Welcome

I take great pleasure and pride in welcoming all readers to this year's edition of *EMJ Hepatology.* Contained within are a selection of peer-reviewed articles detailing key hepatological findings, insightful interviews with several members of *EMJ Hepatology's* Editorial Board, and, of course, our informative congress coverage of the European Association for the Study of the Liver (EASL) International Liver Congress (ILC), this year held in Paris, France.

The EMJ team were fortunate enough to make the journey to Paris for the ILC and, as always, were thoroughly blown away by the astounding array of information on offer, ranging from late-breaking research conferences to the learning centre; the event truly represented a global hub for the discussion of liver disease. Everyone at EMJ has worked hard to create a Congress Review section that captures the spirit of the ILC, and I believe we have achieved that. These pages highlight just a small fraction of the cutting-edge information disseminated in Paris, ranging from the discussion of a web-based lifestyle change intervention to the development of an immunotherapeutic strategy for managing hepatocellular carcinoma. Furthermore, I am excited to share with you a select series of abstract summaries that were presented at the ILC; these summaries were penned by the presenters themselves. If you want to learn more about real-world data on patients with hepatocellular carcinoma treated with transarterial chemoembolisation, or a follow-up strategy for patients with acute liver failure, then I suggest you do not miss this section.

No edition of *EMJ Hepatology* would be complete without a collection of peer-reviewed articles. *EMJ Hepatology's* Editor-in-Chief, Prof Markus Peck-Radosavljevic, has selected a literature review on primary hepatic angiosarcoma as his Editor's Pick for this edition. This paper, by Chen et al., covers the latest work on primary hepatic angiosarcoma, providing pertinent information on epidemiology, aetiology, presentation, diagnosis, pathology, and treatment. Furthermore, Tombesi et al. have provided an excellent summary of the use of thermal ablation for treatment of liver tumours, discussing radiofrequency ablation, laser ablation, and microwave ablation. Additionally, the authors have penned a companion case report, which details their experience using percutaneous laser thermal ablation to treat a patient with 22 liver metastases. There are a number of additional papers contained within the covers of this eJournal and I heartily invite you to read them all.

Finally, I would like to sum up by thanking all of those who contributed to *EMJ Hepatology* this year; it has been a labour of love and I hope you will all agree that the efforts were worth it. Here is to another year expanding the frontiers of hepatology, which EMJ very much looks forward to reporting on!

Spencer Gore

Chief Executive Officer, European Medical Group

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Foreword

Dear colleagues,

I would like to welcome you to the newest edition of *EMJ Hepatology*. After coming back from the International Liver Congress (ILC), it was good to see that the European Association for the Study of the Liver (EASL) is still top of the list when it comes to large international liver meetings. Although the focus on liver disease has shifted a little this year, the ILC was again able to feature many very interesting and scientifically relevant presentations. *EMJ Hepatology 6.1* showcases the latest developments of this impressive congress in its thorough Congress Review, complemented by a much-anticipated selection of peer-reviewed articles on a broad range of topics.

This year, the ILC prominently featured the release of several new clinical practice guidelines, including those combating liver cancer, hepatitis C recommendations, and decompensated cirrhosis and nutrition. While the guidelines on hepatocellular carcinoma (HCC) were a long-awaited update of the 2012 version, the guidelines on decompensated cirrhosis and nutrition are completely new.

Liver cancer was one of the hot topics at this year's conference. The presentation of a third Phase III trial showing no superiority for selective internal radiotherapy (SIRT) versus the current standard of care surely seals the chapter on SIRT for the management of hepatocellular carcinoma, supporting the recommendations of the current EASL clinical guidelines. In a similar vein, within this eJournal, McCulloch et al. examine the literature and provide a significant review on the impact of sarcopenia on HCC, as well as considering the role of staging schemes and nutritional interventions to guide evidence-based practice. Additionally, Chen et al. provide this issue's Editor's Pick with an important review of a rare and deadly hepato-oncological condition: primary hepatic angiosarcoma.

In the field of nonalcoholic fatty liver disease (NAFLD), several mid-stage clinical trials with promising results were presented, suggesting that we are making correct assumptions of the pathophysiology of NAFLD and that we may be able to successfully treat NAFLD through biologically plausible targeted treatments. Within this edition, Goel et al. contribute directly to this discussion with their description of acute fatty liver of pregnancy as an uncommon yet important cause of preventable maternal mortality globally, concluding that further research must be done improve fetal outcomes in this population.

Thus, I am happy to present to you the latest edition of *EMJ Hepatology*, and I also invite you to attend the next ILC in April 2019 in Vienna.

Yours sincerely,

Prof Markus Peck-Radosavljevic

Klinikum Klagenfurt am Wörthersee, Austria

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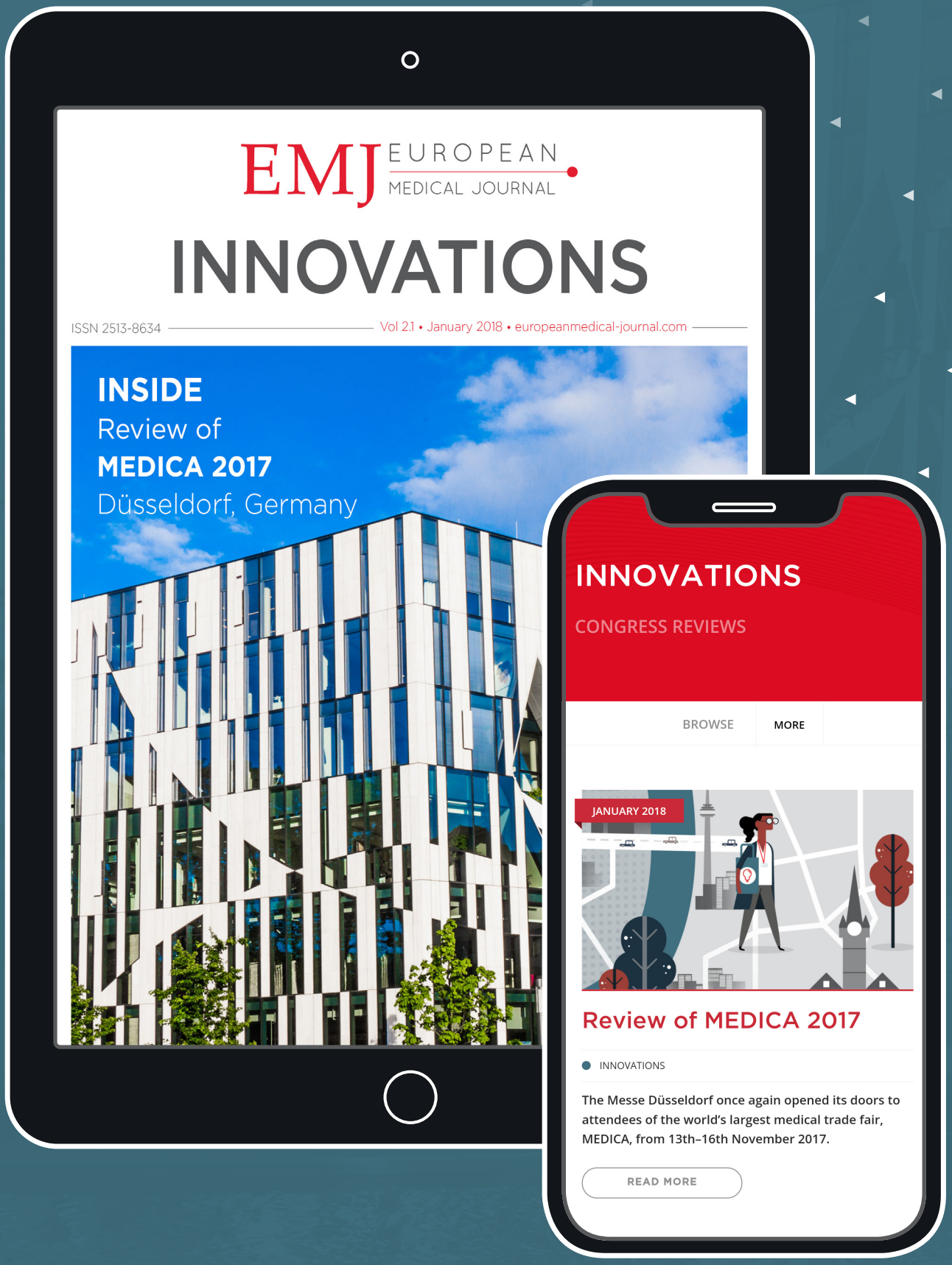

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+ Review of MEDICA 2017 Düsseldorf, Germany, 13th–16th November 2017

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+ A Transition from Disease-Centred to Goal-Directed Individualised Care of Patients with Multiple Morbidities: A Journey to Goal-Orientated Patient Healthcare Katarzyna Rygiel

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Congress Review

Review of the European Association for the Study of the Liver (EASL) International Liver Congress (ILC) 2018

Location: Paris, France – Paris Expo Porte de Versailles

Date: 11.04.18–15.04.18

Citation: EMJ Hepatol. 2018;6[1]:12-28. Congress Review.

Bigger and better than ever before, this year's dynamic EASL ILC was held in Paris, France from the 11th–15th April 2018. This historic city, long recognised as a hub of culture and sophistication, provided a beautiful backdrop for the event as delegates arrived to pursue scientific excellence. Thousands of hepatology scientists, clinicians, and associates flocked to the Paris Expo Porte de Versailles, where >1,500 original presentations would be on offer; however, this was just the tip of the iceberg.

During the opening ceremony, performed before a crowd of delegates from across the globe, Prof Tom Hemming Karlsen, Secretary General of EASL, introduced the ILC as: "truly now an international platform for liver disease." He went on to explain some of the innovations that the EASL had put in place for the meeting, including the inspiration for the congress format, which this year was modelled on the 'everyday work' of its attendees: "...when we do our job as hepatologists we interact with other specialists, we interact with each other, we discuss the patients during the morning rounds... and you will have morning rounds here that you can attend and you can participate in the discussions as if you were in your own centre."

Thousands of abstracts were submitted from all over the world, showcasing a plethora of the greatest hepatological achievements. Each day, a huge variety of presentations took place, from plenary sessions to basic science sessions and late-breaking research conferences. The congress not only offered delegates wonderful opportunities to network with peers, but also to meet key opinion leaders in the field during the corresponding Expert Sessions and to enhance their skillsets in the learning centre. Interactivity was paramount at the ILC, where a hands-on, discursive approach was taken to maximise the learning opportunities for all attendees.

The meeting also included, for the first time, a collaboration between the EASL and the European Society for Medical Oncology (ESMO) in the form of a co-organised symposium to discuss the future of liver cancer. This impressive session highlighted a theme deeply interwoven within the congress itself: a multidisciplinary approach is integral to hepatological research and clinical practice. With discussion ranging from the latest therapies to personalised treatment, this co-operation between two of Europe's titanic societies surely ensured that the future of liver cancer research will be standing on the shoulders of not one, but two giants.

Thousands of hepatology scientists, clinicians, and associates flocked to the Paris Expo Porte de Versailles, where >1,500 original presentations would be on offer; however, this was just the tip of the iceberg.

Throughout the meeting, social media was ablaze as attendees shared photos from presentations, discussions on the late-breaking research, and, naturally, many stunning photos of the Eiffel Tower. As a platform for rapid communication, social media is second to none, and at the ILC it was used to its full potential to instantaneously disseminate crucial information to scientists around the world.

In the following Congress Review section, we bring you highlights from across this captivating meeting, including the latest research on topics such as cirrhosis, viral hepatitis, primary biliary cholangitis, and nonalcoholic steatohepatitis. For those of you lucky enough to have attended the ILC this year, we hope this section reignites your memory of the incredible event; for those of you who sadly missed out, we hope this brief overview can convey even a fraction of the passionate spirit at the very heart of this event.

Next year's ILC will be held in beautiful Vienna, Austria, home of the Wienerschnitzel, the Habsburgs, and the harmonies of Wolfgang Amadeus Mozart. We look forward to seeing you there.

Presentation of New Liver Dialysis Technology

NEW and developing technologies were presented at the ILC, with the BioTech Village home to a myriad of product showcases. One such technology that premiered was DIALIVE. As part of their product launch, the ALIVER consortium published a short, animated video, as reported in a EASL ILC press release dated 11th April 2018.

This video communication was intended to increase public awareness of liver failure and the challenges associated with its management. Indeed, around 170,000 people die annually across Europe as a result of liver cirrhosis, with >1 million deaths attributed to the condition globally each year. There are also high numbers of individuals living with liver disease: 650 million globally, with 29 million of these being citizens of the European Union (EU).

The results from the first clinical trials are now eagerly anticipated in order to see if DIALIVE will represent a step on the path to a new outlook for patients with liver failure.

The video began by presenting a number of the factors that put people at risk of liver disease, including long-term, excessive alcohol consumption and malnutrition. Having briefly summarised the risk factors, the video then stated that liver transplant was currently deemed the only plausible treatment for patients with liver failure; however, transplant rates were limited by the number of viable organs available. It is this treatment gap that DIALIVE, a liver dialysis device, aims to fill. The technology was developed by researchers at University College London, London, UK, and is currently undergoing clinical trials. As the video explained, the intention behind the technology is to extend the lives of patients with cirrhosis for a long enough period of time that either a transplant becomes available or their liver starts recovering. The results from the first clinical trials are now eagerly anticipated in order to see if DIALIVE will represent a step on the path to a new outlook for patients with liver failure.

Immunotherapy Beneficial in the Management of Hepatocellular Carcinoma

T CELLS engineered to present hepatitis B virus (HBV)-specific T cell receptors (TCR) have been shown to be an effective immunotherapy, preventing further lung metastases after liver transplant in hepatocellular carcinoma (HCC) patients, according to study results reported in a EASL ILC press release dated 12th April 2018. After 6 months of treatment with this novel immunotherapy, computed tomography (CT) imaging showed a volumetric reduction of nearly all lung lesions detected in HCC liver

transplant patients, with no new lesions detected in the lung or liver up to the time of the congress. All tumour lesions in the lungs remained stable until January 2018.

HCC is the second most common cause of cancer mortality worldwide, with a poor prognosis and limited therapeutic options.[1] Liver transplantation is an option for some HCC patients; however, HCC reoccurs in up to 20% of cases, with metastases to the lungs being the most common.[1–3]

"Further development of this new immunotherapeutic strategy may offer new hope of a cure for HCC."

Lead author, Dr Anthony Tan, Emerging Infectious Disease Programme, Duke-NUS Medical School, Singapore, explained: "We first had to test if short, integrated HBV DNA fragments in the tumour cells can produce HBV epitopes recognised by cytotoxic T cells." The characterisation of the short, integrated HBV DNA fragments allowed researchers to identify HBV epitopes that were presented on the cell surface. Further analysis identified a region of the HBV envelope encoded by integrated HBV DNA fragments derived from the primary HCC of liver transplant patients with hepatitis B surface antigen (HBsAg)-negative HCC metastasis in the lungs. TCR specific for the HBV envelope were incorporated into T cells and, over a 6-month period, multiple adoptive transfers of the HBV-specific TCR into patients were performed, presenting with these encouraging CT results.

With >50% of all HCC cases thought to be associated with chronic HBV and up to 20% of HCC cases reoccurring in liver transplant patients, the potential impact of this novel treatment method could be vast.[1,4] Dr Tan concluded: "Further development of this new immunotherapeutic strategy may offer new hope of a cure for HCC."

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Web-Based Lifestyle Intervention Programme Improves Liver Health

WEB-BASED lifestyle change intervention has been shown to be as effective as group-based, face-to-face programmes for improving weight loss and overall liver health in nonalcoholic fatty liver disease (NAFLD) patients. These results, highlighted and reported in a EASL ILC press release dated 13th April 2018, may present a more feasible and achievable management option for many NAFLD patients.

With structured lifestyle intervention programmes now recommended in NAFLD management guidelines, the team, led by Prof Giulio Marchesini, University of Bologna, Bologna, Italy, set out to develop a successful web-based lifestyle modification programme aimed at younger, busier patients who may not be able to attend traditional group intervention programmes. "Weight loss has long been recognised as an effective therapy for NAFLD, but the challenge has been creating the infrastructure to achieve it," elucidated Prof Philip Newsome, Queen Elizabeth Hospital and University of Birmingham, Birmingham, UK, and EASL Governing Board Member. The Italian study included 716 NAFLD patients divided into two intervention groups: a 5-week, intensive, group-based programme (n=438) or a web-based series of five modules (n=278). NAFLD severity markers were evaluated at 6, 12, and 24 months follow-up and the percentage of patients who achieved a 10% weight loss was used as the study's primary outcome.

"Our study has shown that a web-based lifestyle modification programme is a feasible and practical way of achieving a clinically meaningful level of weight loss in our NAFLD patients..."

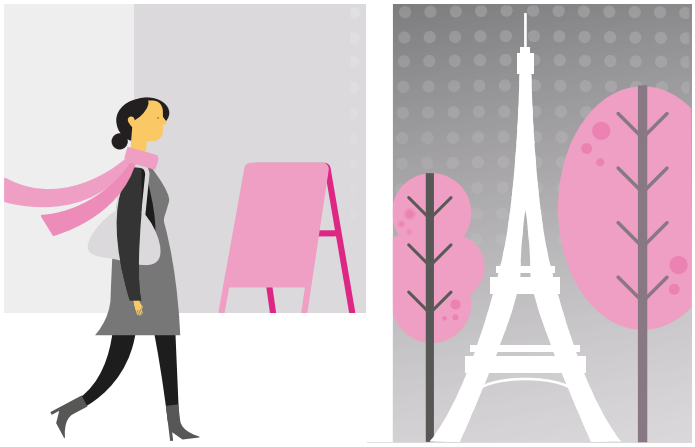
According to Prof Marchesini, the primary outcome was achieved by >1 in every 10 patients in both intervention groups, and this coincided with a decrease in the surrogate markers of fibrosis. "This weight loss threshold has been associated with resolution of nonalcoholic steatohepatitis and regression of fibrosis in studies that have evaluated NAFLD histology," explained Prof Marchesini. The team reported significant decreases in liver enzyme levels in both intervention groups; however, patients using the web-based programme were more likely to have a normal alanine aminotransferase level at both 6 months and 12 months (odds ratio: 2.34 and 2.22, respectively).

"Our study has shown that a web-based lifestyle modification programme is a feasible and practical way of achieving a clinically meaningful level of weight loss in our NAFLD patients," concluded Prof Marchesini. For the estimated 25% of adults across the globe who have NAFLD, lifestyle modifications aimed at weight loss, improved dietary habits, and increased physical activity are essential, and the team hope to initiate similar web-based intervention programmes at other liver units.

Obeticholic Acid Treatment Demonstrates Antifibrotic Effects for Primary Biliary Cholangitis Patients

PRIMARY biliary cholangitis (PBC) is a rare autoimmune disease with numerous detrimental effects on the liver that ultimately lead to the development of fibrosis, cirrhosis, and hepatocellular carcinoma. Typically, the disease is treated with ursodeoxycholic acid (UDCA), but up to 40% of patients treated with UDCA have an insufficient response. Now, researchers have demonstrated that long-term treatment with obeticholic acid (OCA) can lead to the reversal or stabilisation of fibrosis in PBC patients who had an incomplete response to UDCA.

The POISE study, the results of which were reported in a EASL ILC press release dated 13th April 2018, was a biopsy substudy that examined patients who had undergone liver biopsies before and after 3 years of treatment with OCA. These biopsies were assessed via a six-tier staging system, ranging from no fibrosis to cirrhosis. Thirteen patients receiving UDCA at baseline ultimately had paired biopsies suitable for analysis; nine (69%) of these patients had pre-cirrhotic fibrosis and four (31%) had cirrhosis.



Paris Expo Porte de Versailles

Venue for the ILC 2018

"Relevant changes are on the way for the management of patients with PBC, for which ursodeoxycholic acid has been the only treatment option for a long time..."

After 3 years of treatment, the results of these biopsies showed that most patients had an improved (n=6; 46%) or sustained (n=5; 38%) histological stage. Two patients (15%) had deteriorated. In addition, three of the four patients with baseline cirrhosis had improved to fibrosis while receiving this treatment.

"Eighty-five percent of the patients with PBC in this study with an incomplete response to UDCA had regression or no worsening of their fibrosis or cirrhosis after 3 years of OCA treatment, a period of time during which we would have expected some degree of fibrosis progression," explained Dr Christopher Bowlus, University of California, Davis, California, USA.

These results represent a positive step in the management of this rare and complicated condition, providing a new and promising option for treatment. "Relevant changes are on the way for the management of patients with PBC, for which UDCA has been the only treatment option for a long time," concluded Prof Marco Marzioni, University Hospital of Ancona, Ancona, Italy. Further research on the use of OCA is already underway.

New Therapeutic Option for Primary Sclerosing Cholangitis

A POTENTIAL therapeutic option for prolonging transplant-free survival in primary sclerosing cholangitis (PSC) patients has shown promise, according to the results of a Phase II multicentre study that was reported in a EASL ILC press release dated 13th April 2018. The development of new treatments for PSC is of importance because, although liver transplantation is an effective option for the treatment of advanced stages of the condition, there are presently no treatments that facilitate increased transplant-free survival.

The researchers investigated NGM282 therapy for PSC patients. NGM282, a nontumourigenic engineered analogue of fibroblast growth factor 19, has previously been shown to have potential as a viable treatment for nonalcoholic steatohepatitis. In this new study, 62 patients with PSC and an elevated alkaline phosphatase level were randomised to one of three study arms: placebo, daily 1 mg subcutaneous injection of NGM282, or daily 3 mg subcutaneous injection of NGM282.

"This study provides good evidence of relevant clinical activity for NGM282 in individuals with PSC and highlights the need to explore NGM282's impact on liver fibrosis in larger studies of a longer duration."

While there were no significant reductions in serum alkaline phosphatase levels at 12 weeks in either active treatment study arm when compared with placebo, which was the study's primary endpoint, there were significant reductions in several other variables. Patients in the 3 mg NGM282 study arm presented with significant reductions in alanine aminotransferase and aspartate aminotransferase serum levels at Week 12 ($p<0.01$ versus placebo). Furthermore, patients in both NGM282 study arms displayed reduced serum levels of 7α-hydroxy-4-cholesten-3-one when compared to the placebo study arm.

The researchers also reported that there were no differences between study arms in PSC-related clinical events. Adverse events in the NGM282 study arms were predominantly mild and able to be resolved while the patients were on treatment; the most commonly reported events were frequent stools, injection site reactions, and diarrhoea.

Summing up the key impact of the research, Prof Gideon Hirschfield, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK, who was presenting the results, explained: "This study provides good evidence of relevant clinical activity for NGM282 in individuals with PSC and highlights the need to explore NGM282's impact on liver fibrosis in larger studies of a longer duration."

Rapid Automated Analysis of Nonalcoholic Steatohepatitis Histological Features

Automated analysis of nonalcoholic steatohepatitis (NASH) has been shown to be highly accurate at predicting histological patterns relating to hepatocellular ballooning and lobular inflammation in liver biopsy samples, according to data presented in a EASL ILC press release dated 13th April 2018. The deep-learning system created to detect and analyse these features was able to predict cell histological patterns with accuracies of 98% and 91% for ballooning and inflammation, respectively.

"Our automated scoring system for ballooning and inflammation showed a high correlation with expert evaluation and it is ready to be used for high-throughput activity scoring in preclinical studies or, in the near future, as a companion diagnostic tool for clinical application."

An expert histopathologist determined the ballooning and inflammation scores for liver biopsy samples from animal models of rats or mice fed on a choline-deficient, L-amino acid-defined diet supplemented with cholesterol. The deep-learning models were then constructed to detect and analyse these histological features. The initial training set of 31 animal models was used to train the device, which was then used to predict the histological features in four independent cohorts (n=271).

The resultant data showed excellent agreement between the histopathologist and the fully automated scores for ballooning and inflammation for each cohort at a cellular level (kappa coefficient: 0.84 and 0.81, respectively), with the full tissue samples (kappa coefficient: 0.71), and between whole slide imaging-based automatic scoring of inflammation on the training cohort (Rho=0.907).

Mr John Brozek from GENFIT, Lille, France, the company developing the deep-learning system, commented on the reasoning behind the study: "The histological evaluation of NASH by microscopy is time-consuming and limited by inter and intra-observer variability." With liver biopsy remaining essential for establishing a NASH diagnosis and the severity of the disease, the aim of this novel diagnostic technique was to eliminate the subjectivity of interpreting histological images.

Commenting on the encouraging results, Mr Brozek explained: "Our automated scoring system for ballooning and inflammation showed a high correlation with expert evaluation and it is ready to be used for high-throughput activity scoring in preclinical studies or, in the near future, as a companion diagnostic tool for clinical application." These results, coupled with the fact that the number of individuals with NASH is increasing rapidly worldwide, could revolutionise the diagnostic strategy for this disease.

The Beneficial Effect of a Mediterranean Diet

A MEDITERRANEAN diet, rich in fermented foods such as yoghurt and vegetables, has been linked to greater gut bacterial diversity and a lower risk of hospitalisation in patients with liver cirrhosis when compared with patients consuming a predominately Western diet, according to a EASL ILC press release dated 12th April 2018.

Alongside the primary drivers of liver cirrhosis (alcohol consumption and hepatitis B and C infections), gut microbiota has been linked to the pathogenesis and progression of cirrhosis: bacterial biodiversity has been shown to be significantly reduced in healthy individuals, compensated cirrhosis patients, and decompensated cirrhosis patients. Dr Jasmohan Bajaj, Virginia Commonwealth University, Richmond, Virginia, USA, explained that the aim of the study was to investigate the impact of diet on the progression of liver cirrhosis.

"This is an important study stressing that an antioxidant-rich Mediterranean diet has a protective effect not only in the early phases of chronic liver disease but also in its more advanced phases."

The research team recruited 157 liver cirrhosis patients from the USA, who predominately consumed a Western diet high in coffee and carbonated drinks, and 139 liver cirrhosis patients from Turkey exposed to a Mediterranean diet. Dietary and stool microbiota analysis revealed that the Turkish patients had a significantly greater level of gut bacteria diversity compared to patients from the USA. Analysis also showed that there was no difference between the biodiversity in healthy and cirrhosis individuals in Turkey, while in the USA there was a vast contrast in the biodiversity of the gut microbiome. Furthermore, there was a significantly higher number of liver-related hospitalisations in the USA population compared to the Turkish population.

Further work needs to be carried out to establish whether a change in diet can be used as a nonpharmacological alternative for protecting against liver disease, which, according to this investigation, could be likely. Prof Annalisa Berzigotti, University of Bern, Bern, Switzerland and EASL Governing Board Member concluded: "This is an important study stressing that an antioxidant-rich Mediterranean diet has a protective effect not only in the early phases of chronic liver disease but also in its more advanced phases."

Promising Results for Hepatitis C Elimination from Iceland and Georgia

ENCOURAGING results have been reported in a EASL ILC press release dated 13th April 2018 from Iceland and Georgia regarding the efforts of both nations to eliminate the hepatitis C virus (HCV) at a population level. Despite the adoption of different approaches, both the Icelandic and Georgian initiatives have recorded high levels of engagement in the programmes, initiation of the use of direct-acting antiviral agents (DAA), and, ultimately, cure of chronically infected HCV patients; this suggests both countries are on track to achieve their HCV elimination goals.

Georgia was home to the world's first HCV elimination programme, which operated in collaboration with the US Centers for Disease Control and Prevention (CDC). Initiated in April 2015, the programme's positive results are now being revealed for the first time. Of 43,989 people diagnosed with HCV, 33,673 patients had begun DAA treatment and 24,273 individuals had already achieved a sustained virological response. "In the first 2 years of this programme, we have diagnosed more than one-quarter of our HCV-infected adults in Georgia, we have treated 77% of those diagnosed, and cured over 95% of those completing treatment," explained Prof Tengiz Tsertsvadze, Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia.

These two programmes represent a positive step towards HCV elimination. The Icelandic results were particularly exciting, given its nature as an island and the programme's defined target population.

January 2016 marked the start of the Icelandic programme, which focussed on those who inject drugs, prisoners, and those who had advanced liver disease, as well as those who quickly become reinfected. "Encouragingly, even in individuals with recent intravenous drug use, DAA treatment, although challenging, resulted in an 87% cure rate, including in those that did not complete the treatment regimen," explained Dr Valgerdur Rúnarsdóttir, Vogur Hospital, Reykjavík, Iceland. He added: "We would like to emphasise and encourage collaboration between addiction treatment centres in both screening and treating HCV. This is key to success in reaching the population in focus."

These two programmes represent a positive step towards HCV elimination. The Icelandic results were particularly exciting, given its nature as an island and the programme's defined target population. "If elimination of HCV is possible without a vaccine, it will surely be possible in Iceland," explained EASL Governing Board Member Prof Markus Cornberg, Hannover Medical School, Hannover, Germany.

Treatment of Rare Liver Diseases

PROMISING PROGRESS in the development of therapies for three rare liver diseases was reported in a EASL ILC press release dated 14th April 2018. The developmental therapies, namely sebelipase alfa, givosiran, and ARO-AAT, were designed to treat lysosomal acid lipase (LAL) deficiency, acute intermittent porphyria (AIP), and alpha-1-antitrypsin (AAT) deficiency, respectively. The importance of this work was highlighted by Prof Marco Marzioni, EASL Governing Board Member, who declared: "Rare diseases are a greater challenge than you might expect, as apart from the difficulties in reaching a full diagnosis, there are often no effective treatments available."

"Rare diseases are a greater challenge than you might expect..."

The first study examined the long-term use of sebelipase alfa, which was approved in 2015 for the treatment of LAL deficiency. The study followed 31 patients with LAL deficiency treated with a 1 mg/kg intravenous sebelipase alfa infusion every other week. The patients were followed for up to 96 weeks and the results demonstrated reductions from baseline across several measured variables: -44.4% in alanine aminotransferase, -38.4% aspartate aminotransferase, -17.6% liver volume, -14.9% liver fat content, and -16.5% spleen volume. Furthermore, most adverse events were mild-to-moderate in severity.

Also reported were the results of a three-part Phase I study assessing the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of givosiran in patients with AIP. One of the studies found that patients who were treated with 2.5 mg/kg givosiran on a monthly basis had a mean decrease of 83% in the annualised attack rate and, additionally, an 88% decrease in the number of haemin doses. During the course of the study, there were no serious adverse events reported.

Finally, the results of ARO-AAT pharmacological activity in non-human primates were announced. It was shown that, after two doses of 3 mg/kg ARO-AAT administered 4 weeks apart, non-human primates presented with a 89–91% mean decrease in serum AAT. This decrease was sustained for >7 weeks following the second of the doses, which the researchers argued lent support to the case for less frequent dosing with ARO-AAT.

Glecaprevir/Pibrentasvir First Real-World Study: Effective Treatment of Hepatitis C Virus Infections

ENCOURAGING results from the first real-world studies assessing the efficacy and safety of the combination drug glecaprevir/pibrentasvir (G/P) for the treatment of hepatitis C virus (HCV) infections were presented at this year's ILC meeting and reported in a EASL ILC press release dated 12th April 2018. Sustained virological response (SVR), defined as undetectable HCV RNA 4 and 12 weeks after

treatment, was measured in both Italian and German studies of HCV patients treated for 8–16 weeks.

The G/P combination is a relatively new direct-acting antiviral treatment, with the drugs inhibiting NS3/4A protease and NS5A, respectively. Until now, no real-word studies with G/P have been reported, despite initial Phase II and III trials reporting high SVR12 rates and a favourable safety profile.

"...G/P has the potential to expand the treated population and support the goal of HCV elimination."

The Italian study presented an interim analysis of 723 consecutively G/P-treated patients. Analysis of the data revealed that HCV RNA was undetectable in 74% of patients at Week 4 and in 98% at the end of treatment. Treatment-related adverse effects were few and were of mainly mild severity. Only three patients discontinued treatment prematurely.

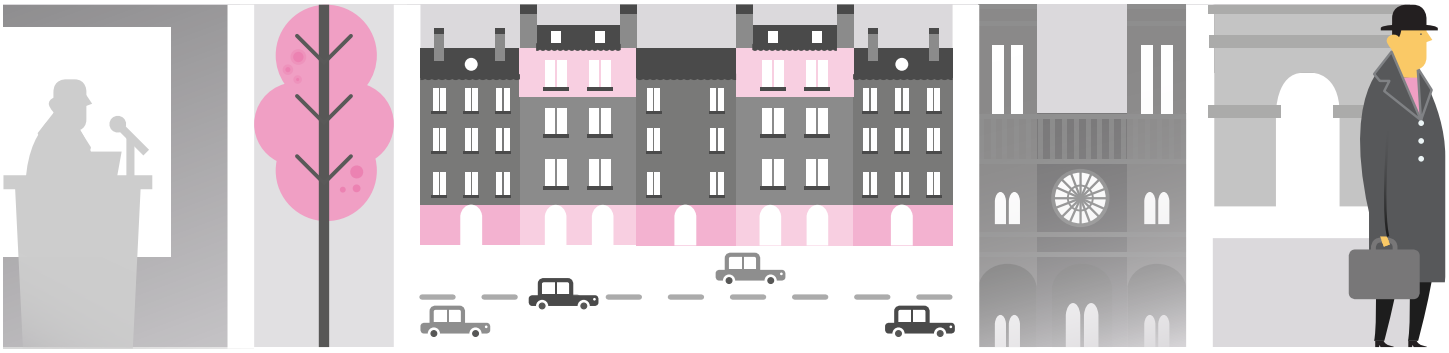
Dr Roberta D'Ambrosio, University of Milan, Milan, Italy, commented: "Our real-world study involving more than 700 patients with chronic HCV infection confirmed that the effectiveness and safety profile of G/P were excellent across a range of different patient types."

The ongoing German study assessed 638 patients treated with G/P for 8 weeks. Adult patients with HCV genotypes 1–6, with or without compensated cirrhosis, who were treatment-naïve or treatment-experienced were included. Of the 49 patients with data at the time of presentation, 100% had achieved SVR12; this excluded those who had discontinued treatment prematurely (n=4), two of whom due to adverse effects.

Commenting on the real-world benefits of G/P combination treatment, Prof Thomas Berg, University of Leipzig, Leipzig, Germany, said: "We have found G/P to be a very useful addition to our HCV treatment armamentarium as it simplifies treatment decisions for the majority of patients; G/P has the potential to expand the treated population and support the goal of HCV elimination."

Novel Primary Biliary Cholangitis Treatments Show Promising Results

PHASE II studies presented at EASL ILC 2018 and reported in a EASL ILC press release dated 13th April 2018, show encouraging preliminary efficacy, safety, and tolerability of tropifexor and seladelpar for the treatment of primary biliary cholangitis (PBC). These results may pave the way for effective treatment of the 3–5% of PBC patients who cannot tolerate ursodeoxycholic acid (UDCA), the current standard of care.



In the first study, PBC patients with a poor response to UDCA were randomly allocated to receive once daily placebo or tropifexor, a selective non-bile acid farnesoid X receptor agonist, at doses 30 µg, 60 µg, or 90 µg for 4 weeks. Decreases in markers of cholestasis and hepatocellular damage were observed in a dose-dependent manner, including a 72% reduction in gamma-glutamyltransferase and a 41% decrease in alanine aminotransferase at Day 28 in the 90 µg/day tropifexor group versus placebo ($p<0.001$). The team concluded that these results show the potential benefit of farnesoid X receptor agonism for the treatment of PBC, and the lack of reports of increased itch with tropifexor is a major advantage of this drug.

"Clinical research in PBC is very active at present and these two studies indicate how much scientists are engaging in designing studies aimed at providing patients with effective treatments."

The second study involved patients who expressed an inadequate UDCA response; the patients were randomised to receive 2 mg, 5 mg, or 10 mg per day of the peroxisome proliferator-activated receptor-delta agonist seladelpar. The study's primary efficacy outcome was a change from baseline in alkaline phosphatase (ALP) levels; at Week 26, 69%, 67%, and 79% of patients in the 5 mg/day, 5–10 mg/day, and 10 mg/day groups, respectively, had an ALP <1.67-fold higher than upper limit of normal. Overall, normal ALP levels at 26 weeks were reported in 29% of patients. The team concluded that seladelpar demonstrated an impressive level of activity over the study period: "In the absence of a transaminase safety signal, the doses of 5 mg/day and 10 mg/day appear to represent an appropriate risk/benefit profile," stated Prof Gideon Hirschfield, University of Birmingham, Birmingham, UK. Like tropifexor, seladelpar also demonstrated no associated increase in pruritis, and some patients even experienced a decrease in itch, suggesting anti-pruritis activity.

These Phase II studies show promise for longer-term studies in PBC patients and may suggest alternative treatments for the progressive disease that do not involve UDCA. Commenting on these encouraging results, Prof Marco Marzioni, University Hospital Ancona, Ancona, Italy and EASL Governing Board Member, added: "Clinical research in PBC is very active at present and these two studies indicate how much scientists are engaging in designing studies aimed at providing patients with effective treatments."

Phylogenetic Analysis Identifies the Origins of Hepatitis A Outbreak

Phylogenetic analysis of the viral agents behind a recent hepatitis A outbreak in Italy has linked the culprit pathogens to disease outbreaks in other European nations, as reported in a EASL ILC press release dated 12th April 2018.

The genetic sequencing of a virus, such as hepatitis A, can aid in the identification of the source of the disease and can highlight the population groups that are most at risk. Dr Massimo Iavarone, Fondazione IRCCS Ca' Granda Maggiore Hospital, Milan, Italy, led a study that prospectively analysed 244 acute hepatitis A cases from the Lombardy region of northern Italy between January and May 2017.

The rarely fatal hepatitis A infection was shown to predominately affect men who have sex with men, leading to the number of infected persons increasing from 0.750 and 1.069 per 100,000 inhabitants in 2015 and 2016, respectively, to 9.512 per 100,000 inhabitants during the study period. Of these patients, 80% were hospitalised, with a median stay time of 7 days, and, while no patients required liver transplant, 14% of the patients were diagnosed with severe liver disease.

"Efforts to increase hepatitis A vaccine coverage in high-risk groups must be taken to strengthen population protection from HAV [hepatitis A viruses]."

Molecular analysis of the patients revealed that 93% were infected with the hepatitis A 1A virus genetic variant, with the remaining 7% were diagnosed with hepatitis A 1B. The 1A hepatitis variant was identified to be the cause of hepatitis A outbreaks in the UK, Germany, and the Netherlands. Phylogenetic comparisons between the outbreaks showed that, of the 1A cases in Italy, 54% shared genetic origin with the UK viral variant, 45% with the Dutch variant, and 1% with the German 1A variant. Further analysis showed that the Netherlands strain accounted for 100% of the January hepatitis A cases in the Lombardy region, while the UK strain caused 68% of the cases in May. Dr Iavarone concluded: "Efforts to increase hepatitis A vaccine coverage in high-risk groups must be taken to strengthen population protection from HAV [hepatitis A viruses]."

New Information on Hepatitis E Virus Infection

INFORMATION that challenges the contemporary understanding of hepatitis E virus (HEV) infection was shared at the EASL ILC 2018 and reported in a EASL ILC press release dated 12th April 2018. The studies showed that blood-borne HEV transmission was more common than previously thought and that HEV infections can be fatal in both immunocompromised and immunocompetent individuals. With the reported incidence of HEV infection on the rise across Europe and >21,000 cases reported from 2005–2015, this information is of high importance and could ultimately lead to a change in the management paradigm of HEV infection.

The first study presented was a retrospective analysis of 150 HEV RNA-positive individuals; 69 of the patients were immunocompetent. Five years after HEV infection diagnosis, the mortality rate of immunosuppressed patients was 10%, and 3 out of 8 of these deaths were believed to be related to the HEV infection. Approximately half (53%) of the immunocompetent individuals were hospitalised for a combined total of 74 days; furthermore, 2 of these individuals who had pre-existing liver disease died as a result of developing acute-on-chronic liver failure. The researchers noted that their findings showed HEV infection could be associated with significant morbidity and mortality in both immunocompromised and immunocompetent individuals.

"Both studies emphasise the severity of HEV infection in vulnerable patients."

The second study examined the transmission of HEV infection. A retrospective analysis was conducted on the data of 37 patients with HEV infection who were immunosuppressed. Of the 30% of patients who later developed chronic HEV infection, 36% had originally been infected as a result of a HEV-positive blood donation. Of the 4 patients infected due to blood donation, 2 had been recipients of a heart transplant and had been treated for humoral rejection with a combination of rituximab and plasmapheresis. Based on their results, the study authors recommended that blood products for transplant or immunosuppressed patients should be screened for HEV RNA.

Prof Markus Cornberg, EASL Governing Body Member, spoke on the significance of the results, commenting: "Both studies emphasise the severity of HEV infection in vulnerable patients." He went on to note: "These studies will lead to further discussions around if and how HEV screening of blood products should be carried out."

Direct-Acting Antiviral Agent Therapy Slowing Hepatitis C Progression in Scotland

NATIONAL records of hepatitis C virus (HCV) patients in Scotland have been used to examine the efficacy of direct-acting antiviral agent (DAA) therapy in preventing the progression of the disease to decompensated cirrhosis. The results of this analysis were reported in a EASL ILC press release dated 12th April 2018. DAA cure HCV, providing a sustained virologic response, and are effective in >90% of HCV patients.

Scotland has an estimated 34,500 people chronically infected with HCV and is considered a world leader in the battle against viral hepatitis, making it an excellent source of HCV data. "Scotland's national surveillance of HCV treatment and disease means we are ideally placed to examine the early impact of DAA treatment on HCV-related disease progression at a population level," explained Prof Sharon Hutchinson, Glasgow Caledonian University, Glasgow, UK.

Data from the Scottish HCV Clinical and Diagnosis databases, combined with figures from the national inpatient hospital database, were analysed to examine what therapies were used to treat HCV patients from April 2014–March 2017. Additionally, the number of patients admitted to hospital with decompensated cirrhosis for the first time between 2000 and 2016 was recorded. From a cohort of 4,800 people, 83% were treated with DAA and 94% achieved a sustained viral response. In comparison to the previous 3 years, this scale-up of therapy was linked to a 29% and 39% reduction in first-time presentation of decompensated cirrhosis in patients previously diagnosed with HCV and those with HCV at the time of admission, respectively.

"We have been able to show that scale-up of therapy has resulted in substantially fewer patients presenting with decompensated cirrhosis, but has highlighted the need to address comorbidities that pose a continued risk of liver disease progression in those clear of the virus," commented Prof Hutchinson.

EASL Governing Board Member Prof Markus Cornberg, Hannover Medical School, Hannover, Germany, concluded: "Recently, the value of DAA therapies has been challenged by a Cochrane review. These data are therefore very important in documenting not only sustained virological response, but also the prevention of morbidity and mortality."

"We have been able to show that scale-up of therapy has resulted in substantially fewer patients presenting with decompensated cirrhosis..."

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Interviews

A selection of insightful interviews from the esteemed *EMJ Hepatology* Editorial Board

Featuring: Dr Amedeo Lonardo, Dr Diego Calvisi, Ms Fiona Fry, and Dr Dhiraj Tripathi

Dr Amedeo Lonardo @LinkedIn

Azienda Ospedaliero-Universitaria, Italy

What first motivated you to pursue a career in medicine and what, or who, inspired you to specialise in the field of hepatology?

Initially, I enrolled at the medical school in Naples where I was born, with the idea of becoming a psychiatrist. However, when I started my fourth year of studying medicine, my father gave me the gift of a subscription to an esteemed medical journal. It was reading this that convinced me that there were areas of medicine that were probably more advanced than psychiatry at the local medical school. Following a selective examination, I was accepted for three different postgraduate specialisation classes in Naples: internal medicine, infectious diseases, and gastroenterology; I chose gastroenterology. Incidentally, my father was also diagnosed with a chronic liver disease; maybe I chose my hepatological studies as an unconscious reaction to his illness.

Could you describe your main responsibilities at the Azienda Ospedaliero-Universitaria in Modena? Are there any challenges that you currently face in your position?

I am a full-time hospital physician and vice director of the Unit of Internal Medicine. Our ward is, to a large extent, populated by patients admitted via an emergency route from the Accident and Emergency Unit. Some physicians of our medical team are also engaged in the specialised activity of diagnosis and management of metabolic disorders for outpatients. For our group, it is not always easy to conciliate these two different 'souls', generalist and specialist, but we do our best.

"...maybe I chose my hepatological studies as an unconscious reaction to his illness."

"A call to action is necessary to prevent the development of NAFLD and MetS, not only in developed countries but on a global basis."

You have published multiple papers on nonalcoholic fatty liver disease (NAFLD). What first interested you about this fascinating disease?

In 1988 I moved from Naples to Modena. After serving as a doctor in the Accident and Emergency Unit of the local public hospital, I gradually started performing liver ultrasound scanning as a part of my medical duties in the internal medicine ward. In doing so, I was impressed by the very common occurrence of 'bright' (i.e., fatty) livers in asymptomatic outpatients. Concurrently, by reading many different medical sources, I also developed an interest in the cluster of metabolic derangements commonly alluded to as metabolic syndrome (MetS). What was incredible to me were the similarities linking 'bright' liver patients to those with MetS. Despite such an evident similarity, only a few authors in the published literature had dared to identify or hypothesise a causal link between 'bright' liver and MetS at that time. Such a link was formally reported by three different authors, including myself, in 1999. Many years later, we now appreciate that NAFLD and MetS are, indeed, closely and mutually bidirectionally linked to each other.

In developed countries, NAFLD is a very common liver disorder due to increasing obesity rates; what initiatives have emerged in recent years to combat the condition's high prevalence? What actions are necessary to prevent NAFLD developing into nonalcoholic steatohepatitis and ultimately cirrhosis?

The 'diabesity' epidemic we are witnessing clearly shows that no effective initiatives have emerged so far to fight metabolic disorders in the general population. A call to action is necessary to prevent the development of NAFLD and MetS, not only in developed countries but on a global basis. The public should first become aware of this necessity and implement it through political and health authorities on a global scale; however, this is a very complex action that involves the diffusion of healthier lifestyles as an individual initiative. Even more difficult to achieve is a reduction in the sale of junk food, including beverages enriched with fructose. In the final analysis, a healthy lifestyle seems to be incompatible with the 'obesiogenic' and sedentary milieu we all live in, and possibly even in conflict with certain economic interests.

What aspect of NAFLD are you and your team currently working on and how does this relate to global research regarding NALFD?

There are some chief aspects we are interested in, such as the search for noninvasive markers of presence and severity of disease, investigating the utility of innovative drugs in preventing the fibrotic progression of disease, and better characterising the determinants of hepatic versus extrahepatic features of the natural history of NAFLD. The first line of research goes back to the initial interests of our medical school in Modena. The second is more 'trendy' and new for us given that, until recently, no specific drugs were available for specifically treating nonalcoholic steatohepatitis (i.e., the more evolved form of NAFLD). The last promises to be the basis for a more personalised approach to the follow-up of these patients.

Recently there have been new insights into the role that dysbiosis of the microbiota plays in NAFLD pathogenesis. Do you think the microbiota will be a new area of interest for researchers tackling NAFLD progression, since both have links to obesity?

There has been a growing interest in the role that altered gut microbiota play in the development of metabolic disorders in humans. Although manipulation of gut microbiota promises to effectively treat such diseases, present evidence, in my opinion, strongly supports microbiota replacement as being effective in treating infections such as recurrent

Clostridium difficile. Of course, this is not to confute the conceptual pathophysiological importance of this particular line of research but rather to highlight that any other indications, such as treatment of MetS in humans, remain to be further evaluated.

What other innovative developments do you hope will emerge in the near future to aid in the early diagnosis and treatment of NAFLD?

I think biomarkers will increasingly be used, and I am concerned that this will detract from the role of ultrasonography, which plays a major role in suggesting the presence and anticipating the metabolic and histological severity of NAFLD, further to ruling out focal liver disease. Clearly, novel drugs are in the pipeline and will probably be marketed soon. Again, I am concerned that our society will have to pay an expensive 'drug bill' as a remedy to unhealthy lifestyles rather than eating less or better, and performing some physical activity whenever possible.

We understand that you are a member of the Italian Association for the Study of the Liver (AISF). Is there anything that the society is currently focussing on that you are particularly excited about? What role do national organisations such as the AISF play in improving the management of hepatic conditions?

What I appreciate most about the AISF is its open attitude in promoting the scientific development of young hepatologists. Further to this, the AISF is a very active scientific society, which is engaged in a variety of different disorders spanning the entire gamut of liver disease, not only NAFLD.

Further to bringing about the constant education of medical hepatologists, again with a specific interest being shown for residential classes offered to young hepatologists, scientific societies such as AISF also address key links in the chain of knowledge and collaboration among specialists and general practitioners to the advantage of the individual patient.

As well as being a member of the AISF, you regularly author research papers and complete reviews for several international journals. How do you prioritise your workload when undertaking such different roles?

As a hospital physician and vice director, my absolute priorities are assisting inpatients and collaborating with my director in many aspects of his responsibilities, not only providing assistance to patients but also directing the medical team, under the clinical governance of the managers of the public hospital we work in. When such priority duties are finished, I am very busy serving as a referee for medical journals, completing >100 reviews per year. Finally, if there is some time left, I dedicate it to writing medical articles. Thus, I work a lot and have little time to attend medical congresses, so I go only to those where I am invited as a speaker. Luckily, my partner has been quite understanding so far.

Finally, you teach general practitioners and tutor medical students. What advice would you give to medical students who would like to pursue a career in the world of hepatology?

In the past, I have been more involved in teaching young general practitioners but, for various reasons, I eventually relinquished my position. Now, I spend more of my time teaching medical students and even more time teaching fellows in internal medicine. With this last group of young colleagues there is daily contact and close collaboration in routine medical activity, which is the basis of their post-doc education.

I would warmly recommend young hepatologists to preserve a high ethical standard, both when dealing with patients and, importantly, with colleagues and researchers. In this respect, I feel a strong sense of responsibility because I think that teaching becomes credible if the teacher is credible. With regard to the technical contents, hepatology is, by its very nature, a multidisciplinary area and many different medical specialities converge in the scientific formation of a modern hepatologist. The particular proportion of each of the individual competences acquired will make each hepatologist unique. Finally, I would highlight to

young hepatologists that, while an individual's enthusiasm to work may wane with age, one can have their best research ideas and projects in their mature years. This simple consideration implies that different generations of researchers are destined to collaborate by law of nature.

Dr Diego Calvisi

University of Sassari, Italy

What or who encouraged you to become a specialist in hepatological medicine? Was there a particular aspect of your medical education that was influential in your career path?

During my medical studies at the University of Sassari, I became more and more interested in cancer research; thus, I started to work as a volunteer in the Institute of General Pathology directed by Prof Francesco Feo, a renowned expert in liver carcinogenesis. After graduation, he gave me the opportunity to join the Laboratory of Experimental Carcinogenesis at the National Cancer Institute (NCI) (part of the National Institutes of Health [NIH]), directed by Dr Snorri S. Thorgeirsson, one of the most innovative and prominent researchers in liver cancer internationally. These two unforgettable and very constructive experiences convinced me to pursue my career in this specific field.

What does a typical day in the laboratory involve for you and your research team?

Generally, the day in the lab starts with a discussion on the planned experiments and eventual problems to overcome. Subsequently, I continue my work in the office, where I take care of generating new ideas and writing manuscripts and grants, as well as analysing our results and comparing them with the known information from the literature. Being part of a relatively small research group, I have to focus on these duties and have no time to work in the lab; instead, my collaborators dedicate most of their working time to the lab.

"...our knowledge of the mechanisms responsible for HCC development and progression needs to be deepened and the oncogenic process better understood."

As the most common type of primary liver cancer, how has research progressed in recent years to improve the prognosis of hepatocellular carcinoma (HCC)?

A mounting body of information regarding the molecular mechanisms leading to HCC development and progression has been generated in the last decade, leading to a better and deeper understanding of this highly malignant tumour. However, this progress in the knowledge of the molecular pathogenesis of HCC has not been successfully translated into effective therapies (and, thus, a better prognosis for the patients) to date. Indeed, with the exception of sorafenib and regorafenib, two multikinase inhibitors, molecularly targeted therapies have been shown to be ineffective in unresectable HCC. In addition, the benefits of sorafenib and regorafenib are almost negligible in terms of survival of HCC patients. Obviously, our knowledge of the mechanisms responsible for HCC development and progression needs to be deepened and the oncogenic process better understood. For instance, it is clear that an intricate network of compensatory mechanisms takes place when we inhibit a pathway that seems to be important in HCC, thus precluding the effectiveness of our therapy attempt. It is crucial to identify and characterise these mechanisms in order to circumvent their

deleterious effects on treatments. Furthermore, it is widely accepted that HCC is a highly heterogeneous disease in terms of molecular features, indicating that the same drug or treatment might be either effective or unsuccessful depending on the features of each tumour. Thus, it is crucial to identify in the tumour or, even better, in the serum the molecular markers that could predict adequately whether a patient would find a treatment beneficial or not.

One aspect of your research focusses on the pathological molecular mechanisms responsible for HCC development, and you have published substantial work on many of the different pathways. Which pathway is of main interest to researchers currently and why?

Since the discovery of signalling pathways and their roles in liver carcinogenesis is a growing and dynamic field, it is difficult to select a favourite pathway to investigate. Nevertheless, two particular pathways have attracted most of my attention lately. The first pathway is the lipogenic cascade; HCC cells, like many other tumour types, rely on lipids (especially fatty acids) for many of their metabolic needs. Of note, some of the crucial players in this pathway are not active in the adult liver and could be specifically targeted by drugs that are already used for obesity. Another interesting pathway is the heat shock cascade. The heat shock response is one way in which normal cells respond to a variety of stressors in order to survive. This response is highly induced in liver cancer cells, where it is orchestrated by the heat shock factor 1 (*HSF1*) gene. Of note, recent studies from our laboratory have shown that suppression of *HSF1* is able to completely abolish liver carcinogenesis in various mouse models without significant side effects, thus implying that *HSF1* might be a promising target for the treatment of human HCC.

What do you think the future holds for HCC treatment, and what particular treatment advances would you like to see?

I believe that the future holds great hopes for the treatment of HCC due to constant new discoveries in the field. A better understanding of the molecular features of HCC will allow the design of novel therapeutic regimens characterised by combinations of specific drugs able to synergistically delay or inhibit the growth of HCC cells and their spread to other organs. Of course, the appropriate treatment strategies will be employed following the stratification of the patients based on the molecular features of each tumour; this approach is known as precision medicine.

You recently published a paper supporting the use of MEK inhibitors for the treatment of intrahepatic cholangiocarcinoma, another common primary liver tumour. What steps need to be taken to further validate these results and progress this treatment into clinical practice?

It is well established that mutations in the Ras/MEK/ERK pathway play a critical role in cholangiocarcinogenesis. Thus, it was not surprising to detect a beneficial effect of MEK inhibition on the growth of cholangiocarcinoma lesions in mice. Similarly promising results have been shown in a limited number of human cholangiocarcinoma cases recently. However, the fact that cholangiocarcinoma development was delayed but not abolished in mice, together with the molecular complexity of the human disease, suggests that MEK inhibitors should be used in combination with drugs that are able to suppress other critical pathways.

You are a strong advocate of personalised treatment methods. What makes tailored treatment so important for hepatology patients?

As previously mentioned, it is clear that HCC is not a single tumour entity, but rather a number of tumour subtypes with peculiar molecular features and, presumably, specific molecular vulnerabilities. Thus, a tailored therapeutic approach would be necessary for the treatment of each patient in order to achieve a significant benefit. On one hand, providing a molecular treatment for all HCC patients without any molecular discrimination would 'dilute' the eventual positive effects of the drugs. For instance, clinical trials with inhibitors of the mTOR pathway have been conducted

and showed no benefits on the treated cohorts. However, the mTOR inhibitors were administered universally (without determining whether the same liver tumours displayed activation of the mTOR pathway), thereby making the evaluation of the results difficult to interpret. On the other hand, administering a drug to non-responders could be dangerous for the patients and create a significant, avoidable cost for the healthcare system.

In your opinion, what do you think the biggest challenges will be for hepatologists in the next 5 years?

A hepatologist should orientate their focus more on the molecular aspects of liver tumours in order to be prepared for the development of innovative therapies against this deadly disease.

What moment from your career so far are you most proud of?

I am glad to have contributed to the discovery of pathways and mechanisms that, when inhibited, could be beneficial for the treatment of HCC patients.

Finally, if you could give one piece of advice to your younger self as an aspiring medical student, what would it be and why?

Doing research and having a career as a researcher is difficult due to the limited funding and high competitiveness. However, it is a passion and a truly challenging job. Thus, it should be pursued with all our efforts and without hesitation.

"I believe that the future holds great hopes for the treatment of HCC due to constant new discoveries in the field."

Ms Fiona Fry

Royal Devon and Exeter NHS Foundation Trust, UK

We recently spoke to one of the *EMJ Hepatology* Editorial Board members, Ms Fiona Fry, about a number of pertinent topics in the field. Ms Fry is a very important voice in the liver nurses' community, holding the position of Lead Hepatology Nurse at the Royal Devon and Exeter NHS Foundation Trust, Exeter, UK as well as being co-chair of the recently amalgamated British Liver Nurses Association (BLNA), a special interest group of the British Association for the Study of the Liver (BASL).

With a wealth of experience in this area of medicine, Ms Fry was able to provide us with excellent insights into the evolving role of nurses in hepatology and the crucial point of contact they have become for liver patients. We also talked about the importance of the recent establishment of the BLNA last year, a process Ms Fry was heavily involved in, and some of the vital work they are currently undertaking. Additionally, we analysed a number of challenges in the field and the ways they can be addressed to help improve care for these patients. These include combatting stigmatism of liver conditions such as hepatitis C and the work of outreach services in helping a patient group that is often difficult to treat via the usual methods.

Increased Skillset

Ms Fry firstly outlined how her own role and that of nurses in general has broadened over the years, requiring an increasingly greater skillset and knowledge than ever before. "I have been a hepatology nurse specialist for nearly 18 years and my role has developed significantly in this time. When I started in this role, I just saw hepatitis C patients that were referred by the consultant for treatment," she explained. "That has completely evolved and is now a truly

nurse-led service, especially for viral hepatitis patients. My week is very busy with clinics in both the hospital and outreach settings, hepatocellular carcinoma (HCC) screening, and also inpatient and telephone support (to patients, their family/carers, and general practitioners). The complexity of patients seen has changed dramatically over the past 17 years: whereas previously all complex liver patients would be seen solely by the consultants, they are now being seen in the nurse clinics. I now see anyone from a decompensated cirrhotic patient to a new hepatitis B patient in the clinic."

These multifaceted skills have had a significant impact in relieving the pressure on consultants, according to Ms Fry, who also explained that nurses tend to hold certain attributes that make them particularly suitable for such a prominent role in patient care. One of these is their organisational skills and systematic mode of operation that prevents people missing crucial stages of their care. "I think nurses are very good at putting in those systems," she added.

Relationship with Patients

In addition, nurses are particularly adept at communicating with patients, something that is well-recognised as being crucial for ensuring the best possible outcomes. With consultants often constrained by time, nurses have a pivotal role in explaining each step of diagnosis and subsequent treatment to hepatology patients, for example, the reasons why they are undergoing a particular scan or procedure, which makes them more likely to turn up for appointments. Building relationships through familiarity with the nurses also enhances the trust patients have with the care they are receiving, and this improves adherence to management and treatment plans. "A good example is the establishment of really effective nurse-led services for the delivery of hepatitis C treatment. Adherence to treatment is essential to ensure the best outcomes and nurses have clearly proven their skills in delivering this," stated Ms Fry.

"I now see anyone from a decompensated cirrhotic patient to a new hepatitis B patient in clinic."

"A good example is the establishment of really effective nurse-led services for the delivery of hepatitis C treatment. Adherence to treatment is essential to ensure the best outcomes and nurses have clearly proven their skills in delivering this."

Creation of the BLNA

With the aim of giving nurses a more unified and stronger voice in hepatology in the UK, Ms Fry, who was chair of the BASL Nurse Forum, and Ms Michelle Clayton, previously chair of the British Liver Nurse Forum, merged the two organisations to set up the BLNA in September 2017. It is hoped that in addition to providing a more coherent message from liver nurses in the UK, unique knowledge and skills from both bodies will be brought together to engage in new projects that enhance the provision of care. One that has been embarked upon since the inception of the BLNA is a scoping exercise to establish a database of all the liver nurses working in the UK. Ms Fry explained that, currently, many nurses are isolated in underfunded regions and not receiving sufficient support. They hope that a record of every liver nurse will ultimately ensure they each have access to full levels of support and educational opportunities.

Stigmatism in Liver Disease

Ms Fry was drawn to specialising in hepatology whilst working in gastroenterology wards during the early part of her career. She observed that some healthcare professionals tended not to treat liver patients with as much compassion as that being given to other gastroenterology patients. She quickly developed a lasting empathy and concern for these patients, finding that they didn't always have as much of a voice as other patients and often relied on nurses to advocate for them; this is partly due to the complex issues many of these patients tend to have, such as alcohol misuse and mental health problems. These issues, as well as the commonly held perception that liver disease

is self-induced, has meant that stigmatism has always been a major problem in this area of medicine. Ms Fry believes these sorts of attitudes have improved over time with education, but there is clearly still a lot of work to be done.

Hepatitis C Testing

As part of an Operational Delivery Network (ODN) through which hepatitis C treatment is delivered, Ms Fry and her colleagues are playing a major part in the effort to implement the recent NHS England plan to eliminate hepatitis C in England by the year 2025. With massive strides being made in this direction, particularly treatment-wise over recent years, Ms Fry is confident of this being achieved; however, she sees the biggest challenge in this respect to be finding all the undiagnosed hepatitis C patients out there, believing a major advertising campaign that encourages more people to be tested would be highly beneficial. In particular, she feels that the 'baby boom' generation (born between 1946 and 1964) who may, in the past, have been exposed to hepatitis C after receiving blood transfusions or blood products, recreational drug use, tattoos with unsterilised equipment, etc., should be made aware of these risks and be offered testing. "We are seeing patients aged in their 50s and 60s that are presenting with cirrhosis or liver cancer that didn't know they had hepatitis C," commented Ms Fry. "I think we're good at testing drug users and prisoners and all those at really high risk, but we also need to look at going back to people who may have had a risk many years ago."

Outreach Services

The liver nursing service at Royal Devon and Exeter NHS Trust currently delivers outreach services to both the local prison and GP surgery for the homeless and vulnerably housed. "We are now able to see and treat patients with hepatitis C in these outreach settings which has proved hugely successful in engaging with patients by offering a 'one stop shop'; this has been made even more effective with the availability of a portable fibroscan. Patients can be assessed, monitored, and treated without the need to travel to the hospital for appointments and investigations which had previously often been a barrier to accessing care," she explained. Another big step forward that she informed us about has been in obtaining the funding for an ultrasound machine to enable vascular access in the outreach clinics. Vascular access teams have provided best practice for taking bloods from people with poor venous access within the hospital setting for many years, and by securing a portable ultrasound machine they have been able to remove another barrier to accessing treatment.

There are similar hepatitis C outreach services being delivered across the UK, and these could be replicated to see patients with other types of liver disease, such as fatty liver disease. Fatty liver disease is the condition that Ms Fry sees as being the next major area that needs addressing in hepatology. She believes nurses will have an important role in combatting this and other liver conditions by reaching out to at-risk patients in the community. This could be achieved by developing more outreach services in the community that enable education, monitoring, and treatment to take place in general practice surgeries rather than requiring patients to travel to local hospitals.

The next stage is to provide a business case for the creation of more outreach services, something Ms Fry is working towards. It is successes such as this that show the voice and positive influence on decisions that nurses can have in the field of hepatology.

Optimism

Overall, there are undoubtedly grounds for optimism in regard to the care of liver disease patients. The efforts being put into the elimination of hepatitis C are highly positive for example, and Ms Fry reiterated that nurses are able to make a difference and help bring about changes that benefit patients, although she acknowledged this can be time-consuming and, at times, frustrating.

"We are now able to see and treat patients with hepatitis C in these outreach settings which has proved hugely successful in engaging with patients by offering a 'one stop shop'..."

Dr Dhiraj Tripathi

Queen Elizabeth Hospital Birmingham, UK

The EMJ team interviewed Dr Tripathi at the European Association for the Study of the Liver (EASL) International Liver Congress (ILC) 2018 in Paris, France. What follows is a transcription of that interview.

Could you please give us an update on the progression of the CALIBRE trial, for which you were a leading investigator?

The CALIBRE trial stands for carvedilol versus variceal band ligation in prevention of bleeding in patients with liver cirrhosis. This is a National Institute for Health Research (NIHR)-funded national randomised control trial.

Some may remember the trial in patients with alcoholic hepatitis called the STOPAH trial; this was also an NIHR-funded trial. We are going to be approaching all of the centres that recruited for the STOPAH trial, 66 centres in total. We are at the stage of set-up, so we are doing the protocol and all the regulatory bits and pieces, including the trial registration, MHRA, and approaching all the pilot centres; we have approached 20 centres to pilot. Over the next 6 months, we are hoping that the setup process will be complete, and then we will be ready to recruit. When we start the will depend on the approval of the different sites, research and development approvals, and contracts.

What are you hoping the outcomes will be from the trial, and what changes do you hope this will lead to in terms of care for patients?

CALIBRE is an NIHR-funded trial and came about due to a need for a large trial to investigate patients who have medium-to-large varices and have not bled. In the UK at the present time, there is a disparity between national guidelines. On one hand, the National Institute for Health and Care Excellence (NICE) have said to do one thing, and the British Society of Gastroenterology (BSG) have said something similar but not quite the same. As a result, there is a bit of a disparity, and the resource implications for band ligation are enormous; this is an expensive procedure requiring patients to come not just for one but for four to five endoscopies. Carvedilol has been shown to be very effective, better than other beta-blockers in portal hypertension, but it has not been studied in a large trial.

The primary endpoint of this trial is variceal bleeding, and there are a number of secondary endpoints, which include survival and mortality. Additionally, there is a lot of interest in the role of beta-blockers in advanced liver disease and whether it can reduce decompensation. As such, liver decompensation is one of the secondary endpoints, along with other associated complications, like liver cancer and infections. There is also a qualitative element of this in the pilot: we want, for example, to learn why the patients did not consent to the trial, and also health economics, with a cost-effectiveness analysis. The trial is powered to show that carvedilol is better, which is why the sample size is so large (>2,500). However, the results from the trial will be a way off in the future; we are talking recruitment for 4 years and a 1-year follow-up, so it is a 5–6 year recruitment period and about 8 years in total.

If the trial shows improved survival and less bleeding that would be fantastic, but it is only powered to show differences in bleeding If it shows less bleeding with carvedilol, then carvedilol can replace band ligation as the primary therapy, and this would have enormous resource implications. Obviously, we would have less bleeding and less need for endoscopy, because once you have somebody on carvedilol, you do not need to do any further routine endoscopies. If somebody is having band ligation, then the number of endoscopies is indefinite. So, you can work out the maths there: a procedure that costs hundreds of pounds versus a tablet that costs less than a pound a month. There are huge resource implications for the NHS if carvedilol is shown to be more efficacious in regard to the primary endpoint.

Is variceal bleeding in cirrhosis patients a growing problem at the moment?

In total, estimates from NICE state that there are around 60,000 patients per year with liver cirrhosis; this may be a slight underestimate. Half of those patients will develop varices, equating to 30,000 patients with varices, and maybe about 10% of those will develop varices that will bleed. The overall prevalence of cirrhosis is probably slightly on the rise, and there will be an increasing problem with variceal bleeding. I think it is a very relevant, and a very cultural problem. It is one of the most dramatic complications of liver cirrhosis; there are no ifs or buts, if somebody presents with a variceal bleed it is a major, life-threatening emergency.

Which sessions of this year's ILC were you most looking forward to attending or have attended?

The programme is fantastic, and I particularly enjoy using the app. It really helps to plan the conference. It is a long conference, and because there is so much, you do need to focus, otherwise you will not make the most out of it. I downloaded the app in advance and planned ahead. I have a particular intertest in portal hypertension and also in vascular liver diseases. There is a special interest group, the vascular liver disease interest group (VALDIG); the chair is Prof. J.C. Garcia Pagàn. I am a member of this group and they had a session early Wednesday morning, as well as the general assembly meeting.

I am looking forward to some of the interesting parallel sessions on portal hypertension, and I am particularly interested in the clinical aspects. There are also 'Meet the Experts' sessions; however, the rooms are quite small, so you need to get there well in advance to get a seat. There is also a session where they were looking at the literature as well, dissecting landmark papers from the 1970s, which I am hoping to attend. There is also a session on the clinical aspects of portal hypertension and a very interesting session on intensive care for patients with liver disease.

"...if somebody presents with a variceal bleed it is a major, life-threatening emergency."

Do you regularly learn lessons from colleagues from other countries at the ILC that inform your own research and clinical practice?

Very much so. Obviously, I have talked about the sessions, but there are lunch breaks and coffee breaks, so it is these breaks throughout the conference that give ample opportunities to learn from colleagues and to network, which is very important. People say: "You do not need to go to a conference, you can just look it up all online" and you can, all of the ILC is recorded, and you can log on if you are a member and access multiple talks, but it is not the same as being there, and actually interacting with colleagues. You need to attend the meetings and interact, there are things about talking to someone that is so different; for instance, you can share research ideas. The VALDIG group are proposing a number of studies and I am collaborating with them; I would not have had that opportunity to if I had not attended the ILC. You cannot get everything just by emails, you need to be there in person and attend the meetings and the committee meetings. I have found all these factors so important over the many years I have been attending these conferences, which is exactly why I make a point to attend, so that I can really interact.

This must be very important for you to create research collaborations over the years?

Very much so. I am a member of the UK Portal Hypertension research group and we had a very informal meeting, where some of the members got together to discuss clinical trials.

Have you noticed any changes in the prevalence and severity of liver patients in recent years at your role at The Liver Unit, The Queen Elizabeth Hospital, Birmingham, UK, and if so, why do you think this might be the case?

That is a very good question. I started at The Queen Elizabeth Hospital about 10 years ago as a consultant hepatologist, in a liver transplant position. At that time, we were doing 140 transplants a year. In the last financial year,

we were doing about 200. So, in the last 10 years we have increased the number of transplants from 140 to 200, which gives you an idea of the increasing burden of liver disease. Perhaps the numbers have not shot up, but there is an increase in burden in terms of the severity of liver disease; we are seeing more severe liver diseases, which is reflected by the growth of our clinics; there is about 10% growth every year of clinical activity. As there have been so many advances in the last 10 years and because the unit is so research active, we are actually attracting patients from outside the region. There is also a national rare diseases initiative to improve the care of these patients, and I think Birmingham has the only national rare diseases centre for adults. I am seeing more and more patients with particular liver diseases, and my colleagues are attracting more patients as well, a lot of them are involved in clinical trials.

Do you think that because a liver transplant has become a much safer procedure, has that increased the prevalence of cases?

It is about awareness. At the moment we still have a way to go in terms of improving access to transplantation. I think things are a little bit better now; in the past the areas where patients needed transplants the most were the ones who had worst access to transplantation. There have been some measures to improve transplant access; for example, I run an outreach clinic in Liverpool, UK, where I see patients who do not have to come all the way to Birmingham to start the transplant process, we can do it there and then. I am one of many colleagues in liver transplant centres who do this kind of outreach work. That has really grown over the last few years, and it is so important. I do not think the number of liver transplant centres is going to increase, but we are reaching out to areas where there may not be optimal access to transplantation.

"In some parts of the country, the mortality rate is almost 8-times higher than in other parts, and some patients die 9 years earlier in some parts of the country than other parts..."

Do you believe there is a disparity in care received by liver patients across the UK? And if so, how do you think this issue can be addressed?

That is a very good topical question. You may be aware that there have been a couple of papers released recently on the subject, with the first one published in 2013. The research showed that there is quite a marked geographical disparity. If you look at years of life lost to a patient <75 years old, liver disease comes fourth in male patients and second in female patients. In some parts of the country, the mortality rate is almost 8-times higher than in other parts, and some patients die 9 years earlier in some parts of the country than other parts; there is quite a disparity. This is particularly notable as liver disease is largely preventable; the main contributors are alcohol, viral hepatitis, and fatty liver disease. There are a number of initiatives to try and reduce the burden of alcoholic liver disease, with successful initiatives in the Merseyside area. Also, liver units need to be accredited, so there are a number of quality standards. This is all being rolled out nationally now, so if you want to be a liver unit you have to be accredited. Also, there is increased public health initiatives such as minimum unit pricing, which is now active across the border, in Scotland, UK. There is also the sugar tax; it is up to the manufacturers and has not been enforced, but it is going some way towards reducing the huge burden. We are talking billions of pounds of tax revenue lost because of loss of days work for these patients that have morbidity from liver cirrhosis.

What first inspired your interest in portal hypertension?

When I was a junior trainee all those years ago, I was interested in gastroenterology; I liked the procedures. Then one of the consultants introduced me to hepatology, and he had links with the Edinburgh Liver Unit, a liver transplant unit. I was appointed there as a specialist registrar, and I started to look into research opportunities and got really interested in portal hypertension. I liked endoscopies and varices, and got really interested in that, and Prof Peter Hayes, a Professor of Hepatology at the University of Edinburgh, really inspired my

interest and supported my research; he was my supervisor for my research on portal hypertension. My interest in the topics really stemmed from there because he was so enthusiastic. He kept everybody really interested and was very supportive.

What are the areas that require the most attention in portal hypertension in your view?

Complications of cirrhosis, including variceal bleeding, there is a lot of interest in transjugular intrahepatic portosystemic shunts (TIPS) and early TIPS, early on after variceal bleed to try and reduce the risk of re-bleeding and death. A trial published a number of years ago showed dramatically improved outcomes in patients using TIPS rather than the usual standard of care. Real-life data is a bit variable, but I know that there is a trial that is about to finish recruitment in Scotland on early TIPS. We need more data, particularly for patients who have gastric varices, where there is a lack of studies because these studies are very difficult to recruit for as there are not many patients and some patients are not eligible. There is also interest in ascites; there is now interest in TIPS for ascites, not just for end-stage but for intervening early so the patients do not have to come back for repeated drains. It is mainly about early prevention, once you get to a certain point and you develop a number of complications in liver cirrhosis, the only option then is a liver transplant. We want interventions well before this point, so we need to stratify patients so patients with early stages of disease are treated differently to those with more advanced liver disease. This is the area we need more insight into and more studies looking into it.

What advice do you have for young medical students who want to get the most out of their time at the ILC?

Plan! It is so easy now because you can get access to the app about 2–3 weeks before the conference where you can see the full programme. You can download the electronic posters, and you can even communicate with poster presenters through the app, so you can really plan ahead. Also, if you are having any meetings, plan those well in advance because it is such an opportunity to network. It is more than just going to the presentations, it is about networking, make the most of that time. The collection of important people here is through the roof, there are top, top hepatologists here, make the most of it, if you have questions to ask, think about them beforehand.

What have been the main personal highlights for you personally from your years of attending the ILC?

There have been a lot of landmark studies presented at the congress over the years on portal hypertension, viral hepatitis, and cholestatic diseases. These congresses are an opportunity to see these studies and interact with the presenters. If I did not attend these conferences I would not have been aware of the VALDIG group and have been able to be as involved and interact with the individuals. It is about the interactive component; it is so important.

Are there any new areas of research that you are looking to move into in the future?

CALIBRE is taking up a lot of my time. There is a lot of interest in prevention of variceal bleeding by the NIHR and NHS because it requires huge NHS resource. If someone has a variceal bleed they end up in intensive care for days. Each day in intensive care is very expensive. Furthermore, the mortality is up to 20%, 1 in 5 will die. As a result, there is a lot of interest in variceal bleeding, and also prevention of variceal bleeding and starting early on in the process. At the slightest hint of a varices, you could start treatment. We do not know how early to start treatment in cases of varices, so I have also applied for a grant for a study to look into that. The theme is ‘get there early’ because by the time a patient requires a transplant they have missed the boat, so it is about getting in early and stratification. Also, with the vascular liver disease network, we have collaborated on a number of studies in this very, very niche topic with very small numbers of patients. The only way we can study this group of patients is with international collaborations.

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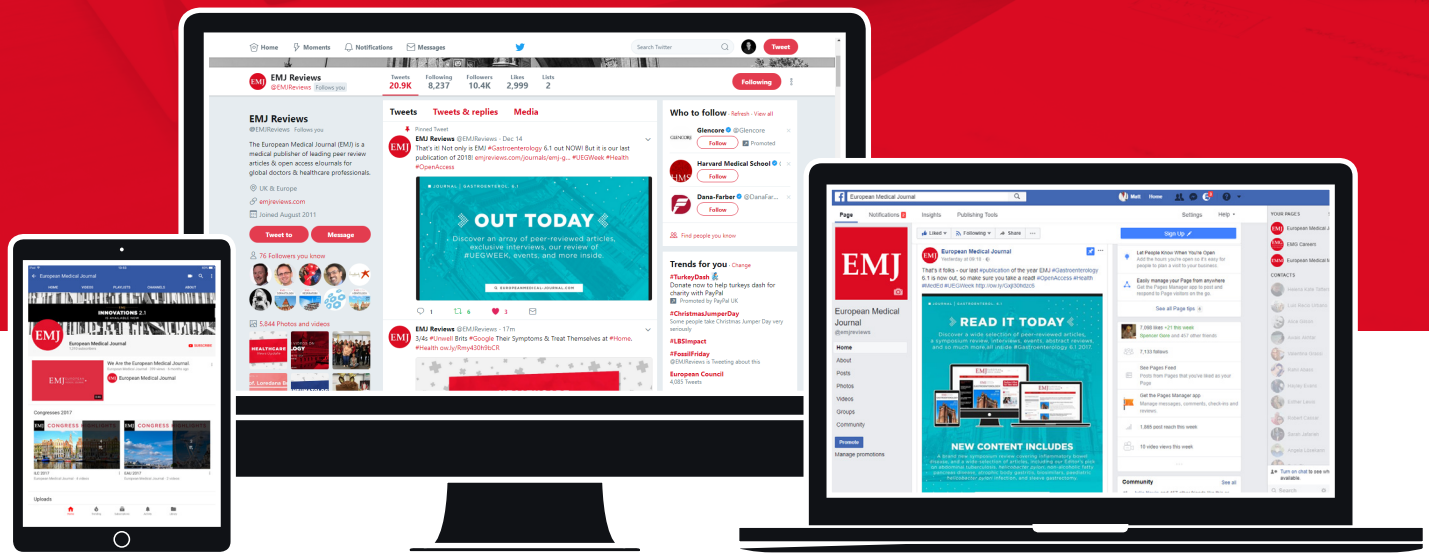

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Abstract Reviews

A series of abstract reviews from the EASL ILC 2018, presented and summarised by the researchers themselves

Resolution of Ascites, Hepatic Encephalopathy, and Variceal Bleeding in Patients with Hepatitis C Virus and Decompensated Cirrhosis Who Received All-Oral Direct-Acting Antiviral Treatment

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Disclosure: Dr Sims has received research support from the National Institute on Alcohol and Alcoholism (NIAAA). Dr Massoud has received grants from Gilead Sciences. The remaining authors have declared no conflicts of interest.

Keywords: Decompensated cirrhosis, hepatitis C virus (HCV), treatment.

Citation: EMJ Hepatol. 2018;6[1]:55-56. Abstract Review No. AR1.

INTRODUCTION

Worldwide, 185 million people are infected with the hepatitis C virus (HCV);[1] approximately 12% of these individuals have manifestations of decompensated cirrhosis.[2] No studies have examined sustainable reductions in specific forms of decompensation in patients with HCV and decompensated cirrhosis after treatment. Thus, the primary aim of this study was to examine changes in the proportion of patients with HCV and decompensated cirrhosis with ascites, hepatic encephalopathy, and variceal bleeding at pretreatment compared to 3 and 12 months post sustained virological response (SVR). Secondary aims were to compare pretreatment and post-SVR Model for End-Stage Liver Disease (MELD) scores, Child–Pugh (CP) scores, and α-fetoprotein (AFP) levels.

METHODS

A retrospective chart review was performed on all patients with HCV and decompensated cirrhosis who began direct-acting antiviral (DAA) treatment between November 2014 and January 2016 at a tertiary medical centre. Measures of central tendency and frequency distributions were used for univariate analysis. Pretreatment proportions of patients with ascites, hepatic encephalopathy, and variceal bleeding were compared to 3 and 12 months post-SVR proportions using the McNemar–Bowker test. Changes in median MELD score, CP score, and AFP levels were compared using the Wilcoxon signed rank test.

RESULTS

Thirty-seven patients met the inclusion criteria. Most patients were male (57%), Caucasian (84%), treatment-naïve (60%), and genotype 1 (78%). The median patient age was 60 years. Ascites was resolved in 29% of patients at 3 months (65% versus 36%; p<0.01) and in 35% at 12 months (65% versus 30%; p=0.07). Hepatic encephalopathy was resolved in 54% at 3 months (70% versus 16%; p<0.01) and in 48% at 12 months (70% versus 22%; p=0.03). Variceal bleeding was resolved in 32% at 3 months (35% versus 3%; p<0.01) and in 27% at 12 months (35% versus 8%; p<0.01). Median AFP levels were significantly reduced (6.45 [range: 0.81–39.70] versus 4.24 [range: 0.60–15.70], respectively; p<0.01), with a median decrease of 2.21. There were no differences in statistical significance between pretreatment and post-SVR median (interquartile range) MELD scores (15 [8–28] versus 15 [6–36]; p=0.20) and median CP scores (7 [5–11] versus 7 [5–15]; p=0.60).

DISCUSSION

We demonstrated that patients who achieve SVR with DAA can also achieve reductions in manifestations of hepatic decompensation. These findings were clinically significant. Ascites and hepatic encephalopathy are the most common reasons for hospital admission among patients with advanced liver disease. Although there were no improvements in MELD and CP scores in this study, MELD and CP scores did not worsen with DAA treatment. This will allow clinicians to pursue DAA treatment in patients currently on liver transplantation waitlists with the potential benefit of improving morbidity and mortality without affecting their ability to obtain a transplant. Finally, the significant reductions in AFP scores after treatment can perhaps improve the clinical utility of AFP as a screening modality for hepatocellular carcinoma in patients with advanced liver disease.

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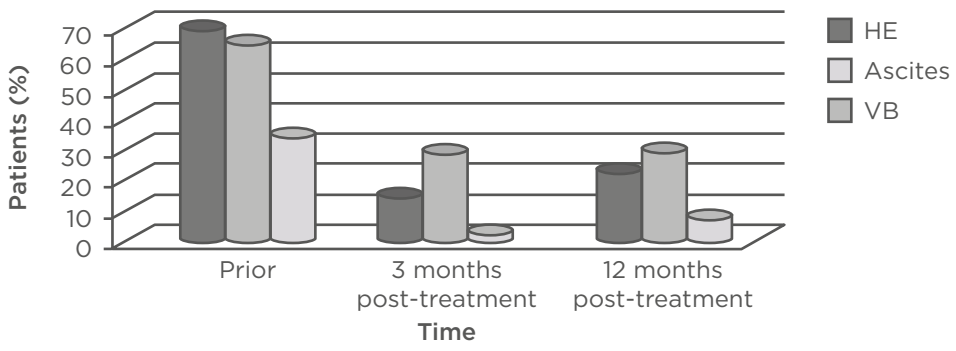

Figure 1: Manifestations of decompensated cirrhosis prior to treatment and 3 and 12 months post-sustained virological response.

HE: hepatic encephalopathy; VB: variceal bleeding.

A Comparison of Renal Function Before and After Treatment of Chronic Hepatitis C Infection in Patients Who Achieve Sustained Virological Response with Direct-Acting Antivirals

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Keywords: Hepatitis C (HCV), renal function, treatment.

Citation: EMJ Hepatol. 2018;6[1]:57-58. Abstract Review No. AR2.

BACKGROUND AND AIMS

Approximately 3 million people have chronic hepatitis C virus (HCV) infection in the USA.[1] However, there are limited data with regard to the effect of direct-acting antivirals (DAA), a treatment for HCV, on renal function. The purpose of this study was to examine changes in renal function after HCV treatment with DAA.

METHODS

This study was a single centre, retrospective analysis of patients with chronic HCV infection who were treated with DAA. Patients were included if they were seen in the clinic between December 2014 and December 2015, had confirmed diagnoses of chronic HCV infection by RNA polymerase chain reaction test, and achieved sustained viral responses after treatment. Post liver transplantation patients were excluded, as were patients with incomplete data. Data were collected on demographics, presence of cirrhosis, presence of hepatic decompensation, presence of hepatocellular carcinoma, genotype, treatment experience, treatment regimen and duration, and pre and post-treatment glomerular filtration rate (GFR) and serum creatinine. Measures of central tendency and frequency distributions were used for univariate analysis. The paired sample t-test was used to compare serum creatinine levels before and after treatment.

RESULTS

A total of 306 patients were included in the study. Patient characteristics included a mean age of 57 years, 53% male, 70% non-Hispanic white, 84% HCV genotype 1, 70% treatment-naïve, 49% with cirrhosis, 14% with hepatic decompensation, and 5% with hepatocellular carcinoma. Eighty-three percent of patients received ledipasvir or sofosbuvir. The mean baseline serum creatinine level was 0.96, and 90% of patients had normal baseline renal function. Of those with normal baseline renal function, GFR stayed the same in 97% of patients and worsened in 3%. Of those with baseline renal impairment, 23% of patients had improvement in GFR, 68% had stable GFR, and 10% hadworsened GFR. Altogether, GFR improved or stayed the same in 96% of patients (Table 1). There were no statistically significant differences between serum creatinine levels before and after treatment (0.96 versus 1.00; p=0.15).

Table 1: Proportion of hepatitis C patients treated with direct-acting antivirals with renal improvement, no change, or worsening at 3–6 months post-sustained virological response (N=306).

Renal function before treatment	GFR improved at follow-up	GFR stayed the same at follow-up	GFR worsened at follow-up
Normal (GFR >60 mL/min/1.73m^2) n=275 (90%)	0 (0%)	266 (97%)	9 (3%)
Impaired (GFR <60 mL/min/1.73m^2) n=31 (10%)	7 (23%)	21 (68%)	3 (10%)
Total (N=306)	306 (100%)	287 (94%)	12 (4%)

GFR: glomerular filtration rate.

CONCLUSION

In this study, DAA have been shown to be safe from a renal function standpoint. Furthermore, nearly one-quarter of patients in this study with baseline renal impairment had improvements in renal function after DAA treatment. Early DAA treatment may not only prevent chronic liver disease but also prevent and/or treat chronic renal insufficiency. Larger prospective studies are needed to further investigate these findings.

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OPTIMIS: Real-World Data in Patients with Hepatocellular Carcinoma Treated with Transarterial Chemoembolisation: Second Interim Analysis

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Keywords: Hepatocellular carcinoma (HCC), selection for transarterial chemoembolisation (TACE), TACE.

Citation: EMJ Hepatol. 2018;6[1]:58-59. Abstract Review No. AR3.

Transarterial chemoembolisation (TACE) is the most common treatment modality for hepatocellular carcinoma (HCC) worldwide, often used to treat cases of unresectable HCC in particular.[1] However, TACE is a treatment modality that only marginally improves patient survival and, at the same time, can cause significant harm to the cirrhotic liver if used in patients who are unsuitable for TACE treatment.[2] The OPTIMIS study aimed to characterise TACE utilisation and outcomes in a real-world setting using a multinational observational trial of patients with Barcelona Clinic Liver Cancer (BCLC) Stage B HCC or higher, who were scheduled to undergo TACE at study entry. TACE usage as well as post-TACE treatment

were documented, including the use of sorafenib following TACE treatment.

At the International Liver Congress (ILC) 2018 in Paris, France, the second interim analysis of the OPTIMIS trial, comprising the final analysis of 977 of the total 1,630 patients included, confirmed the overuse of TACE. According to international guidelines, 44% (431) of patients were ineligible for TACE at study entry and before the first TACE treatment. The fraction of ineligible patients was lowest in Japan (15%) and highest in China (82%), with other European and Asian countries ranging between these values. One major reason for ineligibility was that patients were already at BCLC Stage C before the first TACE procedure; this applied to 23% of patients. Again, this finding was lowest in Japan (5%) and highest in China (67%).

Upon analysing the deterioration of individual factors of liver function, albumin (25% of patients) followed by prolonged increased aspartate aminotransferase (22%) indicated worsening liver function and ischaemic liver damage.[2] Liver function deterioration occurred more often in BCLC Stage C patients than in BCLC Stage B patients and slightly more often in TACE-ineligible patients at baseline than in TACE-eligible patients, but the difference was not major.

With regard to treatment received after TACE failure, sorafenib was administered to 27% of the patient population. In most parts of the world, sorafenib was used in approximately one-quarter of patients, except in Korea where only 12% of patients received sorafenib after TACE failure, and China, where 50% of patients received the drug. A reason for the low rate of sorafenib treatment after TACE discontinuation in Korea could be due to the country having the largest fraction of patients (59%) who received ≥3 cycles of TACE before starting drug treatment with sorafenib, which was the case in only 32% of patients in the overall study population. Another reason could be that the rate of treatment-emergent adverse events was highest in Korea (88%), while the rate was only 55% in the overall patient population.

Overall, these data confirm the suspected overuse of TACE in patients who are not TACE-eligible at baseline, but also in patients who become TACE-ineligible during the course of TACE treatment. The results also indicate that not all patients who required drug treatment after becoming TACE-ineligible received it. Whether this was due to the severe deterioration of liver function by (over)application of TACE or simply due to withholding drug treatment by the attending physician remains to be seen in the final analysis of the OPTIMIS data. Even without this information, this study gives an impression of the real-world practice of TACE usage in intermediate and advanced stage HCC patients.

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Acute Liver Failure: Time to Review Our Follow-Up Policy?

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Abstract Review No. AR4.

Acute liver failure (ALF) is a rare but serious clinical condition resulting from sudden, massive loss of hepatic parenchyma and metabolic functions. In the UK, ALF is estimated to affect 1–8 people per 1 million population.[1] In Scotland, UK, all patients with severe acute liver injury or ALF are managed in a single national centre: the Scottish Liver Transplant Unit (SLTU). Here, the patient is managed supportively until effective liver regeneration occurs or until liver transplantation, if this is deemed necessary and the patient is a suitable candidate. The assumption has long been that if an ALF patient survives without liver transplantation (a group known as spontaneous survivors [SS]), they return to healthy liver function and experience no lasting associated morbidity and mortality. However, results of two recent studies have suggested that this assumption may not be true.[2,3]

Our research group aimed to evaluate long-term healthcare use (as a surrogate marker for morbidity) of ALF SS following discharge from their index hospital admission with ALF and to compare this with the healthcare resource use of an age, sex, and postcode-matched sample of the general Scottish population. Studies have previously shown an increase in mortality in SS of ALF compared with this matched control cohort. The study cohort of ALF SS consisted of >700 patients admitted between November 1992 and December 2014 who survived to hospital discharge without transplantation. The matched control cohort was composed of >3,400 matched healthy controls from the general Scottish population.

Overall, it was found that the SS of ALF utilised a significant amount of healthcare resources following discharge from their index hospital admission with ALF. By 20 years of follow-up, >90% of patients had required a readmission to hospital. The majority of these readmissions were unscheduled emergency admissions and were associated with the majority of days spent in hospital and the accompanying healthcare costs. The total cost for all hospital admissions in the SS of ALF cohort exceeded £9 million. Predictors of the number of readmissions in the SS of ALF cohort were identified and included age, aetiology of ALF, and the number of admissions to hospital in the preceding 5 years.

Comparing the healthcare resource use of the SS of ALF and the matched controls, the cumulative incidence of readmission was significantly higher in the SS of ALF cohort than the matched controls, continuing from 30 days post discharge to 20 years of follow-up. Overall, the SS of ALF were 50% more likely to experience any readmission and over twice as likely to experience an emergency readmission. Considering the health economics impact, the SS of ALF accrued an excess cost of >£9,000 per 10 person-years compared to the control cohort.

Identifying the causes of readmissions in SS of ALF will be important to guide the development of follow-up and review strategies. The main cause of readmission may be medical or psychiatric, both of which would require different follow-up protocols. It should be noted that the majority of ALF patients in this study (>80%) had paracetamol-induced ALF and the causes of readmission may be different between the paracetamol and non-paracetamol-induced ALF cohorts. Within the non-paracetamol cohort, the number and cause of readmissions may vary between different ALF aetiologies; however, the number of patients in this current study is too small to draw any significant conclusions. Our work suggests that any new follow-up strategy should initially focus on those patients identified to be at a high risk of readmission. In time, we hope that our work will help reduce the burden, both in terms of morbidity and mortality, of surviving ALF.

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Monocyte-Derived Hepatocyte-Like Cells in Combination with Proteomics Identify a Potential Biomarker for Drug-Induced Liver Injury by Diclofenac

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Drug-induced liver injury (DILI) is the leading cause of acute liver failure leading to death or requiring transplantation in the European Union (EU) and USA.[1-3] Due to the potentially severe consequences of DILI, it is also among the most frequent causes for regulatory actions on drugs, such as marketing restrictions, clinical hold of studies, and market withdrawal.

DILI can occur in a dose-dependent fashion, with acetaminophen (paracetamol) being the prime example. This dose-dependent or intrinsic DILI is a characteristic of the drug causing liver injury in every patient exposed to an overdose exceeding a given threshold (e.g., 150 mg/kg bodyweight for acetaminophen). Dose-dependent DILI is usually detected in preclinical testing using cell and animal models. In patients presenting with dose-dependent DILI, the history (intake of overdose) and toxicological investigations are used to identify the cause of liver injury. The other form of DILI, idiosyncratic DILI (iDILI), is a result of the incompletely understood interaction of an individual patient with a given drug; therefore, iDILI occurs without a clear dose-relationship. Furthermore, iDILI only occurs in a few susceptible patients. The diagnosis of iDILI is a diagnosis of exclusion. There is no preclinical model that can reliably predict iDILI, let alone the possibility to predict susceptibility to a given drug in an individual patient.

Since iDILI is a rare event most often encountered when a drug is used by a number of patients (e.g., in a late-stage clinical trial or post-marketing), much effort is being made to identify biomarkers that help to predict severe iDILI and/or identify susceptible patients. This would allow marketing of innovative drugs by protecting patients at risk while granting access to the patients who tolerate the drug.

The search for biomarkers identifying an individual patient's risk of developing iDILI due to a given drug is impeded by the difficulty of iDILI diagnosis. The misdiagnosis of other causes of liver injury as iDILI and, more importantly, unclear drug causality in cases of patients taking several drugs, leads to ill-defined patient sets for biomarker studies.

In order to provide proof-of-concept for a novel approach to the development of drug-specific iDILI biomarkers, a combination of

monocyte-derived hepatocyte-like (MH) cells and proteomics was used.[4] MH cells were generated from patients and exhibited donor-specific characteristics, most importantly, an enhanced toxicity response *in vitro* towards drugs that have been shown to cause iDILI in the respective patient. The 22 subjects included in this study[5] gave informed consent, after which blood was withdrawn for MH cell testing and data were collected for causality assessment by Roussel Uclaf Causality Assessment Method (RUCAM) and clinical adjudication.

MH cells were generated from 22 donors. Twelve were exposed to diclofenac (3 tolerators; 4 patients with iDILI caused by diclofenac [RUCAM 7–9]; 2 patients with iDILI caused by another drug; and 3 patients with acute liver injury [ALI] of non-drug origin). Ten donors who had not been exposed to diclofenac were used as additional controls (3 tolerators; 4 iDILI caused by other drugs; and 3 non-drug origin ALI). MH cells were either kept under baseline conditions or exposed to diclofenac *in vitro*. Afterwards, mass spectrometry-based proteomics were performed using the cell lysates.

Following the proteomics analysis, >2,700 proteins were quantified in all of the samples. The protein expression pattern from patients with diclofenac-induced DILI differed from the other groups as shown by principal component analysis. Moreover, there was a distinct shift in protein expression upon diclofenac exposure, only seen in MH cells of patients with DILI caused by diclofenac. From the proteins influenced by diclofenac exclusively in the patients with DILI caused by diclofenac, we identified integrin beta 3 (ITGB3) as a promising biomarker because it showed marked upregulation and can be measured from peripheral blood samples since it is an extracellular protein.

As a result, ITGB3 expression in whole-blood samples from patients with diclofenac DILI and control groups was investigated by flow cytometry. The experiments revealed a decrease in ITGB3 expression only in diclofenac DILI patients. To further explore the discrepancy between the proteomics results (upregulation of ITGB3) and the flow cytometry analysis from patient blood (downregulation of ITGB3), the expression of ITGB3 in liver biopsy samples of one patient with iDILI caused by diclofenac, one patient with iDILI caused by another drug, and one patient with ALI of non-drug-cause was investigated. Here, an increase in ITGB3-positive inflammatory sites was present only in the liver of the patient with diclofenac DILI.

Thus, ITGB3 seems to be a promising candidate for an iDILI-biomarker specific for diclofenac. The hypothesis that the inflammatory event evoked by diclofenac DILI leads to recruitment of ITGB3-positive cells from the blood to inflammatory sites in the liver was developed as a result of these investigations. The results provide evidence that the novel approach combining MH cell testing with omics technologies will be helpful to identify iDILI biomarkers with increased specificity. These results warrant further investigation.

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Primary Hepatic Angiosarcoma: A Brief Review of the Literature

EDITOR'S PICK

I have chosen this paper by Chen et al. as my Editor's Pick because primary hepatic angiosarcoma is a rare, malignant, mesenchymal tumour of the liver, and the diagnostic challenges and its rapidly progressive nature contribute to the poor prognosis seen in clinical practice. There is little published literature on primary hepatic angiosarcoma and therefore this paper reviewing the challenges posed by the tumour will be a helpful guide for clinicians across the globe.

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Abstract

Primary hepatic angiosarcoma (HAS) is a liver tumour of endothelial cell origin. It is the most common malignant mesenchymal tumour of the liver, but is nonetheless rare, accounting for approximately 0.1–2.0% of all primary liver malignancies. Historically, 25% of HAS cases were associated with occupational or medicinal exposure, but most cases are now considered idiopathic. Patients present with vague signs and symptoms of liver disease, often resulting in late diagnoses; patients may present with acute liver failure or spontaneous rupture of the tumour, but this is rare. Preoperative diagnosis of HAS is difficult because laboratory and radiological findings are often non-specific or unable to discern malignant masses from benign growths. Obtaining a biopsy for histopathological diagnosis of HAS is also difficult because of its vascular and haemorrhagic nature, and reports of death from closed biopsies have been noted. Prognosis is poor because of the disease's diagnostic challenges and the tumour's rapidly progressive and early metastatic nature. The reported median survival is approximately 6 months, with only 3% of patients living >2 years. This paper will review and summarise new and existing publications in the English language literature to provide a better understanding of the challenges posed by HAS.

INTRODUCTION

Primary hepatic angiosarcoma (HAS) is a rare liver tumour of endothelial cell origin. Diagnostic challenges and its rapidly progressive nature contribute to the poor prognosis of the tumour. Historically, 25% of cases were associated with occupational exposures or medicinal carcinogens,[1] but today the majority of cases have no known aetiology. Patients present with vague symptoms and signs of liver disease, and many cases are diagnosed incidentally or at autopsy.[2] Liver resection is the most effective treatment for HAS, but appropriate management requires early work-up and diagnosis.[3] This paper will review and summarise new and existing literature to provide a better understanding of the challenges posed by HAS.

EPIDEMIOLOGY AND AETIOLOGY

Angiosarcomas are rare tumours of endothelial cell origin. Most cases occur in the head and neck, with 6% arising in the liver, making it the fifth most common site for angiosarcomas.[4] HAS is rare and accounts for approximately 0.1–2.0% of all primary liver malignancies, but it remains the most common malignant mesenchymal tumour of the liver.[2,5] HAS predominantly occurs in males in the 6th and 7th decades of life, and most studies have shown a male-to-female ratio of 3–4:1; however, a lower ratio of 2:1 in Asian countries has been reported.[6]

Historically, up to 25% of HAS cases were linked to a known aetiology.[1] Previously, Thorotrast was used as a radioactive contrast and was responsible for a large number of HAS cases during its widespread use between 1928 and 1955.[7] It was mostly deposited in the liver's reticuloendothelial system and has a long biological half-life of 200–400 years.[8] Since the cessation of Thorotrast use in the 1950s, the number of Thorotrast-induced cases has decreased. The latest case report described 65 years of latency, far beyond the average latency period of Thorotrast, which is approximately 20 years.[7] Vinyl chloride monomer, which is involved in the manufacture of plastic, is another well-studied aetiology of HAS and was first described by Creech and Johnson in 1974.[9] It was shown to increase the risk of HAS 10–15-fold, with a latency period of 9–35 years.[10,11] Cases of vinyl chloride monomer-induced HAS are now less common, with worldwide regulations on its emission having been established.[12] In addition, arsenic is also known to cause HAS and is found in contaminated drinking water and pesticides, or used in Fowler's solution to treat asthma, psoriasis, and other conditions.[13] Other proposed associations with HAS include androgenic anabolic steroids, cyclophosphamide, phenelzine, and copper,[12] but these cases are rare and lack a definitive causal relationship.

Some conditions thought to be associated with HAS include von Recklinghausen disease and haemochromatosis.[12] There have been two cases of HAS that have occurred in young patients with dyskeratosis congenita, a bone marrow disease associated with an increased risk of various malignancies.[14] In addition, some studies have considered the relationship of HAS with chronic viral hepatitis; however, results from a study in Taiwan, a hepatitis-endemic country, suggested that there is no increased risk of HAS in patients with a background of hepatitis B or C.[6] The phosphatase and tensin homolog (*PTEN*) tumour suppressor gene and the alternative lengthening of telomeres (ALT) mechanism have also been found to have some association with HAS, but no extensive research is available in this area.[15,16]

PRESENTATION

While 9% of individuals present with manifestations secondary to metastasis,[2] most patients present with non-specific symptoms of liver disease, often resulting in late diagnoses. Abdominal pain, fatigue, weight loss, and anorexia are common symptoms of HAS, but many cases can be asymptomatic.[5] Examination findings include hepatomegaly, jaundice, ascites,[17] and, rarely, hepatic bruits may be audible on auscultation due to the vascular nature of the tumour.[18]

Haemoperitoneum secondary to spontaneous tumour rupture occurs in 17–27% of cases,[17] and should be considered an indicator of a malignant tumour because benign tumours rarely undergo spontaneous rupture.[19] There have also been cases of patients presenting with haemothorax

from diaphragmatic tumour invasion,[20] as well as a case of bleeding oesophageal varices in a patient with no previous carcinogenic exposure or liver disease.[21] Acute liver failure or fulminant liver failure with encephalopathy and coagulopathy are rare initial presentations.[22,23] Other rare presentations include high-output cardiac failure[24] and disseminated intravascular coagulopathy (DIC), which occurs in <5% of cases.[17] Few reports have been associated with Kasabach–Merritt syndrome, which is DIC occurring in any vascular tumour.[25]

INVESTIGATIONS

Serology can be normal in some HAS cases,[26] but results usually show non-specific elevations indicative of liver disease, with 97% of patients displaying at least one raised liver enzyme;[17] alkaline phosphatase is the most commonly elevated liver enzyme.[18] Hyperbilirubinaemia may also occur, particularly in progressive disease.[27] Thrombocytopenia occurs in approximately 54% of patients,[17] secondary to local destruction of platelets within the tumour. The sequestration of platelets and local intravascular destruction of clotting factors[28] both contribute to the pathophysiologic process of DIC, which has been well-described in the literature.[28,29] Anaemia is another common finding in patients with HAS, with 8% of patients developing microangiopathic haemolytic anaemia as a result of the use of blood components by the tumour.[17] Anaemia can also be explained by spontaneous tumour rupture. Leucocytosis has also been described, with one particular case reporting extreme leucocytosis secondary to a leukemoid reaction, with a white cell count of 74.7×10^9.[30] Hypercalcaemia can be elevated in cases associated with bone metastases.[17] There are no specific tumour markers associated with HAS; carcinoembryonic antigen,[23] alpha-fetoprotein, and cancer antigen 19-9 may show mild elevations,[11] but none are specific to HAS.

Appearances on conventional ultrasound are non-specific, and masses typically demonstrate different echotextures depending on the presence of necrosis and haemorrhage.[22,31] Colour Dopplers may display minimal blood signals in large masses only.[31] In contrast-enhanced ultrasound, HAS can present with peripheral nodular or rim enhancement without centripetal filling and there can be a reticular or chaotic pattern of arterial enhancement.[32] A non-enhancement area in the centre of the large mass is another commonly reported feature in contrast-enhanced ultrasound.[31]

Similarly, appearances on unenhanced computed tomography (CT) are non-specific, and the hypervascular and heterogenous nature of HAS is better depicted in dynamic CT and magnetic resonance imaging (MRI). Currently, there are no known pathognomonic features for HAS.[33] Tumours generally display heterogenous enhancement on the late arterial phase with progressive enhancement on the portal venous and delayed phases.[34-36] The pattern of progressive centripetal nodular enhancement mimicking cavernous haemangioma has been previously described but is now thought to be atypical for HAS.[35] The nodular foci found in HAS can be distinguished from benign haemangiomas because they tend to be more bizarre in shape, even if centripetal enhancement is observed, and with ring enhancement;[34,35] some cases may demonstrate a centrifugal enhancement or 'reverse haemangioma' pattern.[34] Arterioportal shunting is not commonly seen in haemangioma and its presence favours the diagnosis of HAS,[37] while diffuse HAS can rarely present as pseudo-peliosis with infiltrating micronodules filled with contrast.[35]

This progressive enhancement pattern is even more noticeable in dynamic contrast MRI due to the availability of delayed phase images.[35,38] On delayed phase images, dominant masses exhibit progressive but incomplete enhancement. In addition, nodules can appear uniformly hyperintense due to complete filling and lesions show peripheral rim and central septal-like or linear progressive enhancements in comparison to progressive centripetal nodular enhancement found in haemangioma.[38] In comparison to haemangiomas, the enhancement of angiosarcomas is usually less than that of the aorta.[39]

On unenhanced T1-weighted images using MRI, dominant masses can present with decreased signal with focal areas of high signal intensity, suggesting the presence of haemorrhage.[36] On T2-weighted images, dominant masses display

increased signal intensity and are generally hyperintense relative to the surrounding hepatic parenchyma.[34,38] Nodular cases mostly display moderate-to-high signal intensity with varying intralesional areas of low signal intensity. Elevations of the apparent diffusion coefficient level have also been described when compared to other hepatic malignancies, but the values are lower compared to those seen in benign cysts and haemangiomas.[38] Foci with varying amounts of signal intensity, progressive enhancement, and intratumoural haemorrhages mirror the heterogenous architecture of hepatic angiosarcomas.[39] Lastly, hepatic angiography is another tool used for diagnosis and the contrast medium routinely migrates into small vascular lakes with central areas of hypovascularity and peripheral contrast staining. The most characteristic angiographic feature is the intense peripheral stain late in the arterial phase lasting 30–40 seconds.[40]

DIAGNOSIS

Diagnosis of HAS is challenging due to non-specific presentations and investigation findings, which often overlap with the findings from other vascular tumours. Preoperative diagnosis is important for the planning of management; for example, tumours such as epithelioid haemangioendothelioma may be treated with an orthotopic liver transplant, whereas HAS is a contraindication for transplantation.[41]

Obtaining tissue samples for histopathological diagnosis is difficult in HAS. Haemorrhage due to the vascular nature of the tumour remains the most controversial complication, occurring in approximately 27% of patients and 5% of cases result in death.[17] The incidence of haemorrhage after percutaneous biopsy in HAS is much higher than biopsies for other liver tumours, such as hepatocellular carcinoma.[42] This is substantiated by two notable cases of death secondary to haemorrhage, both from fine needle biopsies.[43,44]

Many reports recommend open rather than closed biopsies because of better visualisation and easier haemostasis.[17,40] The diagnostic yield is also reported to be higher with open biopsies than closed biopsies (65% versus 25%, respectively; $p<0.01$).[17] Reports on fine needle aspiration vary, with some supporting their use[45] while others report inconclusive results with all fine needle aspiration cases (N=4), requiring further biopsies to validate diagnosis.[32] Percutaneous biopsies are reported to be more sensitive because samples contain larger core tissues without fragmentation.[42] They are also safer and faster to perform, without significant complications or mortality.[35,42] Koyama et al.[35] reported that 78% (7 out of 9) of their biopsies yielded diagnostic specimens without complications. Similarly, Kang et al.[42] performed a multicentre study in South Korea and concluded that 96.9% (32 out of 33) of cases were diagnostic on first biopsy, while the remaining 3.1% (1 out of 33) were diagnosed on the second biopsy. They reported bleeding occurrences in 9.1% (3 out of 33) of patients, which were managed by transfusion (2 out of 3) and hepatic artery embolisation (1 out of 3), resulting in no mortality.[42] Transjugular liver biopsies are reportedly safer and have fewer complications than percutaneous biopsies, but have less diagnostic power.[40]

Regardless of the approach used, false-negative biopsies remain an issue due to a high frequency of necrotic and haemorrhagic foci within the tumour.[17,32,46] Occasionally, non-malignant changes, such as portal tract and sinusoidal fibrosis, are identified without malignant foci;[17,46] as a consequence, as many as one-third of patients are diagnosed during autopsy,[17] while others are diagnosed after liver transplant.[11,46]

PATHOLOGY

Macroscopically, HAS is characterised by four growth patterns: multiple nodules, large dominant mass, mixed pattern of dominant mass with nodules, and, rarely, a diffusely infiltrating micronodular tumour.[35,38] Lesions can vary in colour, ranging from pale white-yellow-grey[5,22,31] to red-brown,[26] and the margins are usually poorly defined,[2] but well-demarcated borders have also been reported.[5] The tumours are described as spongy[24] and are usually heterogenous in appearance, with alternating areas of haemorrhagic foci, large intraparenchymal cystic spaces filled with thrombotic content,[29] and gross necrotic areas.[5,26,31]

Microscopically, HAS is composed of malignant atypical endothelial cells[47] that are pleomorphic and may be round, irregular, or spindle-shaped.[8,18] Tumour cells contain prominent chromatin[33] and atypical hyperchromic and elongated nuclei with frequent mitoses.[18] Erythrophagocytosis has also been described.[8,45] Neoplastic cells proliferate in single or multiple layers[37,47] and infiltrate along preformed vascular channels, such as dilated sinusoids,[18] as well as central veins and portal vein branches.[24,29] Tumour cells may also form their own disorganised[34] anastomotic vascular channels,[2,28,46,47] shape solid nodules or nests,[2,38] or form cavernous spaces due to the loss of adjacent hepatocytes,[18] which may mimic cavernous haemangiomas.[35] More than one vascular pattern may be found in a single patient[46] and the predominating pattern differs in each case.[35] Surrounding hepatocytes may be hyperplastic[18] or atrophic.[27] Separation of atrophic hepatocytes and sinusoidal dilatation is sometimes mistaken as a sign of peliosis hepatitis.[11] Areas of haemorrhage, necrosis, infarction, and calcifications are also frequently described;[18,31,33] for example, one case series reported that 83% (10 of 12) of specimens contained necrosis, while 82% (9 of 11) contained haemorrhage.[35]

While atrophic hepatocytes are thought to be indicative of progressive HAS,[48] hyperplastic hepatocytes and cells lining irregularly dilated and atypical sinusoids are believed to be early changes.[8,48] Precancerous changes have been described by several authors in the 1970s and 1980s when the relationship concerning HAS and occupational exposure was brought to light. Popper et al.[49] reviewed 117 cases and believed sinusoidal dilatation and fibrosis were the predominant early changes. They also concluded that the pattern and evolution of HAS were the same irrespective of aetiology, which was confirmed in another review several years later.[14] el Zayadi et al.[50] performed a retrospective review of cases associated with agricultural pesticides and reported no histopathological differences between idiopathic cases and those patients exposed to pesticides.

There are no specific markers suggestive of HAS and, while some are more sensitive than others, they should only be used alongside other investigations to assist diagnosis. It may also be possible for the expression of markers to be variable within the tumour.[18] Wang et al.[47] tested a cohort of HAS samples and concluded that *ERG* expression was the most sensitive and specific marker, with a 100% sensitivity (n=24), followed by CD34 (87.5%), CD31 (87.2%), and FVIII-rA (41.7%). Other immunohistochemical, tumour, and protein markers that have been reported include CD10,[22] CD117,[51] cytokeratin,[47] FLI-1,6 D2-40,[6] and Ki-67.[22]

TREATMENT

The most promising HAS treatment to date is surgical resection of the tumour;[2,3,12] currently, radical surgery with R0 resection is the only curative treatment.[51] Combining adjuvant chemotherapy with surgery gives the highest chance of cure, with a reported median survival of approximately 17 months.[3] In comparison, liver transplant is contraindicated due to high recurrence rates and poor survival post-transplant; the median survival after transplant is <7 months and no patient has survived >23 months.[41] The intrinsic radioresistant property of HAS means that radiotherapy has largely been abandoned as a treatment option.[52]

Many chemotherapy regimens have been described in the literature but no routine has proven to be notably superior to the others. Kim et al.[52] demonstrated improved survival in 50% (n=2) of patients with 5-fluorouracil/carboplatin/doxorubicin/ifosfamide and reported that paclitaxel may be used as a salvage chemotherapy based on its antiangiogenic properties.[52] Others have demonstrated partial response with the mesna/doxorubicin/ifosfamide/dacarbazine regimen.[53] Newer molecular therapies, including bevacizumab, sorafenib, and sunitinib, have demonstrated limited efficacy and cannot be recommended without further studies.[51,52] Single-agent chemotherapy regimens, including dacarbazine, cyclophosphamide, and doxorubicin, have also been used with disappointing results.[25]

More recently, the potential role of the Hippo signalling pathway, which regulates cell proliferation and apoptosis, has been explored in the biological treatment of angiosarcomas.[54] *YAP* is an oncogene involved in this pathway and CD31 regulates endothelial cell function and redox status via *YAP*.[55] Angiosarcoma cells

have been subclassified based on phenotypical expression and it was found that CD31^{low} was more common in angiosarcomas than CD31^{high} and was associated with increased YAP, making the tumour more chemoresistant to agents such as doxorubicin.[54] Venkataramani et al.[54] demonstrated *in vitro* that pazopanib, an effective YAP inhibitor in cancer cells, was effective when used with doxorubicin in resensitisation of CD31^{low} to chemotherapy.[54] Also of note, one retrospective paper found that 30% of a primary angiosarcoma cohort used ALT as the telomere maintenance mechanism and this was highly associated with HAS, with two-thirds of the population positive for ALT. They also reported that ALT-positive cells were sensitive to ATR kinase inhibitors. However, further *in vivo* trials of ATR kinase inhibitors and pazopanib with doxorubicin are required to delineate the benefits and efficacies for the treatment of HAS.[16]

Transarterial embolisation is the modality of choice when patients present with an intrahepatic bleed to achieve haemodynamic stability.[56] Transcatheter arterial chemoembolisation may also be effective in the treatment of patients with dominant lesions with or without metastases. Park et al.[56] suggested that a combination of lipiodol and cisplatin may benefit patients with large dominant masses and few or no intrahepatic metastases, after noting a reduction in tumour size in 50% of patients in their study (n=2). Ozden et al.[57] also described the use of prophylactic chemoembolisation with lipiodol, adriamycin, and mitomycin; the study patient had been alive and recurrence-free for 5 years and 4 months at the time of study publication in 2003.

PROGNOSIS

The median survival after diagnosis of HAS is approximately 6 months, with 3% of patients living >2 years.[3,12,17] In patients who undergo local excision with or without adjuvant chemotherapy, the median survival is around 17 months.[3] In resectable cases, positive resection margins correlate more with poor prognosis as opposed to the size of the tumour; other prognostic factors include poorly differentiated tumours, multinodular and diffuse tumours, and haemoperitoneum with tumour rupture.[6,12,52,57] Hepatic failure is the cause of death in approximately 50% of patients and haemoperitoneum in 25% of patients, followed by metastatic disease, infection, and, rarely, renal failure and congestive heart failure (3% of deaths).[3,17,18,25,29] The lung is the most common metastatic site, followed by the spleen and bone.[17,35,52] Other sites reported include brain,[6] adrenal glands,[58] pericardium and myocardium,[58] kidneys,[58] stomach,[22] left gastric vein,[22] small bowel,[58] and ascending colon.[58,59] Distant metastases are evident in >60% of cases post-mortem[17] and spontaneous tumour rupture carries a poor prognosis, even if bleeding is treated with emergent transarterial embolisation or surgery.[27,56] This complication has been reported in approximately 17–27% of patients.[17] The longest survival to date is a 47-year-old woman who was recurrence-free at 10 years post operation.[12]

CONCLUSION

Early diagnosis and management of primary HAS is critical in patients with a potential lesion; however, this is difficult as most patients present with vague signs and symptoms, non-specific investigations indicative of liver disease, and tumour markers that are often unremarkable or misleading if positive. HAS cases have been accurately diagnosed on both dynamic CT and MRI, where the heterogeneous and vascular nature of the tumour are best depicted. However, there are still no pathognomonic imaging features and, in certain cases, the imaging findings of HAS still overlap with those of benign vascular tumours. Ultimately, a histopathological diagnosis is the only way to confirm HAS. While many early papers advised against percutaneous liver biopsies in light of several fatal cases, more recent studies have demonstrated better outcomes with less complications. Regardless, false-negative biopsies will continue to occur given the pathological process of HAS and surgical resection is the only curative treatment available, particularly in patients with large dominant masses. Several reports on biological and chemotherapy agents are available but more research is required to identify which regimen is most effective. Until then, the poor prognosis remains an issue for HAS patients because of the tumour's rapidly progressive nature and tendency to metastasise early.

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Acute Fatty Liver of Pregnancy: Better Understanding of Pathogenesis and Earlier Clinical Recognition Results in Improved Maternal Outcomes

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Abstract

Acute fatty liver of pregnancy (AFLP) is an uncommon disorder affecting women in late pregnancy. It is increasingly recognised as an important cause of preventable maternal mortality across the world. The pathogenic mechanism of AFLP is now better understood; it appears that a compensated defective fatty acid oxidation becomes overt when metabolic stressors are superimposed on the increased energy demands of late pregnancy. The mother tends to rely more on fats as a source of energy in late pregnancy. This phenomenon may have an evolutionary basis and may explain why AFLP typically occurs in late pregnancy. The Swansea criteria have proven to be useful in early diagnosis of AFLP. Attempts to simplify these criteria further have proved helpful in early recognition of the disease. Although liver biopsy showing microvesicular steatosis of hepatocytes is the pathologic hallmark of AFLP, it is neither necessary nor safe in the antepartum setting. Current management strategies revolve around ensuring urgent delivery of the fetus and anticipating and managing complications of acute liver failure. While early recognition and multidisciplinary management have considerably improved maternal survival in AFLP, fetal outcomes remain poor. The authors postulate a therapeutic intervention to improve fetal outcomes in this disorder.

INTRODUCTION

Pregnancy-related disorders comprise several poorly recognised but important causes of liver dysfunction that complicate various stages of pregnancy. These include acute fatty liver of pregnancy (AFLP); haemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; and pre-eclamptic liver dysfunction, which often results in poor maternal and fetal outcomes.[1] With timely recognition, as well as early and aggressive management, the maternal

mortality associated with these disorders can be mitigated. Recent advances have increased the current understanding of the pathogenesis of these disorders, which has translated into better overall management of these patients. This review focusses on the most under-recognised pregnancy-related liver disorder: AFLP.[2] AFLP, first described in 1934, was initially termed (as per gross liver appearance on autopsy) acute yellow atrophy of the liver.[3,4] AFLP is characterised by microvesicular fatty infiltration of the hepatocytes and presents in late pregnancy (late second or third trimester) with rapidly progressive illness.[5]

EPIDEMIOLOGY

AFLP is a rare disease, with a single large prospective population-based study (229 hospitals and 1,132,964 pregnancies in the UK) estimating an incidence of 5 cases per 100,000 pregnancies (3.8–6.5 per 100,000 pregnancies; confidence interval: 95%).[6] The estimated incidence of AFLP was higher in hospital-based studies from the UK[7] (114 cases per 100,000 pregnancies) and India[8] (30 cases per 100,000 pregnancies). Furthermore, a retrospective study from a single hospital in India found that of 285 maternal deaths (in a total of 113,755 pregnancies from 1999–2011), 23 (8%) were secondary to pregnancy-related liver disorders and 17 (6%) had AFLP, histologically proven in 7 patients.[9]

PATHOGENESIS

The Timing of Acute Fatty Liver of Pregnancy: Clues from an Evolutionary Viewpoint

Why does this illness occur in an otherwise healthy woman in late pregnancy? The timing of this disease in late pregnancy may have an evolutionary basis. During periods of starvation, the human body switches from carbohydrate to fat stores as an energy source.[10] Fat stores are the main and preferred energy source during prolonged starvation in hibernating animals (≤100 days hibernation duration) and in migratory birds during long distance, intercontinental, nonstop flights, which can cover 3,000–4,000 km.[11,12] While the specific mechanisms regulating the transition to fat metabolism in migrating birds are unknown, recent studies have focussed on the role of peroxisome proliferator-activated receptors in regulating migratory adiposity.[13] If these animals or birds experience a hindrance or blockage in utilising these fat stores, they could be expected to become energy deficient and fall sick during these annual periods of starvation.

In humans, the pregnant mother relies on fat stores as the main source of energy in late pregnancy.[14] Why would the pregnant mother rely on fats as the primary energy source as the pregnancy advances? It is possible that fats are the preferred energy source for the mother to tide over the reduced dietary intake, as well as the markedly increased energy expenditure during labour (a situation similar to the migratory bird on a long distance flight that does not eat and experiences a markedly increased energy expenditure). It is also possible that the mother redirects dietary carbohydrates to enhance fetal nutrition. Thus, if the pregnant mother has some defect in metabolising or utilising her fat stores, she can be expected to become energy deficient in late pregnancy.[15]

AFLP is characterised by mitochondrial dysfunction causing liver failure, known as mitochondrial hepatopathy.[5] Dysfunction of mitochondria, the power generators of the cell, leads to energy deficiency. In the cell, mitochondria are the sites where fatty acid oxidation takes place to produce ATP. They are also the site where fatty acid oxidation and glucose metabolism merge. A rat model of hepatic microvesicular steatosis (induced by sodium valproate) led to mitochondrial structural changes and oxidant stress in mitochondria and lysosomes of the liver.[16]

Fetal Fatty Acid Oxidation Disorders are Associated with Acute Fatty Liver of Pregnancy in the Mother

In some AFLP patients, fatty acid oxidation disorders (commonly defects of long chain 3-hydroxy acyl coenzyme A dehydrogenase [LCHAD]) in the fetus are noted. The enzymes involved in fatty acid oxidation are located on the inner mitochondrial membrane. Fetal fatty acid oxidation disorders are inherited in an autosomal recessive way with both parents being simple heterozygotes.

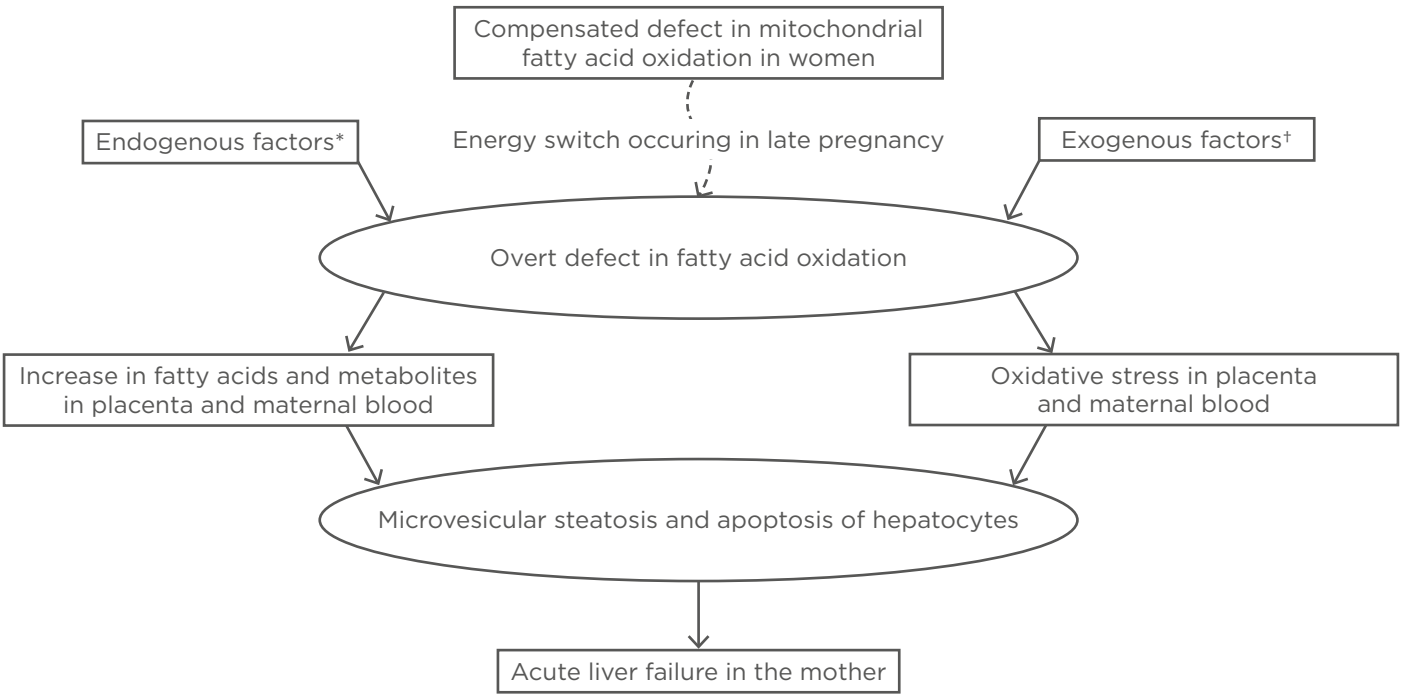

Figure 1: The authors' current understanding of the pathogenesis of acute fatty liver of pregnancy.

There is an unmasking of a hitherto compensated defective fatty oxidation in women as a result of increased reliance on fats as an energy source during late pregnancy (energy switch). This is also precipitated by endogenous factors and/or exogenous factors.

*Fetus being homozygously defective in fatty acid oxidation or coexisting HELLP syndrome or pre-eclampsia; †For example, drug and intercurrent infection.

HELLP: haemolysis, elevated liver enzymes, and low platelets.

In a study comparing 50 infants with fatty acid oxidation disorders to 1,250 healthy control infants, maternal liver diseases (AFLP, HELLP syndrome, or pre-eclamptic liver dysfunction) were noted in 16.0% of fatty acid oxidation defect pregnancies compared with 0.9% observed in the control group (odds ratio: 20.4; 95% confidence interval: 7.8–53.2). Different types of fetal fatty acid oxidation defects were associated with maternal liver disease. While infants with LCHAD defects were 50-times more likely to be associated with maternal liver disease than controls, infants with short and medium chain fatty acid oxidation defects were 12-times more likely to be associated with maternal liver disease.[17] Additionally, an analysis of 63 pregnancies in 18 mothers with a total of 28 LCHAD-deficient children noted AFLP, HELLP syndrome, and pre-eclampsia in 31%, and intrahepatic cholestasis in 10% of pregnancies, but in none of the pregnancies with a healthy fetus.[18] Accumulation of acyl carnitine may be involved in pathogenesis of AFLP in these situations.[19] However, other reports have shown a lack of association between fetal fatty acid oxidation disorders and maternal liver diseases.[20,21]

The Placenta as a Driver of Pathogenesis of Acute Fatty Liver of Pregnancy

The placenta shares a similar genetic profile to the fetus. Mitochondrial enzymes are expressed in the placenta. A dramatic improvement in the health of the mother with AFLP often occurs after delivery of the baby and the placenta. This observation led to studies on the placenta in AFLP patients.[22] Raised levels of free fatty acids were seen in the placenta and serum of AFLP patients compared to controls; arachidonic acid and palmitic acid levels were higher in the placenta and serum of AFLP patients while oleic acid and myristic acid

levels were higher in the placenta of AFLP patients. Serum arachidonic acid levels were 4-fold higher in AFLP patients (80 µm/mL) compared to healthy pregnant controls (20 µm/mL). Mitochondrial dysfunction and increased oxidative and nitrosative stress were also noted in the placenta and serum of AFLP mothers compared to controls.[23]

Arachidonic acid, at the concentration seen in serum of AFLP patients (80 µm/mL), induced mitochondrial dysfunction, oxidative stress in mitochondria, apoptosis (without necrosis), and fat deposition, suggestive of microvesicular steatosis, in Chang liver cell lines.[23] Figure 1 summarises the authors' current understanding of pathogenesis of AFLP.

CLINICAL FEATURES AND INVESTIGATIONS

AFLP is typically limited to late pregnancy and the patient usually presents with vomiting, abdominal pain, and mild jaundice;[24] symptoms of polydipsia and polyuria can be present but are rarely seen.

Though it is more common in primigravida, AFLP can occur in multiparous females with a history of prior uneventful pregnancies.[2] The clinical course often progresses rapidly downhill, with eventual occurrence of encephalopathy, hypoglycaemia, and/or ascites. Rare complications include liver rupture and haemoperitoneum.[2]

At presentation, patients may or may not be clinically jaundiced with a mild-to-moderate increase in aminotransferases. Coagulopathy (prolongation of prothrombin time and/or hypofibrinogenaemia), renal failure, hyperuricaemia, hypoglycaemia, and leukocytosis are commonly observed at presentation or over the next few days. Ultrasound scan showing fatty acid infiltration of the liver is neither sensitive nor specific for the diagnosis of AFLP.[24]

The most specific, gold standard investigation to reach the diagnosis is liver biopsy demonstrating diffuse or perivenular microvesicular steatosis.[25,26] Presence of coagulopathy and difficulties in performing liver biopsy in the antenatal mother are challenges that impact obtaining histological confirmation of AFLP in a suspected patient. Thus, liver biopsy is not advisable before swiftly embarking on management.[27] Post-partum liver biopsy confirmation of AFLP may help in patient counselling (regarding further pregnancies) and individualising management of the child. The transjugular route provides a safe access point for liver biopsy in patients with coagulopathy.[28] The microvesicular fatty infiltration of the hepatocytes is noted to rapidly resolve after delivery and thus liver biopsy, if undertaken, is preferably done within 4 days after delivery.[27] The presentation of symptoms and signs and basic lab investigations form the basis of the Swansea diagnostic criteria for AFLP.

DIAGNOSIS

Realising the lack of diagnostic criteria for AFLP, Ch'ng et al.,[7] based on retrospective analysis of multiple case series, proposed the Swansea criteria for AFLP diagnosis. These criteria are based on typical presentation, absence of an alternate explanation, and laboratory parameters noted in patients with AFLP.[7] These criteria were later prospectively validated against a clinical diagnosis by Knight et al.[6] In a retrospective study of 24 patients with suspected pregnancy-related liver disease, the Swansea criteria were validated against the gold standard for diagnosis, i.e., diffuse or perivenular microvesicular hepatic steatosis on liver biopsy, obtained in either the immediate postnatal (n=19) or post-mortem (n=5) period.[27] Thus, antenatal application of the Swansea criteria (even without liver biopsy; i.e., presence of 6 of the 13 remaining criteria) is useful in diagnosing clinical AFLP and also predicts presence of microvesicular steatosis on liver biopsy.[29] These diagnostic criteria have increased the ability to suspect AFLP (as they have very high negative predictive value) and to institute early management of these patients.[30-32]

The simplified criteria to diagnose AFLP states that the disease should be suspected in all women presenting in the late second or third trimester of pregnancy with unexplained acute liver failure (i.e., jaundice with coagulopathy often accompanied by encephalopathy and/or hypoglycaemia).[33] The time required to evaluate other causes of liver failure has to be balanced against the urgency of delivery.

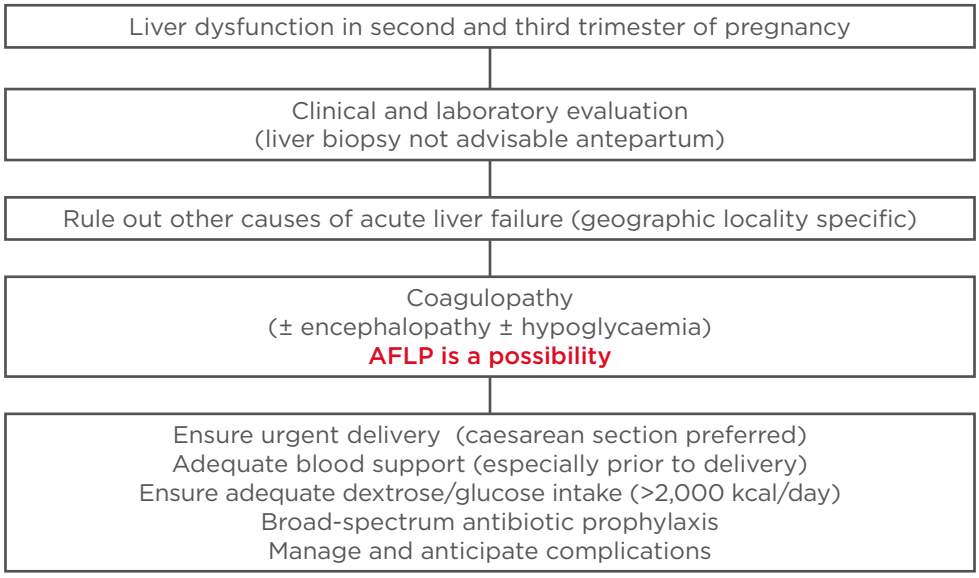

Figure 2: Management protocol in patients suspected to have acute fatty liver of pregnancy.

AFLP: acute fatty liver of pregnancy.

Box 1: Basis for the proposed management strategy to reduce perinatal mortality in acute fatty liver of pregnancy.

Normal pregnancy • Increased caloric demand in later stages of pregnancy. • Switch to fats as preferred energy substrate.
Acute fatty liver of pregnancy • Accumulation of fatty acids that have not undergone beta oxidation at the required rate. • Inability to generate energy at the rate required to sustain a healthy pregnancy and labour. • Energy deficiency in the fetus. • Consumption of available glucose resulting in damaging hypoglycaemia.
Proposal for treatment • Provide sufficient glucose or dextrose as substitute substrate to meet energy requirements of the fetus.

The cause(s) that needs to be evaluated depend on the aetiology of acute liver failure within that geographic locality and needs to be individualised for each patient (e.g., taking into account hepatitis A and E virus infection in endemic areas).[34,35] Clinicians evaluate for drug-induced liver injury (by obtaining history of ingestion of potentially hepatotoxic drugs), malaria (peripheral smear examination), and acute viral hepatitis B before a diagnosis of AFLP is entertained.[3,36]

Overlap of Diagnosis with Other Pregnancy-Related Liver Disorders

In most patients, the diagnostic criteria for AFLP and HELLP syndrome show a considerable overlap.[27,37] Typical changes on liver biopsy may help in differentiating these conditions. Whether this overlap is due to the non-specific nature of the diagnostic criteria or are secondary to overlap in pathogenesis is unclear and needs further clarification. Xiao et al.[38] suggested that HELLP syndrome is caused by endothelial dysfunction leading to secondary thrombotic microangiopathy. It is likely that the initial event (either AFLP or HELLP) contributes additional stress that stimulates the other in a genetically predisposed individual (Figure 1). The management of disease likely remains unaltered, and so it may not be essential to make such a differentiation at present.

MANAGEMENT

Prompt suspicion, early recognition, and emergent careful delivery of the baby remain the cornerstones of management of patients with AFLP.[4,39,40] Maternal survival is to be prioritised and any delay either in recognition of AFLP or in instituting delivery of the baby can adversely affect maternal outcome. It is important to recognise and categorise these patients as seriously sick and consider the intensive care or high dependency unit for disease management. The general principles guiding management in patients with acute liver failure (e.g., intravenous mannitol infusions for cerebral oedema) are also applicable for patients with AFLP, but these are beyond the scope of this article.

Constant involvement of various specialities, such as obstetrics, hepatology, intensive care, anaesthesia, haematology, and neonatology, is a must for optimal care of an AFLP patient. The mode of delivery is to be decided on a case by case basis by an obstetrician. Vaginal delivery possibly entails a longer delivery duration, as well as possible worsening of liver and/or multi-organ dysfunction, because added energy is needed for vaginal delivery in an AFLP patient who is already in a state of systemic energy deficit consequent to mitochondrial hepatopathy; in comparison, caesarean section poses an increased risk of bleeding and anaesthesia-related complications. Most centres prefer delivering the baby by caesarean section with careful anaesthetic management.[41,42] Adequate blood product replacement to address coagulopathy is a prerequisite for both modes of delivery. Hypoglycaemia and postpartum haemorrhage are common complications that need to be anticipated;[43,44] additionally, the use of broad-spectrum antibiotics as a prophylaxis should also be considered.

Intensive monitoring and continued management in the complication of acute liver failure is often required in the post-partum period. Occasionally, patients may require prolonged supportive management, including plasma exchange, and only rarely is liver transplant warranted.[45-47] Managing AFLP patients demands an intensive multidisciplinary team approach, and thus early recognition and referral to an experienced and equipped centre is often required. Instituting early and aggressive management protocols (Figure 2) has reduced maternal deaths at most institutions across the world.[2]

PROPOSED THERAPEUTIC INTERVENTION TO IMPROVE FETAL SURVIVAL IN ACUTE FATTY LIVER OF PREGNANCY

In AFLP, the postulated underlying defect is an inability to meet energy requirements in the face of a) the extremely high calorie requirements of late pregnancy (additional 500 kcals per day is typically required during the third trimester)[48] and b) genetic defects restricting the rate at which fatty acid oxidation can proceed. The baby's energy requirements cannot be met by beta oxidation of fatty acids, and therefore dextrose or glucose supplementations are necessary to counter the deficiency in substrate metabolism and energy release. We propose that upon diagnosis of AFLP, the mother should be fed with dextrose in amounts that provide the required 2,000 calories per day or more.[48] This proposed intervention, aimed at improving fetal survival in AFLP, needs to be tested in clinical studies (Box 1).

Care of the Baby

The management of babies born to mothers who have AFLP needs specialised inputs from a neonatologist and clinical geneticist. The baby must be assessed for fatty acid oxidation defects.[25,49] Specific fatty acid oxidation defects (e.g., *E474Q* mutation in LCHAD component of mitochondrial trifunctional protein) are noted in some populations.[49,50] Some authors suggest universal screening for LCHAD deficiency (by acyl-carnitine assay) in all children born to mothers with AFLP,[51] as LCHAD defects are the most common identified defects.[52,53]

The presentation of children with fatty acid oxidation defects can be extremely variable, from being asymptomatic to severely ill with encephalopathy, cardiomyopathy, or even sudden infant death. The symptoms tend to be precipitated by catabolic stress and all efforts

are required to mitigate this complication at the earliest signs.[54]

Outcome

Most AFLP mothers show rapid improvement within a few days after delivery and on follow-up liver functions revert to normal.[55] The prognosis in these patients is determined by the severity of liver dysfunction (serum bilirubin and prothrombin time), serum creatinine, and delay in delivery.[56-58]

Consequent to protocol-based urgent delivery (simplified criteria to diagnose AFLP), a steady decline in the contribution of AFLP and other pregnancy-related liver disorders to maternal mortality has been reported in one centre (maternal mortality due to pregnancy-related liver disorders decreased from 13% between 1999 and 2003 to 5% between 2004 and 2011).[9] Similar results have been noted across various studies, with the maternal mortality due to pregnancy related liver disorders now expected to be <10%.[2,4,5,59] The fetal outcome remains a challenge and improvement in management strategies may be required to address this important issue.

Future Pregnancies

There is a small but definite risk of recurrence during subsequent pregnancies, especially if there is a demonstrable defect in fatty acid oxidation.[60,61] This should be discussed in detail with the patient.

CONCLUSION

Improved understanding of the pathogenesis of AFLP, early recognition using clinical diagnostic criteria, and aggressive management has resulted in improvement in maternal mortality in many centres across the world. Continued efforts are required to increase awareness regarding this preventable cause of maternal death. Further focus should be directed towards the improvement of fetal survival in mothers with AFLP.

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Targeting the Relaxin Pathway for Liver Disease Treatment

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Abstract

Hepatic fibrosis is a progressive disease with few treatment options outside of transplantation. Relaxin is a member of the insulin/relaxin superfamily of peptide hormones. Originally known for its roles in pregnancy, relaxin promotes reproductive tissue remodelling and regulates vascular changes, including increased arterial compliance and reduced vascular resistance. Outside of pregnancy, relaxin plays a major role in the protection of organs from excess extracellular matrix accumulation, as demonstrated by the relaxin-null mouse, which develops widespread fibrosis with ageing. Relaxin reduces scarring due to excess collagen deposition by inhibiting collagen production while simultaneously promoting its degradation and can reduce established fibrosis in several animal models of extracellular matrix-associated disease, including liver fibrosis. Treatment with relaxin reduces the myofibroblastic phenotype of activated hepatic stellate cells, the major hepatic collagen-producing cell in fibrosis and cirrhosis. Relaxin also has haemodynamic effects, including vasodilation, and can reduce portal hypertension associated with cirrhosis. In this review, a brief overview of hepatic fibrosis and the role of the hepatic stellate cell will be presented, followed by an introduction to relaxin and its actions. The use of relaxin to treat preclinical models of fibrotic diseases, including liver diseases, will also be discussed. Finally, the completed, current, and ongoing clinical trials of relaxin in human disease will be described, followed by the limitations and future directions for the use of relaxin for disease treatment.

INTRODUCTION

Hepatic fibrosis is a progressive liver condition resulting from many causes of chronic liver injury, including viral and autoimmune hepatitis, nonalcoholic fatty liver disease (NAFLD), steatohepatitis, and alcoholic liver disease.[1] The production of fibrillary collagen is necessary for the normal response to injury. Following acute damage, injury-related collagen is rapidly removed, and the liver structure and function are restored; however, with chronic disease, unabated collagen production leads to fibrosis, which disrupts liver architecture and function.

Chronic liver disease affected 29 million individuals in Europe and 30 million in the USA in 2013.[2,3] Chronic liver disease often progresses to cirrhosis, a condition that caused 37,000 deaths in the USA, making it the 12th leading cause of death in the USA in 2013, and caused 170,000 deaths in Europe in 2013.[2,3] Currently, treatment options for hepatic fibrosis and cirrhosis are limited to the removal of the injurious stimuli, implementation of antiviral approaches, or organ transplantation.[1] Therefore, new approaches toward treatment of these conditions are sorely needed.

HEPATIC STELLATE CELLS

Chronic liver injury leads to hepatic fibrosis, characterised by excess extracellular matrix deposition, loss of liver function, and ultimately cirrhosis and liver failure.[4] The increased matrix formation is not only due to accelerated synthesis and secretion of collagen and other extracellular matrix components, but also to decreased clearance of matrix by matrix metalloproteinases (MMP).[1] This is a co-ordinated response mediated by interactions between the hepatocytes and the nonparenchymal cell population, including the sinusoidal endothelial cells, resident macrophages (Kupffer cells), and the hepatic stellate cells (HSC).

Under normal conditions, the HSC are perisinusoidal cells that resemble both fibroblasts and lipid-storing cells. In the absence of injury, HSC display a quiescent phenotype and function primarily to store lipids in the form of retinoids.[5] A major event in the response to liver injury is the transactivation of HSC resulting in an activated myofibroblastic phenotype. In this state, the stored retinoids are released. HSC begin producing large amounts of extracellular matrix, including collagen Types I and III, and secrete tissue inhibitors of metalloproteinases (TIMP), which serve to decrease matrix degradation.[6] Therefore, HSC are the major source of extracellular matrix in liver fibrosis. Activated HSC are also characterised by increased proliferation and sensitivity to cytokines, expression of smooth muscle actin, and increased contractility in response to endothelin-1.[6] Upon removal of the cause of injury, the activated HSC are removed, either by apoptosis or regression to the quiescent phenotype. However, in chronic disease, the activated HSC continue to proliferate and produce excess extracellular matrix; activated HSC are therefore a prime target for antifibrotic therapy. Ideally, an agent that targets activated HSC would inhibit the activated phenotype and promote matrix clearance to remove the scarring; one such candidate agent is the hormone relaxin.

RELAXIN: AN ANTIFIBROTIC HORMONE

Relaxin is a polypeptide hormone of the insulin/relaxin superfamily.[7] The first identified function for relaxin was its role in pregnancy, during which it is produced by the corpus luteum of the ovary. During pregnancy, circulating relaxin levels in humans peak during the first trimester, but there are variable release patterns in different species.[8] Relaxin is involved in a number of critical events associated with pregnancy, including softening of the cervix and vagina.[8] Relaxin also has potent vascular effects and plays a major role in increasing arterial compliance and reducing vascular resistance, while at the same time increasing renal plasma flow and glomerular filtration rate.[9,10] There is also evidence that relaxin may have vascular effects in males and nonpregnant females, but these results are inconsistent and require further study.[10]

An important mechanism associated with many of the effects of relaxin is the widespread remodelling of extracellular matrix, involving both the altered secretion of extracellular matrix components as well as changes in matrix component degradation.[11] These observations have been extended to nonreproductive tissues, where a role has emerged for relaxin as a general antifibrotic hormone.[12] Relaxin has been shown to reduce collagen production, increase MMP activity, decrease the levels of TIMP in scleroderma cell models, and decrease experimentally induced dermal fibrosis in rodents.[13,14] Similarly, relaxin was effective in preventing or reversing several models of experimental renal fibrosis in rodents, and the hormone-modulated collagen, MMP, and TIMP levels in renal fibroblasts.[15] Relaxin decreased the myofibroblastic phenotype of lung fibroblasts and decreased pulmonary fibrosis in rodents.[11] Finally, numerous studies have implicated relaxin in the prevention or treatment of several models of cardiac fibrosis.[11]

Table 1: Summary of preclinical studies investigating relaxin based therapeutics in liver disease.

Model	Method of hepatic damage	Species	Dose, duration	Major findings	Reference
Normal liver	N/A	Rat	10 µg/day 1–4 days	Changes in liver microcirculation, morphology of sinusoidal cells	Bani D et al.,[20] 2001; Bani D et al.,[21] 2001
Liver fibrosis: Prophylactic models	CCl_4	Rat	0.5–0.6 mg/kg/day 4 weeks	Reduced collagen accumulation	Williams EJ et al.,[23] 2001
	CCl_4	Mouse	0.5 mg/kg/day 4 weeks	Reduced liver collagen and SMA	Bennett RG et al.,[27] 2009
Liver fibrosis: Treatment of established disease	CCl_4	Mouse	0.5 mg/kg/day 1–2 weeks	Reduced collagen and SMA (1 week), no change in collagen at 2 weeks	Bennett RG et al.,[27] 2009
	CCl_4	Mouse	25–75 µg/kg/day 4 weeks	Decreased collagen and SMA, increased collagen degradation	Bennett RG et al.,[28] 2014
	CCl_4, BDL	Mouse	0.5 mg/kg/day 72 hours	Reduced portal pressure; decreased SMA (CCl_4 only)	Fallowfield JA et al.,[26] 2014
	TAA	Rat	Adenovirus 3 weeks	Decreased collagen and SMA	Kim JK et al.,[29] 2016
	CCl_4	Mouse	150 µg/kg/day and rosiglitazone 2 weeks	Reduced ALT, collagen, and SMA	Bennett RG et al.,[30] 2017
Ischaemia-reperfusion injury	IRI	Rat	64 ng/mL (perfusion medium)	Decreased peroxidation, increased oxygen	Boehnert MU et al.,[31] 2008; Boehnert MU et al.,[32] 2009
	IRI, transplant	Mouse	5 µg/kg	Decreased IRI, improved survival	Kageyama S et al.,[33] 2018

ALT: alanine transaminase; BDL: bile duct ligation; CCl^4: carbon tetrachloride; IRI: ischaemia-reperfusion injury; N/A: not applicable; SMA: smooth muscle actin; TAA: thioacetamide.

The hypothesis that relaxin has a role as a general protective agent against fibrosis was strengthened by observations made using the relaxin-null mouse. In addition to the expected difficulties with reproduction, these mice spontaneously developed age-related pulmonary, cardiac, dermal, and renal fibrosis, which was reversible with relaxin treatment.[16] In most cases, male mice developed more severe fibrosis than the females, likely due to testosterone-related effects.[17] Similar effects were seen in mice lacking *Rxfp1*, which codes for the cognate receptor for relaxin. These *Rxfp1* mice developed sex-specific pulmonary fibrosis with age.[18,19]

ROLES FOR RELAXIN IN THE LIVER

Relaxin also has effects in the liver. Rats that received relaxin treatment demonstrated acute changes in hepatic microcirculation; morphological changes were detected in sinusoidal myofibroblastic cells lacking retinoids, most likely activated HSC.[20,21] In addition, the relaxin-null mouse presented with increased liver weight.[22] Relaxin treatment of activated rat HSC decreased overall collagen and TIMP production.[23] Similarly, treatment of activated mouse HSC resulted in decreased total collagen deposition, collagen synthesis, Type I collagen secretion, and reduced smooth muscle actin expression, but had no effect on HSC proliferation or apoptosis.[24] Relaxin also

promoted a matrix-degrading phenotype inhibiting TIMP secretion and, by increasing the expression and activity of MMP13, the major rodent fibrillary collagen-degrading MMP.[24] The expression of the relaxin receptor, RXFP1, was found to be low or undetectable in quiescent mouse HSC or normal liver but increased dramatically upon HSC activation or development of hepatic fibrosis.[25] This finding was later confirmed using rat and human activated HSC and tissues,[26] providing evidence that the activated HSC are a target of the antifibrotic actions of relaxin.

Consistent with the findings using cultured cell models, relaxin has shown effectiveness in *in vivo* models of experimental hepatic fibrosis (summarised in Table 1). While the effects of relaxin were favourable overall, there were differences dependent on the dose and timing of treatment. In a rat prevention model, in which treatment with recombinant human relaxin (infusion of 0.5–0.6 mg/kg/day) began concomitantly with carbon tetrachloride (CCl_4)-induced liver injury for 4 weeks, relaxin decreased total hepatic collagen content.[23] In a similar prevention study using mice, infusion of porcine relaxin (0.5 mg/kg/day) reduced serum liver transaminases, hepatic collagen, and smooth muscle actin content.[27] In a second study using a more clinically relevant mouse model, fibrosis was first established with 4 weeks of CCl_4 followed by porcine relaxin infusion (0.5 mg/kg/day) for 1 or 2 weeks with continued CCl_4 administration.[27] After 1 week of relaxin treatment, hepatic collagen and smooth muscle actin content were modestly decreased, but after 2 weeks only the smooth muscle actin content was significantly reduced.[27] In a later study, mice were made fibrotic by CCl_4 treatment for a total of 8 weeks, with relaxin treatment for the final 4 weeks at lower relaxin doses (25 or 75 µg/kg/day infusion of recombinant human relaxin, also known as serelaxin).[28] The lower doses were used due to evidence that extended treatment of human relaxin at high doses can induce an immune response in rodents.[34] In this model, relaxin at either dose reduced hepatic smooth muscle and collagen content, and at the higher dose the expression of Type I procollagen, TIMP2, and MMP2 was decreased, while expression of MMP3 and MMP13, and overall Type I collagen-degrading activity was elevated.[28] Another study examined the effect of short-term treatment (72 hours infusion of 0.5 mg/kg/day recombinant human relaxin) on rats with either established fibrosis (8 weeks CCl_4 treatment), cirrhosis (16 weeks CCl_4 treatment), or cholestatic disease induced by surgical ligation of the common bile duct.[26] Short-term relaxin infusion had no effect on total collagen content in any of the models but decreased smooth muscle actin in the 8 week fibrosis model. However, in all three models, relaxin significantly decreased portal blood pressure without decreasing systemic blood pressure, providing evidence that relaxin may be effective in reducing the intrahepatic vascular resistance characteristic of cirrhosis. Finally, adenoviral delivery of relaxin was trialled in a thioacetamide model of hepatic fibrosis.[29] Rats were treated for 8 weeks with thioacetamide, then infected with adenovirus after Week 7 and examined either 3 days or 3 weeks later.[29] No significant effects were observed 3 days after infection, but after 3 weeks the adenovirally-delivered relaxin reduced liver collagen and smooth muscle actin content and decreased gene expression of Type I collagen and TIMP2. In summary, these preclinical studies suggest that prolonged relaxin treatment may be effective in treating hepatic fibrosis, while short-term treatment may be useful to reduce portal blood pressure.

CROSSTALK BETWEEN RELAXIN AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA

A second critical regulatory element in HSC activation is the peroxisome proliferator-activated receptor gamma (PPARγ) pathway. PPARγ is a transcription factor activated by antidiabetic thiazolidinedione (TZD) drugs and some prostaglandins.[35] Expression of PPARγ is detectable in quiescent HSC but is lacking in activated HSC and myofibroblasts.[6] Restoration of PPARγ expression, either by treatment of activated HSC with PPARγ ligands or by forced expression of PPARγ, induced a reversion of the HSC to a state that closely resembled the quiescent phenotype, as shown by decreased proliferation; reduced SMA, collagen, and TIMP expression; increased MMP-13 expression; and restoration of lipid storage.[36] Treatment of

experimentally induced fibrosis with PPARγ ligands prevented hepatic fibrosis *in vivo*.[37] These findings led to great interest in the use of TZD for the treatment of hepatic fibrosis because the quiescent HSC have adipogenic properties and PPARγ is a major inducer of adipogenesis. However, recent studies have suggested that TZD treatment may be ineffective for established fibrosis in rodents, casting some doubt on the use of using TZD alone for this purpose.[38,39]

There is recent evidence that relaxin signalling through RXFP1 results in activation of PPARγ. In cultured HSC, relaxin caused increased transcriptional activity of PPARγ.[40] Interestingly, the mechanism was ligand-independent, as inhibitors of PPARγ ligand binding did not block the effect.[40] Relaxin was also shown to activate PPARγ in cerebral arterioles.[41,42] More recently, the mechanism for this stimulation was found to be via increased expression of a coactivator protein, known as PPARγ coactivator 1 alpha, via cAMP and p38-MAPK-dependent pathways.[43] Therefore, relaxin may enhance PPARγ activity by inducing the cAMP/protein kinase A pathway. Other activators of protein kinase A enhanced basal and ligand-induced PPARγ transcriptional activity and enhanced the stability of PPARγ-DNA binding in fibroblasts and adipocytes.[44,45] This raises the possibility that relaxin may sensitise cells to respond to TZD drugs. This was addressed in a recent study that used a combination of relaxin and the TZD drug rosiglitazone on established hepatic fibrosis in mice.[30] The results confirmed that rosiglitazone alone is ineffective but found that the combination of relaxin and rosiglitazone was more effective than either treatment alone.[30] This notion is consistent with the recent finding that the response to airway dilators, including rosiglitazone, was increased when used in combination with relaxin.[46]

CURRENT AND POTENTIAL CLINICAL STUDIES OF RELAXIN

The majority of the human studies not focussing on reproduction have focussed primarily on the haemodynamic properties of relaxin. Most of these have examined the treatment of heart disease, based on the effects of relaxin on vasodilation and improved renal function.[47] A large Phase III randomised, placebo-controlled, double blind trial (RELAX-AHF) was conducted involving 1,161 patients presenting with acute heart failure.[48] Study participants were given a 48 hour infusion of recombinant human relaxin (30 µg/kg/day) or placebo, with the primary endpoints of improved dyspnoea by Likert scale or visual analogue scale. One primary end point was reached (improvement in dyspnoea identified through the visual analogue scale) but not the other. The study found a significant decrease in all-cause mortality and cardiovascular death at 180 days in the relaxin-treated subjects. Failure to secure approval from the U.S. Food and Drug Administration (FDA) led to a second, larger Phase III trial (6,566 participants), known as RELAX-AHF-2, with primary endpoints of reduced cardiovascular mortality and worsening heart failure at 180 days.[49] Although the results have not been published at the time of writing, the sponsor has issued a press release stating that the primary endpoints were not met.[50]

The clinical studies of relaxin for human fibrotic disease have thus far been limited to scleroderma. The earliest studies were conducted in the 1950s, using porcine relaxin to treat scleroderma.[11] The results from these studies were highly variable, and were not followed up until more recent trials were conducted using recombinant human relaxin. After promising results in a Phase II study, a larger Phase III study revealed no significant effects after continuous infusion with 10 or 25 µg/kg/day relaxin for 24 weeks, possibly due to the advanced stage of the disease in many of the subjects.[51] Indeed, relaxin treatment failed to reverse advanced dermal fibrosis in the aged relaxin-null mouse, suggesting that severe scleroderma may be irreversible.[52] It is possible that further studies of relaxin treatment in subjects with less severe scleroderma might yield more positive results. Another possible factor may be reduced expression of RXFP1 in skin from scleroderma patients.[53]

To date, the clinical studies of relaxin for liver disease have focussed on reducing portal hypertension. As described previously, preclinical models suggest that a short infusion of relaxin resulted in reduced portal blood pressure without reducing systemic blood pressure. In a Phase II, open label study of

40 patients with cirrhosis and hypertension, two consecutive 1 hour infusions (1 hour of 80 µg/kg/day followed by 1 hour of 30 µg/kg/day) recombinant human relaxin resulted in increased renal arterial flow, with no significant effect on systemic blood pressure or hepatic perfusion, and the treatment was well-tolerated.[54] A second randomised placebo-controlled study Phase II trial is being prepared.[55] The study will analyse 2 hours of relaxin infusion in patients with cirrhosis and portal hypertension, with the primary endpoint of change in fasting hepatic venous pressure gradient.

Finally, there is evidence that relaxin may act as a hepatic protective agent from ischaemia-reperfusion injury and therefore act as a preservative for liver transplantation tissue. Relaxin added to organ preservation solution for rat liver perfusion decreased markers of oxidative damage and increased the hepatic oxygen supply.[31,32] Recently, a study examining a mouse model of orthotopic liver transplantation after 18 hours cold storage found that relaxin decreased ischaemia-reperfusion damage and improved survival.[33] Therefore, it is possible that relaxin may act to improve the viability of human livers for transplantation.

PERSPECTIVE AND LIMITATIONS

The preclinical studies suggest that relaxin is an effective treatment for a wide variety of fibrotic disease models, including hepatic fibrosis and cirrhosis-related portal hypertension. Thus far, no human studies of relaxin treatment of fibrotic disease have been attempted, apart from scleroderma. This may partly be the result of the failure of the Phase III scleroderma study casting doubt for future long-term studies using relaxin. However, given the severity of the disease and the questions about the relevance of the clinical outcomes used,[56] further studies are needed. Additionally, with the recent failure of the RELAX-AHF-2 study, the future of major clinical studies using recombinant human relaxin is unclear.

One major limitation for the extended use of recombinant human relaxin (or relaxin derived from any species) is its nature as a peptide hormone. This property makes relaxin unstable, with a relatively short half-life (approximately 10 minutes) in circulation.[57] Furthermore, as a peptide, relaxin is unsuitable as an oral agent, and so the hormone must be administered intravenously by frequent injections or by continuous subcutaneous delivery. For these reasons, there is much attention currently aimed at producing modified relaxin-based peptides with improved stability.[57,58] Additionally, as described above, preclinical and clinical studies have used a wide range of relaxin doses and treatment durations, with varying degrees of success. A contributing factor may be related to the 'bell-shaped' responses to relaxin that have been frequently observed.[59] Additional studies are needed to systematically optimise relaxin doses and treatment durations for each target disease.

Small molecule activators of RXFP1 may play an important role in the future use of the relaxin signalling pathway in the treatment of human disease. The best characterised of these small molecule RXFP1 agonists is ML290, which is much more stable than relaxin, and is currently serving as the lead compound in optimisation studies.[60,61] In animal models, ML290 has shown properties similar to relaxin in reproductive tissues and cardiac function and has had relaxin-like effects on HSC.[62] ML290 is a promising lead compound for the development of future small molecule relaxin-mimetic drugs.

Another limitation that may impact the successful clinical application of relaxin relates to tissue expression of RXFP1 in disease states. In the liver, the expression of RXFP1 increases in activated HSC in preclinical models and human disease, supporting the use of relaxin in hepatic fibrosis.[25,26] However, in human scleroderma and idiopathic pulmonary fibrosis, expression of RXFP1 appears to be reduced, suggesting that the ability to respond to relaxin may be reduced in these diseases.[53,60] Additional studies of disease-specific RXFP1 expression are needed to inform the applicability of relaxin to these states.

The interaction of relaxin-RXFP1 signalling with other pathways, such as PPARγ, suggest that combination treatment of relaxin and agents such as the TZD drugs may present an improvement over monotherapy.[30,43] Indeed, crosstalk between relaxin and other pathways, such as the angiotensin II type 2 receptor (AT2R),

has been demonstrated. It was shown that RXFP1 can heterodimerise with AT2R and that AT2R activity was necessary for the antifibrotic effect of relaxin in a model of renal fibrosis, suggesting that simultaneous targeting of RXFP1 and AT2R may be a promising avenue of investigation.[64]

CONCLUSION

In summary, relaxin has shown great promise as an antifibrotic agent in cell culture and animal models of fibrosis. The role of relaxin in the protection from excess collagen accumulation is clear from the studies of relaxin-null and RXFP1-null mice, which developed spontaneous fibrotic diseases with ageing. Due to its antifibrotic and haemodynamic properties, as well as the limited expression profile of RXFP1, the relaxin signalling pathway remains a promising target for the development of new treatment options for liver diseases.

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Thermal Ablation of Liver Tumours: How the Scenario Has Changed in the Last Decade

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Abstract

Surgical resection has long been considered the gold standard for the local treatment of primary and secondary liver tumours. Until recent years, percutaneous thermal ablation (PTA), in particular radiofrequency ablation (RFA), was not accepted as a first-line option for the treatment of liver tumours and was reserved for patients who were unsuitable for surgery. However, in the last decade the scenario has changed: interesting technical developments and innovations have improved the performance of RFA and broadened the availability of other ablative technologies, such as microwave ablation (MWA) and laser ablation (LA). The latest generation of MWA systems can achieve larger ablation areas than RFA and LA, with a multifibre technique that uses very thin needles, allowing physicians to treat nodules in at-risk locations with high flexibility and a very low risk ofcomplications. Nowadays, there is an increasingly accepted consensus on the role of PTA as a first-line option for the treatment of liver tumours <2 cm in size, as well as in patients potentially eligible for surgery, and it is likely that in the near future the 2 cm barrier could also be surpassed and extended to at least 3 cm. PTA is becoming more effective and important in the treatment of primary and secondary liver tumours, and, in the well-established scenario of a multimodal tailoredtreatment, it plays and will continue to play a central role. The aim of this paper is to examine the current role of PTA in such a scenario, focussing on advantages and limitations of the three available ablative techniques: RFA, MWA, and LA.

INTRODUCTION

Primary and secondary hepatic tumours are relatively common and significantly impact the overall survival (OS) of cancer patients. The incidence of hepatocellular carcinoma (HCC) is increasing worldwide; nowadays, HCC is the fifth most common cancer and the third most common cause of cancer-related mortality.[1] Secondary involvement of the liver, particularly from colorectal carcinoma, is even more common.[1,2] More than 50% of patients with gastrointestinal malignancies will develop liver metastases (LM), with significant morbidity and mortality.[3,4]

As a consequence, local treatments of liver tumours have gradually assumed a central role as part of multimodal cancer therapy. Liver transplantation represents the treatment of choice for selected patients with HCC, but it is

only available to a restricted number of patients because of the high cost and shortage of donor livers.[5] Surgical resection (SR) has widely been proven to improve OS in patients with either HCC or LM, particularly those with colorectal cancer liver metastases (CRLM), and for a long time it has been considered the gold standard for the local treatment of liver tumours.[6-8] In the last two decades, percutaneous thermal ablation (PTA) has gained a key role in the treatment of patients who are not eligible for, poor candidates for, or refusing surgery, as well as being a bridge to liver transplantation.[5] Although many studies have widely proven its effectiveness and safety, until very recently PTA was not accepted as a first-line option for the treatment of liver tumours and was reserved for patients unsuitable for surgery.[5,9,10] However, compared with the first experiences, which were mostly based only on percutaneous ethanol injection and earlier devices for radiofrequency ablation (RFA), in the last decade the scenario has changed. Technical developments and innovations have improved the performance of RFA and broadened the availability of other ablative technologies, such as microwave ablation (MWA) and laser ablation (LA), allowing interventional radiologists and oncologists to offer an increasing number of tailored approaches for cancer patients, which have better outcomes. The aim of this narrative review is to examine the current role of PTA in the scenario of the multimodal tailored treatment of liver tumours, starting from the first and most widely used thermal ablation technique.

RADIOFREQUENCY ABLATION: STATE-OF-THE-ART IN THE TREATMENT OF HEPATOCELLULAR CARCINOMA AND LIVER METASTASES

RFA is the best established and most used technique worldwide, and its effectiveness and safety have largely been proven over the last two decades.[5,9-12]

Radiofrequency Ablation and Hepatocellular Carcinoma

Although the role of RFA as a bridge to liver transplantation has widely been established, its efficacy in the treatment of HCC with curative intent is still influenced by tumour location and size. RFA of tumours located close to large vessels can achieve suboptimal results due to the well-known 'heat sink' effect, which causes the partial shunt of thermal energy by the cooler blood.[13] Likewise, the treatment of large lesions can require multiple overlapping ablations to obtain an adequate safety margin, and results in tumours >5 cm in size are still poor.[14-16] As a consequence, for a long time SR was considered the gold standard therapy in terms of OS and disease-free survival, and RFA was reserved for patients who were not eligible for surgery.

Nowadays, however, HCC is frequently diagnosed at an early stage. In 2001, the Barcelona Clinic Liver Cancer (BCLC) staging system and treatment strategy included RFA among curative treatments for very early (<2 cm) and early stage (<3 cm) HCC,[17] and several more recent studies have suggested that RFA of very early stage HCC can achieve the same results as surgery in terms of OS.[14,18-24] Moreover, hepatic resection is not an ideal treatment for very small sized cases of HCC because of the potential loss of liver function and the high risk of complications. Conversely, RFA is a minimally invasive, effective, and cost-effective technique, particularly in patients with poor liver function and related comorbidities. As a consequence, according to many authors, RFA could be considered the first-line option in the treatment of HCC <2 cm in size, as well as in patients potentially eligible for surgery.[18-25]

Radiofrequency Ablation and Liver Metastases

The locoregional treatment of LM must be included in a systemic and multimodal treatment plan because the long-term outcomes are mainly determined by the characteristics and natural history of the primary cancer. Most studies regarding the treatment of LM have been conducted on CRLM. SR is considered the procedure of choice, with 5-year survival rates of 51–58%.[7,8] However, multiple or bilobar LM require major hepatic surgery, and the potential complications of this strategy may outweigh the theoretical benefits in terms of survival rates. Given that surgery is feasible in only 10–15% of patients with LM,[26] nowadays many centres propose RFA as an alternative to surgery since RFA has been reported to improve survival and

quality of life in selected patients, with very low risks of complications.[27] Moreover, RFA can be indicated in patients with resectable lesions as an adjunct to resection, for inoperable lesions that demonstrate response after chemotherapy, or for recurrent or progressive lesions.[28] In patients with a maximum of 5 or 6 LM with a diameter of 5–6 cm, RFA was reported to obtain 3-year and 5-year survival rates ranging 28–46%, with a median survival ranging 30–40 months.[29-32]

Although the efficacy of PTA of LM when compared to chemotherapy alone has, to the best of our knowledge, never been proven by randomised controlled trials (RCT), there are a large number of studies in the literature suggesting that combined multimodal treatments including PTA can achieve better outcomes than systemic chemotherapy alone in patients with CRLM.[27-32] Among the several attempts to organise RCT comparing RFA plus systemic chemotherapy versus chemotherapy alone, just one trial, the CLOCC trial,[33] planned by the European Organisation for Research and Treatment of Cancer (EORTC), was completed, but it was downscaled from a Phase III to a Phase II study because of difficulty recruiting participants. The preliminary results after a median follow-up of 4.4 years showed a significantly longer progression-free survival of the patients in the RFA plus chemotherapy cohort, but no difference in OS between the two cohorts.[33] However, after a longer median follow-up (9.7 years), the OS results were significantly better in the combination cohort, with an observed median OS of 45.6 months (95% confidence interval: 30.3–67.8) for RFA plus chemotherapy versus 40.5 months (95% confidence interval: 27.5–47.7) for chemotherapy alone (p=0.01).[34] To date, we can rationally conclude that the best available evidence points towards a benefit of the combination strategy using ablative treatments and chemotherapy in CRLM. Moreover, in recent years the role of RFA in combination with systemic chemotherapy has also been highlighted by several authors in the treatment of LM from breast cancer, gastrointestinal stromal tumours, and neuroendocrine tumours.[35-37]

MICROWAVE ABLATION: ADVANTAGES OVER RADIOFREQUENCY ABLATION OF THE LATEST GENERATION SYSTEMS

Despite the introduction of more effective devices, such as cluster, expandable, and multitined electrodes, the main limit of RFA remains tumour size. Local control rates >90% have been reported for nodules up to 3 cm, but only rates of 6–10% for tumours >5 cm.[14-16] The capability of microwaves to propagate through tissues with low electrical conductivity, high impedance, or low thermal conductivity, like charred tissues, allows the MWA device to generate very high temperatures inside the lesion in a very short time. Moreover, microwave energy radiates into the tissue through an interstitial antenna, which determines direct heating of the lesion regardless of the closeness to the large vessels.[38] As a consequence, MWA can improve PTA efficacy by obtaining larger ablation volumes and broader safety margins. Several studies have demonstrated that early-generation MWA had comparable effectiveness and safety than RFA, with a shorter ablation time.[38-40] However, in recent years, technical advances in MWA technology have allowed the development of safer and more slender MWA antennas with a similar gauge with respect to RFA electrodes, minimising the drawback of the back heating effect and enabling physicians to achieve larger ablation areas than with RFA.[41,42] The introduction of these latest generation MWA systems could surpass the 2 cm barrier in the treatment of HCC, and extend it to at least 3 cm.[43] Surgery remains the gold standard for nodules >3 cm and MWA could be considered the first-line choice for nodules up to 3 cm, particularly if they are central or deeply located or close to large vessels. Reported 3-year and 5-year survival rates in HCC patients range from 72–73% and 51–57%, respectively, with single HCC <5 cm in diameter or up to three HCC <3 cm in diameter.[44]

Given the typical infiltrative growth and lack of clear margins of LM, the capability of MWA to achieve larger ablation areas and obtain adequate safety margins can play a crucial role in the treatment of LM. Studies on the outcomes of MWA are less numerous and generally involve smaller patient numbers than studies of RFA.

Moreover, most studies investigating the effectiveness of MWA were conducted before the introduction of the most recent advances in MWA technology, so at present the best available evidence suggests similar outcomes for RFA and MWA. MWA of LM has been reported to achieve 3-year and 5-year survival rates ranging 46–51% and 17–32%, respectively, with a median survival ranging 20–48 months.[45-47] However, in the near future we can expect better outcomes from studies based on the use of the latest generation of MWA systems.[22]

LASER ABLATION: ITS ROLE IN THE TREATMENT OF TUMOURS IN AT-RISK LOCATIONS AND MULTIPLE TUMOURS OF DIFFERENT SIZES

LA uses laser optical fibres to deliver high-energy laser radiation to the tissue. As a result of light absorption, temperatures of up to 150°C are reached, leading to coagulative necrosis.[28] Neodymium-doped yttrium aluminum garnet (Nd:YAG [wavelength: 1,064 nm]) and diode (wavelength: 800–1,064 nm) lasers are most commonly used because penetration of light is optimal in the near infrared spectrum. Although it is less frequently investigated than RFA and MWA, LA is currently used in several centres for the treatment of HCC and LM, and the available data on its effectiveness and safety are good and comparable to those of RFA and MWA.[28,48-50] The multifibre technique[49,50] enables clinicians to simultaneously use from one to four 300 µm bare-tip optical fibres to treat the tumour according to its size. Usually, one or two fibres are used to treat nodules up to 1.5 cm, three fibres to treat nodules 1.5–2.5 cm, and four fibres to treat nodules >2.5 cm.[51-53] The main advantage of LA is its feasibility because LA needs very fine needles (21 gauge) to introduce the fibres into the tumour, allowing nodules to be treated in at-risk locations with a very low risk of complications. Moreover, the possibility of using one to four fibres at once allows physicians to achieve different ablation areas according to the tumour size and makes LA the most flexible ablation technique to treat multiple lesions of different sizes in the same session, sparing the noncancerous parenchyma as much as possible.

Most studies on LA are focussed on the treatment of HCC. Complete response rates ranging from 82–97% and cumulative 3-year survival rates up to 73% were reported in Child–Pugh Class A patients with single HCC <5 cm, or up to three nodules <3 cm, treated with multiple bare-tip fibres.[52-55] Moreover, a complete response rate of 95.5% was reported in tumours with high-risk locations.[50] Likewise, good results have also been achieved in the treatment of CRLM with a diameter up to 5 cm, with 3-year and 5-year survival rates ranging from 28.0–72.4% and 10.0–37.0%, respectively.[56-58] Due to its novel features, LA has been proposed as the technique of choice in cases of multiple, bilobar, small, and variably sized LM (for instance, from neuroendocrine tumours), because it provides the ability to better modulate the volume of necrosis and more effectively avoid the liver parenchyma than RFA, achieving similar results.[58-60]

FINAL CONSIDERATIONS AND PROPOSALS

The high incidence worldwide of HCC and LM makes it crucial to plan the best strategy of multimodal treatment.[1-4] Concerning HCC, liver transplantation remains the gold standard in selected patients, and for a long time SR has represented the first-line therapy when patients cannot undergo liver transplantation.[5-8] Until very recently, PTA was restricted to patients unsuitable for surgery or used as a bridge to liver transplantation.[5,9,10] Nowadays, the scenario has changed and the role of PTA as the first-line treatment of very early and early HCC with curative intent is widely accepted. The inclusion of PTA among the curative therapies for single HCC <2 cm or for up to three HCC <3 cm in patients with comorbidities dates back to the early 2000s,[17] and its relevance has got progressively stronger.[6,9] PTA of very early HCC has widely been demonstrated to achieve the same results as SR with lower morbidity and mortality,[15,18-23] and its capability to spare the noncancerous liver parenchyma makes its role even more central in the tailored approach to patients with HCC. Therefore, at present, PTA can be considered the first-line therapy for very early and early HCC.[61,62] The vast majority of studies on PTA of HCC refer to RFA. Despite its efficacy and safety, and the

technical developments that have improved the performance of the more recent RFA devices, the main limitation of RFA remains tumour size, and at present the outcome of nodules >3 cm is still poor. Latest generation MWA systems produce larger ablation volumes than RFA,[41,42] enabling HCC up to 3 cm to be treated with the same efficacy as SR and the same safety as RFA.[62] Moreover, some authors have reported local tumour control rates of nodules up to 5 cm, nearly identical to those achieved for tumours up to 3 cm.[43] However, the question of whether the time has come to increase the 2 cm tumour size barrier to 3 cm is still open for discussion and further randomised studies with longer follow-up are needed. In any case, MWA should be preferred for the treatment of HCC >2 cm, particularly when located close to large vessels. The role of LA is less investigated, but the results reported by several authors are quite similar to those of RFA.[48-55] The multifibre technique makes LA particularly interesting in the treatment of HCC at difficult or high-risk locations, and in the treatment of multiple and very small HCC in order to spare the noncancerous parenchyma as much as possible.

With regard to LM, SR in association with systemic chemotherapy represents the best choice in resectable patients with CRLM.[7,9] However, SR can be offered to a small number of patients and the high risk of complications in major liver surgery makes it inappropriate for use in frail patients and in the treatment of multiple or bilobar LM. In nonsurgical-selected patients, PTA has been demonstrated to improve survival with a very low risk of complications,[27,61] particularly when an aggressive approach is adopted to obtain an adequate safety margin.[63] The efficacy of PTA in CRLM compared with chemotherapy alone, as well as the superiority of chemotherapy alone over PTA, have not been proven by RCT.[64] To date, just one prospective RCT comparing RFA plus systemic chemotherapy versus systemic chemotherapy alone has been published.[33] After an adequate follow-up, the trial reported a significantly improved OS in the combination cohort.[34] Likewise, numerous studies reported that combined therapeutic strategies including PTA provide better results than chemotherapy alone.[27-31] PTA can also be considered for patients with potentially resectable lesions as an adjunct to resection, with nonsurgical lesions that demonstrate response after chemotherapy, or with recurrent or progressive lesions.[28] Moreover, when ablation was applied as the first-line therapy to resectable patients, the 5-year survival rates resulted in very similar results to surgical studies.[65,66]

In this regard, a position paper on PTA of CRLM has recently been published by an international panel of ablation experts.[26] A strong consensus level was achieved for the treatment of nodules up to 5 cm when well located (with easy access), and for up to five nodules. Likewise, a strong level of consensus was achieved for combination strategies with respect to systemic treatments alone. The panel also agreed in considering PTA as potentially curative in resectable patients when used as a first-line treatment. Indeed, although most surgical studies have reported higher local tumour progression following ablation than after SR, the OS was comparable.[67] Of course, it is mandatory that PTA is performed by skilled operators and that adequate safety margins are obtained. The authors' experiences are consistent with those of this position paper, as well as with the assumption regarding the convenience of exploiting the advantages and minimising the limits of all three ablation techniques. In this regard, an algorithm aimed at tailoring PTA to the patient's and tumour's characteristics to obtain the best outcome has recently been proposed.[59] As a consequence of these considerations on the technical characteristics, advantages, and limitations of the three available ablation techniques, the possibility to always select the most suitable option for each single case allows clinicians to optimise the potential of PTA and its expected outcome.

CONCLUSION

In conclusion, PTA has become increasingly more effective in the treatment of primary and secondary liver tumours; for example, in the well-established scenario of a multimodal tailored treatment, PTA now plays a central role. The careful characterisation and selection of patients and nodules, according to size, number, and location, will offer the best chance of treatment for liver cancer patients.

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Percutaneous Laser Thermal Ablation in a Patient with 22 Liver Metastases from Pancreatic Neuroendocrine Tumours: A Case Report

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Abstract

The relatively indolent nature of well-differentiated neuroendocrine tumours (NET) and their proclivity to be hormonally active warrants aggressive multimodal treatment, even for advanced stage disease. Good results have been reported in well-selected patients with a median of 23 liver metastases (LM) from NET treated with surgical resection combined with intraoperative radiofrequency ablation. We report the case of a patient who underwent percutaneous laser thermal ablation (LTA) of 22 small LM from NET, treated over three consecutive sessions. After 2 years, five new LM were detected and treated with LTA. At present, 82 months after the first LTA session, the patient is still alive and disease-free. Due to enabling the use of one to four optical fibres at once to tailor the thermal lesion size to the nodule size, LTA could represent the ablation technique of choice in the presence of multiple, small, and variably sized LM.

INTRODUCTION

Liver metastases (LM) from neuroendocrine tumours (NET) occur with variable frequency depending on the primary disease, ranging from 5–10% for carcinoid tumours to 75% for glucagonoma.[1] The relatively indolent nature of well-differentiated NET and their proclivity to be hormonally active warrants aggressive multimodal treatment even for advanced stage disease. Indeed, aggressive cytoreduction using combined treatments, such as surgery, transarterial chemoembolisation, and thermal ablation, can improve both survival and quality of life, and, to date, many guidelines for the management of NET recommend the removal of at least 90% of LM whenever possible.[2-4] With this aim, surgical resection is considered the gold standard treatment, but only 10–20% of patients with NET are suitable candidates for resection. Ablative therapies can be used in place of, or as an adjunct to, resection. In brief, they can preferentially be used for deep small lesions (diameter <4 cm) and recurrent lesions and can be considered as an adjunct to resection in patients with extensive bilobar disease. Thermal ablative techniques can deliver thermal energy, either cooling (cryoablation) or heating the tissue. Radiofrequency ablation (RFA), microwave ablation, and laser thermal ablation (LTA) raise the temperature of the tissue to between 60°C and 100°C, producing coagulative necrosis. LTA, according to the technique proposed by Pacella et al.[5]

and modified by Di Costanzo et al.,[6] uses 300 µm bare optical fibres introduced into the tumour through 21 gauge needles. The ability to place one to four fibres at once using a multisource device makes LTA the most flexible ablation technique.[7,8] A bare-tip fibre provides an almost spherical thermal lesion of 12–15 mm in diameter, and the lesion size can be increased up to 4–5 cm by using multiple fibres with the pull-back technique.[5,6] In our opinion, such novel features make LTA the ablation technique of choice when treating multiple liver tumours of varying size. This paper reports the case of a patient who underwent LTA of 22 small LM from NET.

CASE REPORT

In November 2007, a 26-year-old woman underwent complete pancreatic resection for insulin-secreting NET. The tumour was well differentiated with a Ki-67 expression level of 3% and a chromogranin A level of 92 ng/mL. Contrast-enhanced computed tomography (CECT) showed multiple (≥20) LM, ranging from 5–14 mm in size. In January 2008, the patient began medical therapy with somatostatin analogues (octreotide followed by pasireotide); in January 2010 she underwent transarterial chemoembolisation. In January 2011, restaging CECT showed stable disease.

Given the low proliferation index of the tumour and the indolent course of the disease, ultrasound (US)-guided percutaneous LTA was planned to remove as many LM as possible. LTA was preferred to other ablation techniques because of the high number of LM and their small size to balance the need of obtaining a good safety margin with the need to spare normal liver parenchyma. Medical therapy with pasireotide was not discontinued and LTA was performed on an inpatient basis using a commercially available system composed of an US device and a laser unit (Echolaser, Elesta Srl, Florence, Italy). The laser source was a semiconductor diode with a wavelength of 1,064 nm, and a multisource device enabled the use of up to four fibres at once (Figure 1). After local anaesthesia with 10 mL 1% lignocaine and conscious sedation with intravenous midazolam and remifentanil, the laser fibres were introduced into the tumour through 21 gauge needles under US guidance.

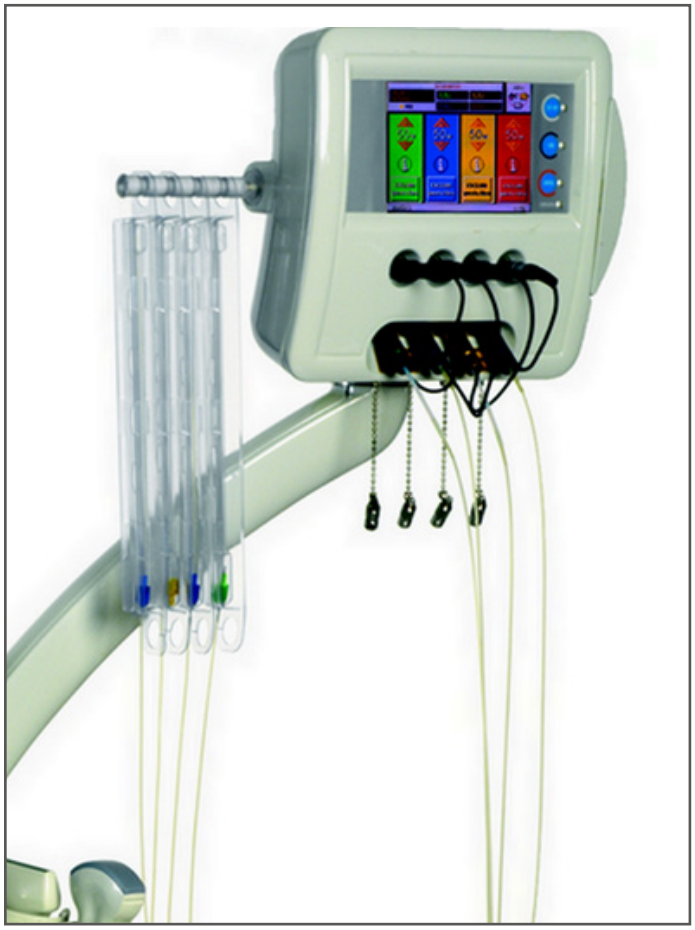

Figure 1: Laser ablation machine with multisource device with four bare optical fibres connected.

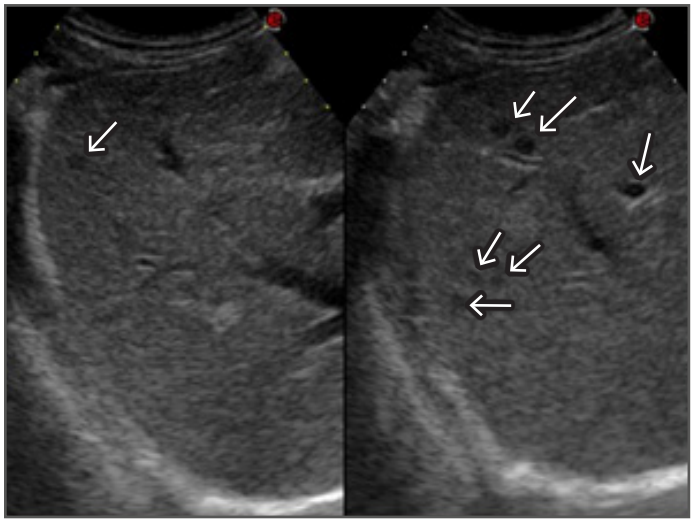

Figure 2: CEUS scan of the right liver lobe before the first session of LTA showing seven small hypoechoic liver metastases (arrows).

CEUS: contrast-enhanced ultrasound; LTA: laser thermal ablation.

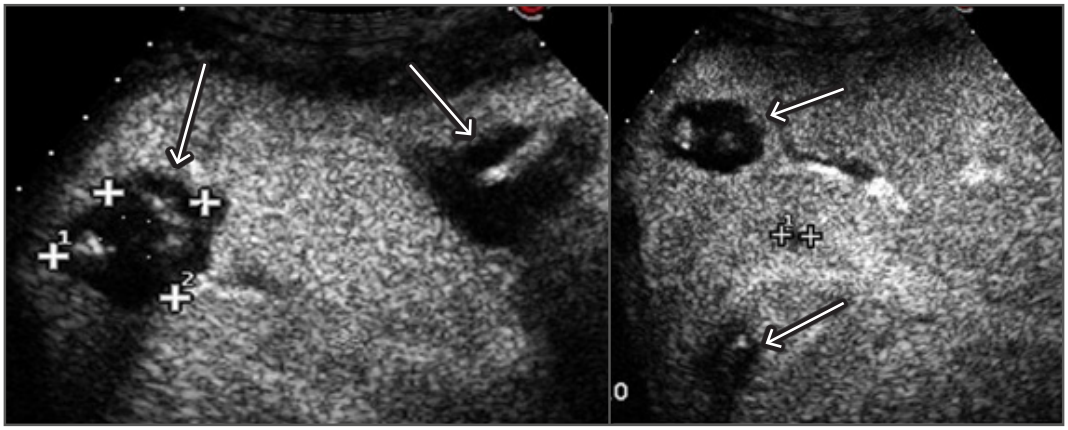

Figure 3: CEUS scan of the right liver lobe after the first session of LTA showing four ablation areas completely covering six metastases. One metastasis was still viable and was treated in the following LTA session (cross-shaped markers).

CEUS: contrast-enhanced ultrasound; LTA: laser thermal ablation.

The laser machine was set at a power of 5 W, and 1,800 J per fibre were delivered in 6 minutes. One, two, or three laser fibres were used for nodules ≤7 mm, 7–12 mm, and 12–14 mm in diameter, respectively; the pull-back technique was used when the anteroposterior diameter of LM exceeded 10 mm.

In total, 22 LM were ablated over three sessions. Primary technical success and 1-month technique efficacy, defined according to the standardisation of terminology and reporting criteria for image-guided tumour ablation,[9] were 100%. In brief, technical success addressed whether the tumours were treated according to the protocol and were covered completely by the ablation zone and was assessed by contrast-enhanced US (CEUS) performed immediately after the end of each treatment session (Figure 2 and 3); 1-month technique efficacy referred to complete ablation of the tumours and was assessed by CECT or CEUS performed 1 month after the end of the third planned session of LTA. The patient was then followed up every 3 months with alternating CECT and CEUS and remained free from disease until October 2013, when five new LM ranging from 5–12 mm were detected. A further LTA session was performed with primary technical success and 1-month technique efficacy of 100%. At the time of writing (30th November 2017) the patient is still alive and disease-free, tumoural hormonal secretion is normalised, and she is being supplemented with insulin because of the previous complete pancreatectomy.

DISCUSSION

Systemic medical therapy is marginally effective in NET, and the reported 5-year survival of patients with LM from NET treated medically ranges from 0–30%.[10] In contrast, aggressive cytoreduction using combined multimodal treatments has been reported to achieve 5-year survival rates ranging from 48–83%.[1,2] Surgical resection is considered the aggressive approach of choice, but it is only recommended when at least 90% of the tumour burden can be removed, and only 10–20% of patients with NET are suitable candidates for resection because the disease is too extensive.[11] Moreover, the 5 and 10-year recurrence rates after resection are 84% and 94%, respectively, with a median time to recurrence of 21 months.[1] Consequently, the need to frequently retreat patients makes less invasive methods of aggressive cytoreduction a quite interesting option, either as a primary therapy or in a multimodal approach, or as an adjunct to hepatic resection.[12] Image-guided thermal ablation offers the possibility of a minimally invasive technique that is usually associated with less morbidity than resection, decreases tumour volume, preserves most of the normal liver, and can be repeated several

times. Among the thermal techniques that can destroy neoplastic nodules by heating the tissue, LTA offers some advantages in clinical settings. The diameter of the needles is considerably smaller than RFA electrodes or microwave ablation antennas, making LTA safer and more suitable for ablating tumours in at-risk locations or in locations that are difficult to reach.[6-8] Moreover, the use of a multisource device that allows the placement of one to four fibres at once enables tailoring of the thermal lesion size to the nodule size, which allows an acceptable safety margin in tumours ranging from 5–6 mm to 3–4 cm in diameter, contemporaneously sparing the normal parenchyma as far as possible.

LM from NET are often multiple, small, variable in size, and very slow growing. Therefore, it is possible to treat patients with indolent disease with numerous and small (≤3 cm) LM, even with multiple treatment sessions over a period of years.[13] In our department, LTA, associated or not with other cytoreductive treatments, such as surgical resection and transarterial chemoembolisation, has become the ablation technique of choice in this subtype of selected patients because of its unique qualities.[14]

Some authors obtained good results adopting a very aggressive approach to LM from NET. In particular, Elias et al.[15] reported a 3-year survival rate of 84% in 16 patients with a median of 23 LM per patient who were treated with surgical resection combined with intraoperative RFA. In four cases this was preceded by preoperative selective portal vein embolisation; however, approximately 60% of the LM was surgically excised, and 40% underwent intraoperative RFA. In our patient, a higher number of LM were successfully treated using only percutaneous US-guided thermal ablation. Twenty-two LM were ablated over three sessions in 2011, and a further five LM were ablated in 2013; at present, the patient is still alive and disease free 82 months after the first ablation session and 128 months after the diagnosis of pancreatic insulin-secreting NET with multiple LM.

A single case is little more than an anecdote and does not allow any conclusions to be drawn, but this case report suggests that a very aggressive approach can yield very effective results in well-selected patients. Many physicians think that aggressive cytoreduction should be reserved for patients presenting with only a few LM,[13,16] but in our opinion the indication for multimodal treatment, including surgery, transarterial chemoembolisation, and thermal ablation, should be expanded in the subgroup of patients exhibiting a low natural tumour burden slope and good general status. In these patients, when thermal ablation is planned to treat a high number of LM, maximum sparing of normal parenchyma is critical, and ablation must be as limited as possible in volume and optimally adapted to the size of the LM to reduce the risk of liver failure. To this aim, due to it enabling the use of one to four fibres according to the size of the LM, in our opinion LTA should be considered the ablation technique of choice.

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Macrophages in Nonalcoholic Steatohepatitis: Friend or Foe?

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Abstract

Nonalcoholic steatohepatitis (NASH) is a subtype of nonalcoholic fatty liver disease that is characterised by steatosis, chronic inflammation, and hepatocellular injury with or without fibrosis. The role and activation of macrophages in the pathogenesis of NASH is complex and is being studied for possible therapeutic options to help the millions of people diagnosed with the disease. The purpose of this review is to discuss the pathogenesis of NASH through the activation and role of Kupffer cells and other macrophages in causing inflammation and progression of NASH. Furthermore, this review aims to outline some of the current therapeutic options targeting the pathogenesis of NASH.

INTRODUCTION

Nonalcoholic steatohepatitis (NASH), a subtype of nonalcoholic fatty liver disease (NAFLD), is one of the most prevalent ongoing liver diseases seen across the world. NAFLD is histologically divided into two types: nonalcoholic fatty liver and NASH. There is an estimated 20–40% prevalence of NAFLD worldwide and approximately 10–20% of those affected progress to the subtype NASH.[1] NASH is histologically characterised by the accumulation of dense lipid deposits in hepatocytes, causing inflammation and hepatic cell injury. This injury leads to hepatic fibrosis, the chief cause of hepatic and extrahepatic complications.

The progression to NASH from its less severe form of NAFLD can be predicted by the amount of inflammation present in hepatic tissue.[2] Severe inflammation can contribute to the progression of other liver diseases, such as cirrhosis, fibrosis, and hepatocellular carcinoma.

The cells' first line of defence against hepatic cell injury is the activation of macrophages. The increased activation of inflammatory macrophages produces inflammatory cytokines, which determine the progress of NASH. In this review, we aim to understand the role of the resident and infiltrating macrophages present in NASH. Additionally, we intend to summarise potential mechanisms and future therapeutic options that aim to reduce the burden of macrophages in NASH.

KUPFFER CELLS

Abundancy

The liver has an abundance of macrophages that spur the development of NASH by means of extensive inflammatory pathways resulting from activated macrophages. It is estimated that for every 100 hepatocytes, there are an additional 20–40 macrophages supplementing the hepatocytes.[3] The majority of macrophages present in liver tissue are the self-renewing, resident phagocytic Kupffer cells (KC). These are split into M1 and M2 subsets that, in a healthy liver, balance each other's functions.

Topology

KC reside in liver sinusoids, the portal tract, and hepatic lymph nodes at the crossroads of capillary-level confluence of the portal vein and hepatic artery tributaries. At that junction they are in an environment that contains various inflammatory agents as a result of hepatic circulation. KC distribution within the liver acinus is correlated with the acinar concentration gradient of immune reactive substrates and other regulatory factors.

Functionality

The main function of KC is to detect and destroy pathogens, cell debris, and bacterial-derived products in the hepatic circulation by phagocytosis, preventing the general circulation of such pathogens. In healthy livers, KC fulfill the dual function of clearing these pathogens while keeping a low and balanced level of inflammation. KC use microbe-associated molecular pathways to bind microbes or microbe ligands. KC function via pattern recognition receptors (PRR) that can be divided into two classes: toll-like receptors (TLR) and NOD-like receptors (NLR).[4] These receptors detect danger signals, including pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP), which leads to the activation of inflammatory pathways. KC are then responsible for clearing these microbes via phagocytosis to prevent them from penetrating general circulation.[5] Additionally, KC may be activated by metabolically driven activated signals.[6]

KC clear microbes whilst keeping the hepatic area at an optimally controlled level of inflammation. This protects the rest of the body from an excessive immune response. KC produce and secrete anti-inflammatory signals to respond to lipotoxicity, including interleukin (IL)-10 in response to lipopolysaccharide (LPS).[7] In this manner, a balanced response is produced by KC. Additionally, KC participate in immunosuppression by expressing high amounts of T cell suppression molecules and low levels of costimulatory molecules.[8]

In conclusion, KC maintain homeostasis and govern inflammation in the liver microenvironment.

Subtypes

There are two main subsets of inflammatory macrophages that are separated based upon their terminal differentiation stage: the proinflammatory M1 and immunoregulatory M2 macrophages. These macrophages perform multiple functions, such as cytokine and chemokine secretion, leukocyte adhesion, phagocytosis, and cellular crosstalk. Later studies[9,10] have shown that the M2 type expands to include many other macrophages with vast differences in their biochemistry and physiology, leading to a classification system based on a full spectrum. M2 macrophages have been subdivided into M2a, M2b, and M2c, each with different regulators, marker proteins, and special functional activity.[9] M1 macrophages are characterised by expression of high levels of proinflammatory cytokines, reactive intermediates, and promotion of a T helper cell (Th)1 response. M2 macrophages exhibit phagocytic activity, tissue remodelling, and tumour progression. A more recent study[10] has shown that the balance of M1 and M2 macrophages regulates inflammation in the liver and is the underlying factor in NASH when the levels of each macrophage subtype are unbalanced (Figure 1).

Activation of M2 Type

M2 macrophages are primarily responsible for wound healing and exhibit anti-inflammatory properties. M2 macrophages are induced and activated by IL-4, IL-10, IL-13, IL-33, tumour growth factor (TGF)-α, TGF-β, peroxisome proliferator-activated receptors (PPAR)-γ, and possibly PPAR-δ.[11-13]

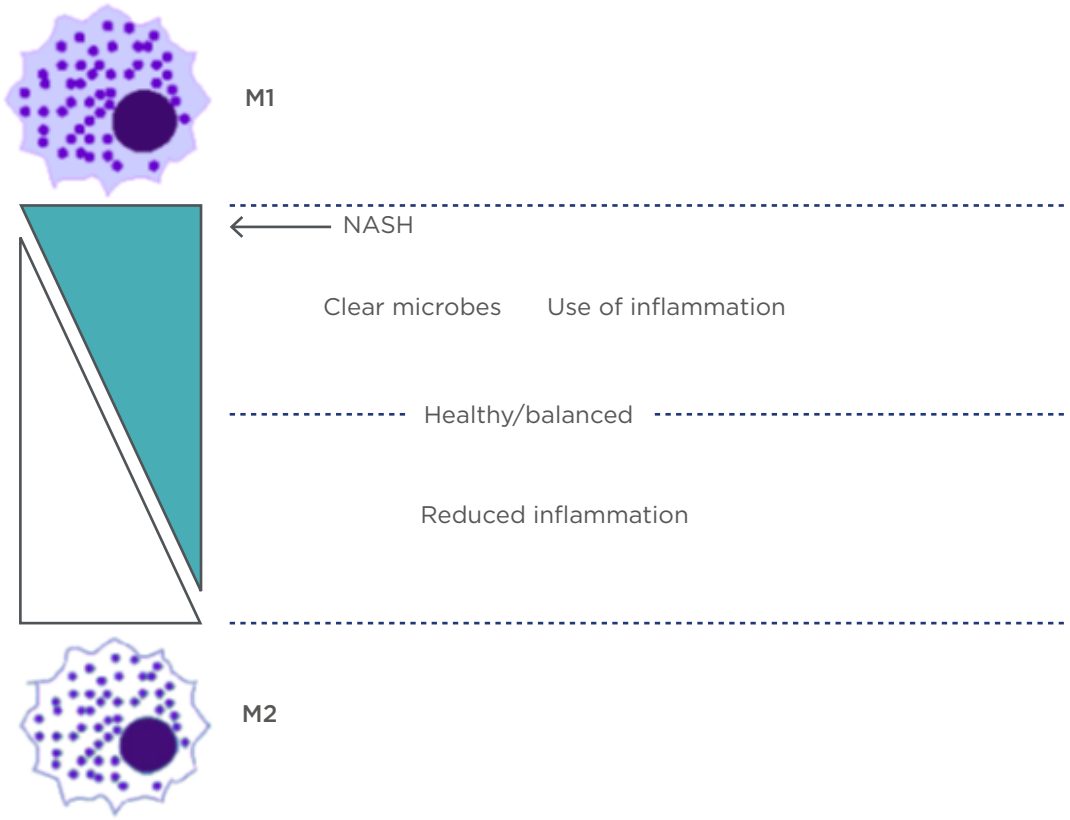

Figure 1: The balance of M1 and M2 macrophage subtypes.

Kupffer cells are in balance between M1 and M2 subtypes to control inflammation. In NASH, the key marker is an imbalance of M1 causing excess inflammation.

NASH: nonalcoholic steatohepatitis.

IL-4 has been shown to promote the expansion of M2 macrophages by initiating Th2 differentiation and downregulating production of proinflammatory chemotactic factors.[14,15] IL-4 is also an inducer of endogenous PPAR-γ ligands.[13] IL-10 downregulates Th1 cytokine expression and suppresses antigen presentation. IL-13 induces secretion of TGF-β and allows for alternative macrophage activation, allergic inflammation, and immunoglobulin (Ig)E secretion.

PPAR-γ primarily controls the expression of gene networks involved in adipogenesis, lipid metabolism, inflammation, and the maintenance of metabolic homeostasis. Activation of PPAR-γ inhibits inflammatory gene expression by preventing the inflammatory signal-specific removal of the corepressor complex.[16] In addition, studies have shown that PPAR-δ regulates an anti-inflammatory switch that proceeds through a ligand activation and genetic receptor depletion.[17] Although PPAR-δ has been shown to promote mouse M2 macrophages, it is still not completely known whether PPAR-δ signals and functions in activation of human M2 macrophages.[18] Studies on human M2 macrophages were performed on atherosclerotic lesions and may give different results in NASH-derived hepatic cells.

M2 macrophages produce anti-inflammatory and profibrotic cytokines. In response to IL-4 and IL-13, M2 macrophages promote Th2 responses.[9] Additionally, M2 macrophages express high levels of arginase, which promotes anti-inflammatory responses and increases mannose receptor expression (Figure 2).[19]

Activation of M1 Type

In NASH, KC are the first macrophages to be activated and thus are of critical importance in the progression of NAFLD.[19] The classically activated macrophages, the M1 type, promote inflammation.

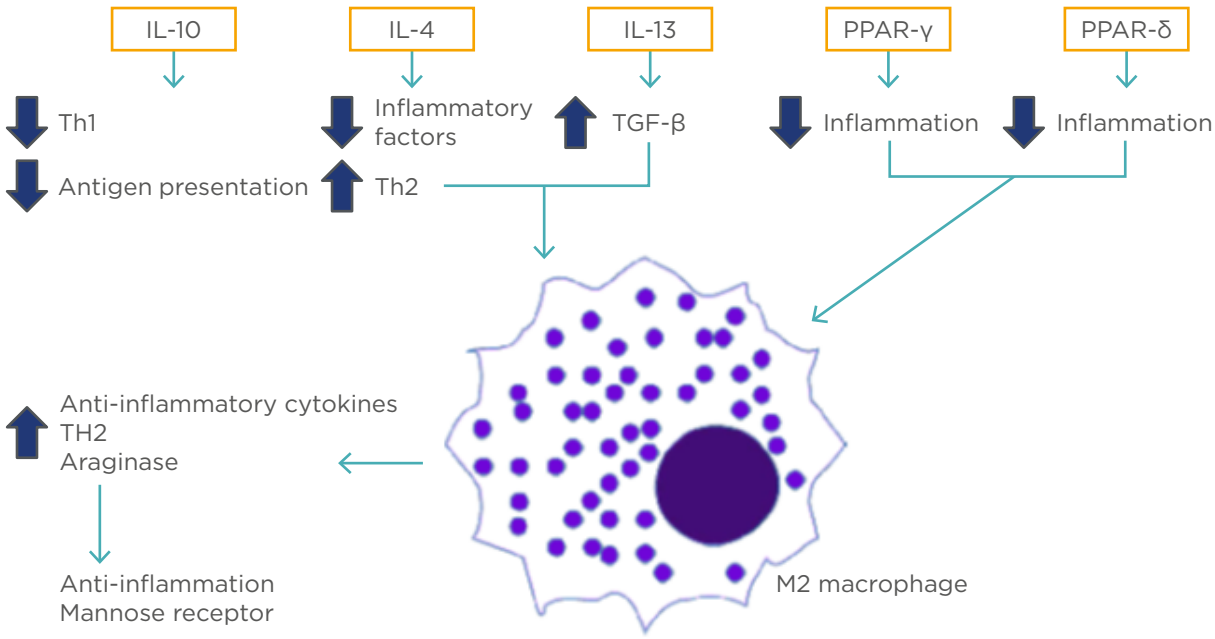

Figure 2: Activation and promotion of M2 type macrophages.

The activation of M2 macrophages is caused by cell signals, including IL-10, IL-4, IL-13, PPAR-γ, and perhaps PPAR-δ. These lead to the reduction of inflammatory factors, downregulation of Th1 cytokine expression, suppression of antigen presentation, and alternative macrophage activation, which ultimately leads to the production of anti-inflammatory cytokines and profibrotic cytokines.

IL: interleukin; PPAR: peroxisome proliferator-activated receptors; TGF: tumour growth factor; Th: T helper cell.

They are induced by proinflammatory signals such as interferon (IFN)-γ, tumour necrosis factor (TNF), and LPS that are present in high fat diets.[18] In NAFLD, LPS levels are raised in portal circulation due to dietary factors. High fructose diets regress NAFLD due to the increase of bacterial levels and intestinal permeability. Disruption of the liver mucosal barrier will allow PAMP to bind to PRR and activate immune cells. Depletion of KC have been shown to protect against the development of steatosis.[20]

Microvesicles released by fat-laden cells undergoing lipotoxicity contribute to the activation of M1 macrophages. It has been suggested that these microvesicles can activate the NLRP3 inflammasome following internalisation by macrophages.[21] In recognising inflammatory substances, KC utilise PRR, including TLR. The various TLR allow for recognition of different microbial products. TLR2 recognises peptidoglycan and results in the release of proinflammatory cytokines,[22] TLR4 recognises LPS, and TLR9 recognises foreign nucleic acids.[4,23] Additionally, toxic lipids stimulate TLR to respond to LPS.[20]

LPS has been shown to activate KC by binding to TLR4 and its associated protein, CD14, as well as myeloid differentiation-2 molecule, thereby activating a cascade of inflammatory signalling pathways.[24] Free fatty acids have also been shown to act on TLR4 via a supplemental ligand.[25] TLR4 deprived mice exhibited less severe hepatic injury and less hepatic lipid accumulation, thus placing TLR4 as an essential mediator in inflammation processes and NASH.[26] Studies in which TLR9-deficient mice were fed a choline-deficient amino acid-defined diet showed less severe hepatic injury and those studies have linked TLR9 signalling to inflammasome activation.[27]

PROGRESSION OF NONALCOHOLIC STEATOHEPATITIS

The hallmark of NAFLD is an excess of fatty acids and lipids in the liver that results in lipotoxicity and hepatocyte injury that initiates inflammation.[28] The immune system attempts to recover from this inflammation through the release of cytokines from KC;

thus, unintentionally furthering inflammation by activating other pathways, leading to steatosis and NASH. Recently, it has been demonstrated that a true lipid signature of NASH exists and is seen spreading into the hepatic parenchyma of selectively accumulated fatty acids.[29] This is caused by a change in the metabolic pathway involved in the synthesis of long-chain fatty acids and very long-chain fatty acids.[29]

Previously, a 'two-hit model' was proposed as the pathogenesis mechanism of NASH. The metabolic syndrome involving triglyceride accumulation is the first hit, and the second hit is defined as the progression to liver inflammation, oxidation, and progression to steatohepatitis via KC.[30] More recently, studies have shown that inflammatory mediators, based on the activation of KC and the release of cytokines, play a central role in the cascade of inflammation and liver injury, and that the inflammation may precede the development of steatosis.[31] The amount of data implicating gut microbiota and genetic factors has led to the development of the 'multiple parallel hits model' that accounts for the observed cases of NASH, even in lean subjects.[32]

In addition, hepatocyte cell death is a key process in the pathogenesis of NASH.[28] Death receptors have been shown to mediate inflammatory signalling.[32] Cells undergoing necrosis and apoptosis release DAMP, which further induces inflammation by activating inflammasomes such as NLR proteins (NLRP). The DAMP associated with M1 macrophages are high motility group box 1, heat shock proteins, breakdown products of extracellular matrix, and nonprotein substrates.[33] The activation of NLRP can cause the assembly of the inflammasome, which contains caspase-1, causing further inflammation and cell death by cleaving prointerleukins into their interleukin form.[34] These inflammasomes are important in the progression to NASH because this cycle can lead to a full inflammatory response, which can result in fibrosis and cirrhosis.

Apoptosis is upregulated in hepatic cells as a result of harmful diets. Hepatic saturated fatty acids found in adipose tissue can be released from lipid droplets via macrolipophagy. In high areas of saturated fatty acids, liver injury occurs by multiple mechanisms. Lipids cause lipotoxicity and lipotoxic stress in the endoplasmic reticulum and mitochondria, thereby causing apoptosis to occur.[35] IRE1, PERk, and ATF6 converge at the C/EBP homologous protein to join with c-Jun to upregulate p53, a modulator of apoptosis, and express B cell lymphoma 2-associated X protein, which results in the release of cytochrome c.[35,36] IRE1 also activates apoptosis signal regulating kinase 1 and c-Jun N terminal kinase (JNK) to form the c-Jun/C/EBP homologous protein complex, further promoting apoptosis and hepatic damage.[37]

Studies have shown that cholesterol crystals are present in the livers of human NASH and murine NASH models.[38] KC can take up cholesterol-rich lipoproteins using scavenger receptors.[39] Lipid droplet-laden KC recruit CD4+ and B lymphocytes.[28] It has been shown that decreasing cholesterol levels causes dissolution of cholesterol crystals and disperse KC structures, helping to resolve NASH.[40] Recently, it has been shown that cholesterol crystals activate NLRP3 in LPS-exposed KC and MCC950 small molecule inhibitor can inhibit the activation of NLRP3.[41] Additionally, current research has shown that HepG2 cells exposed to low-density lipoprotein cholesterol formed cholesterol crystals on the lipid droplet membrane of hepatocytes and activated THP1 macrophage cells that upregulated TNF-α, NLRP3, and IL-1β mRNA (Figure 3).[42]

Neutrophils have exhibited a role in KC activation and NASH progression through the attraction of lymphocytes and the release of myeloperoxidase that increases oxidative stress. In neutrophil-deleted mice, the activation of KC was delayed.[43] Neutrophil elastase is thought to activate TLR2 and TLR4 receptors.[43] Activation of KC and TLR4 has been demonstrated to co-ordinate neutrophil adhesion in liver sinusoids.[44] Neutrophils recruit macrophages using an antigen presenting method. Additionally, neutrophil peptides or α-defensins have the capability to induce fibrosis by recruiting hepatic stellate cell proliferation.[45] Aside from the role of KC in inflammation in NASH, KC also regulate metabolic activities and lipid metabolism of hepatocytes by expressing TNF and IL-1β.[46] In NASH, there is a high uptake of lipids by KC via the secretion of lipases, lipid binding proteins, and bioactive lipids.

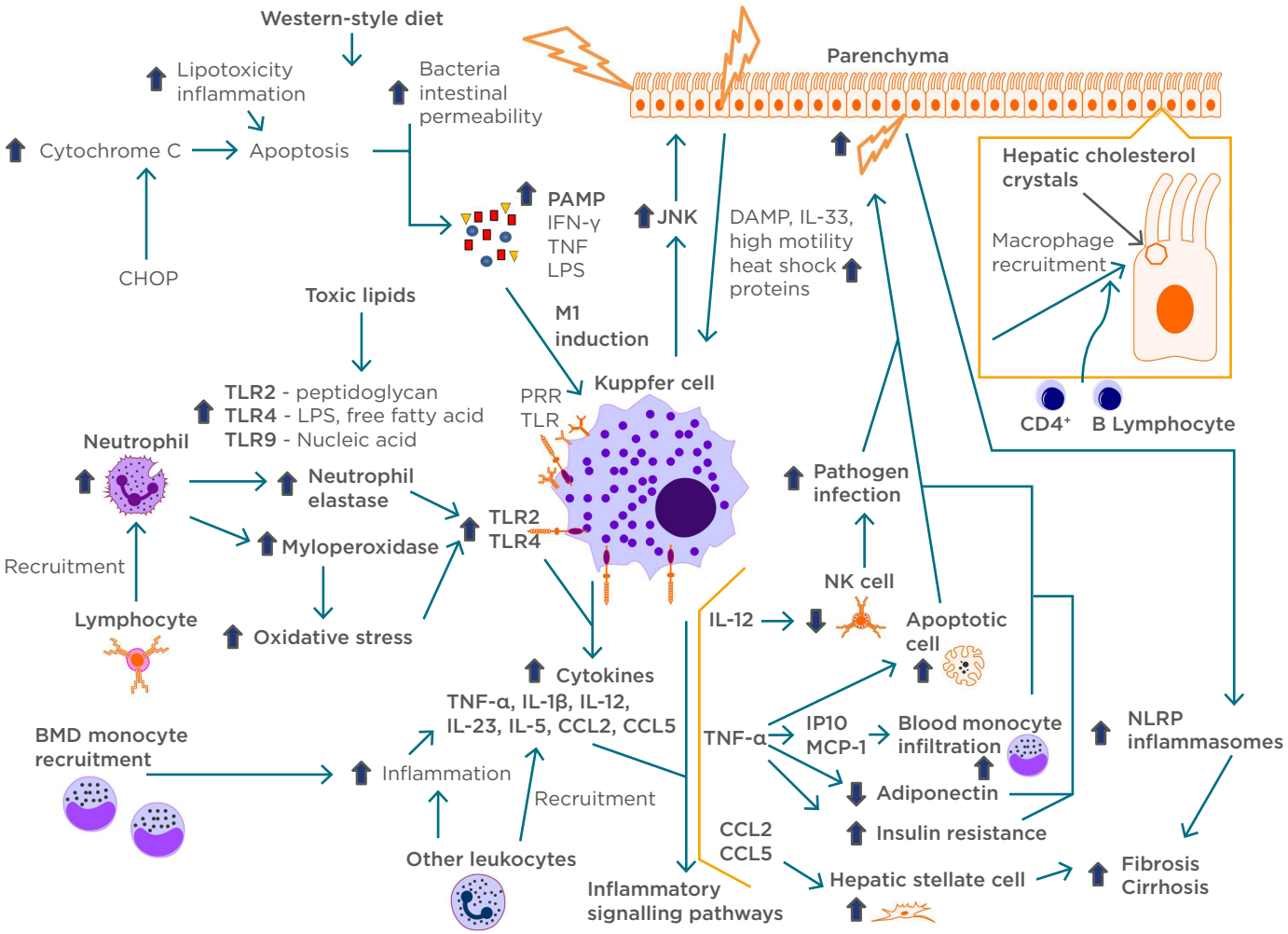

Figure 3: Cycle of inflammation.

A schematic modelling of the pathogenesis of NASH and its inflammatory cycle. Lifestyle factors, such as high-fructose diet and excess saturated fatty acids, lead to lipotoxicity, inflammation, bacterial, and intestinal permeability, causing damage to liver cells. These cells release PAMP, causing the induction of KC into the inflammatory M1 macrophage and recruitment of KC to the damaged sites by JNK. Active scavenging receptors recruit the macrophages with lipid droplets originating from hepatic cholesterol crystals and recruit other lymphocytes. The upregulation of TLR is due to the recruitment of neutrophils by activated lymphocytes and toxic lipids causing an increase in neutrophil elastase, myeloperoxidase, and oxidative stress. In response, KC release cytokines that initiate inflammatory pathways, thereby cascading the area into high inflammation. KC recruit BMD monocytes and cause more inflammation, releasing more cytokines. The inflammation leads to further release of DAMP in the hepatic cells, recruiting more KC. This cycle can eventually lead to fibrosis and cirrhosis.

BMD: bone mineral density; CHOP: C/EBP homologous protein; DAMP: damage-associated molecular patterns; JNK: c-Jun N terminal kinase; IFN: interferon; IL: interleukin; KC: Kupffer cells; LPS: lipopolysaccharide; NASH: nonalcoholic steatohepatitis; NK: natural killer; NLRP: NOD-like receptor protein; PAMP: pattern-associated molecular patterns; TLR: toll-like receptor; TNF: tumour necrosis factor.

KC are activated by CD14, a PRR, when presented with LPS by the mediator TLR4. In cells with a high expression of CD14 there is an increase in sensitivity to LPS.[47] Additionally, KC are activated on NF-κB, MAPK, ERK1, p38, JNK, and IRF3. In NAFLD, there is a high presentation of TLR4 expression resulting in a large release of cytokines, thus contributing to the pathogenesis of liver disease by furthering inflammation and causing fibrogenesis.

In the progression of worsening conditions from NAFLD to NASH, cells may release stress signals. During necrosis, cells release DAMP and chemo-attractants that can recruit various immune cells to the liver, initiating a wound

healing response through fibroinflammatory repair. This can activate a full inflammatory response leading to fibrosis and cirrhosis (Figure 3). The cytokines produced by PRR lead to liver inflammation by the release of several cytokines and chemokines, such as TNF-α, IL-1β, IL-12, IL-23, IL-6, CCL2, and CCL5. The release of these cytokines results in the release of DAMP, promoting additional hepatocyte injury, activating TLR, JNK, and a cycle of vicious inflammation. Cytokines recruit leukocytes to further increase the inflammatory response in the healing process (Figure 3).

The recruitment of Ly-6C^{+} bone marrow derived monocytes via a CCR2-CCL2 recognition event has been shown to be a critical event for the promotion of steatohepatitis and NASH.[48] Other chemokine interactions, such as CCL1–CCR8, CCL5–CCR1/CCR5, and CXC motif chemokine ligand 10 (CXCL10)-CXCR3, have been shown to recruit monocytes as well.[49] These bone marrow derived monocytes can replace the resident KC, promote inflammation, and are identified as Ly-6C^{hi}, CD11b^{hi}, MHC II^{neg}, and CX3CR+.[50] CXCL10 has been shown to enhance inflammation by inducing chemokines and cytokines such as TNF-α and IL-1β.[51] Additionally, chemokine receptor CXCR3, a CXCL10 receptor, mediates inflammatory cytokines and macrophage infiltration (Figure 3).[52]

IL-12 expression leads to the loss of natural killer cells, resulting in a susceptibility to the increase of inflammation due to pathogen infections.[53] CCL2 and CCL5 have overlapping properties that activate hepatic stellate cells leading to fibrosis. Additionally, JNK activation recruits macrophages to the site of hepatic inflammation, thus increasing inflammation and cell death.[54]

TNF-α has been shown to be a key component of NASH by promoting blood monocyte infiltration through the production of IP-10 and MCP-1 cytokines. TNF-α can activate proapoptotic or antiapoptotic signalling cascades, thereby controlling inflammation. TNF-α antagonises adiponectin, an anti-inflammatory adipocytokine, increasing inflammation, and also induces insulin resistance. The increase of TNF-α has been shown to have a crucial role in the development of NASH by promoting this inflammation.[55]

TREATMENTS

Categorisation

On 11th August 2017, there were 218 registered clinical trials under the search term 'NASH' on ClinicalTrials.gov. Macrophage-directed therapies to treat NAFLD and NASH promise to be a worthy intervention strategy. The many different roles and actions in the KC response system allows for different novel therapeutic approaches. These can be categorised into KC activation, KC polarisation, and monocyte recruitment.

Activation of Kupffer Cells

Preventing activation of KC by modulating TLR4 has been shown to ameliorate hepatic inflammation and injury. Transmembrane BAX inhibitor motif-containing 1 promotes the lysosomal degradation of TLR4 to inhibit insulin resistance, inflammation, and hepatic steatosis in mice and monkeys.[56]

M1 and M2 Polarisation

Carotenoids that inhibit lipid peroxidation exhibit antioxidant and anti-inflammatory effects in mice, in addition to regulating M1 and M2 activation, thus suggesting their important value for NASH treatment.[57] Retinoic-acid-related orphan receptor α boosted M2 type in KC by activating Kruppel-like factor-4 and upregulating IL-10 in mice, leading to a reduction in inflammation and protection in NASH.[58]

Preventing the formation of cholesterol with cholesterol-lowering drugs and blocking TLR activation with ethyl pyruvate, phenylmethimazole, or other inhibitors both reduce and antagonise DAMP and PAMP in mice.[40,59] Inhibiting the development of the inflammasome by inhibiting caspase 1, 8, and 9 with GS9450 has been shown in a Phase II trial to be a promising treatment option for patients with NASH.[60]

Blocking the inflammatory signal pathways of KC by inhibiting NF-κB, MAPK, ASK1, ERK1, p38, JNK, and IRF3 can also reduce inflammation in NASH. The ASK1 inhibitor, selonsertib, and the CCR2/CCR5 inhibitor, cenicriviroc, have both been shown to reduce fibrosis in mouse models and early clinical trials.[37,49] Galectin-3 inhibitors have been shown to reduce fibrosis

by inhibiting TGF-β mediated myofibroblast activation in mice.[61] Andrographolide has been shown to inhibit NF-κB and NLRP3 inflammasome experimentally in mice.[62]

Monocyte Recruitment

Inhibiting inflammatory monocyte recruitment to the liver by interfering with the chemokine pathways CCL2–CCR2, CCL1–CCR8, CCL5–CCR1/CCR5, and CXCL10–CXCR3 have been shown to help in clinical trials with the CCR5 antagonist maraviroc and the CXCR4 antagonist plerixafor.[49] Cenicriviroc blocks CCL2 recruitment of monocytes in addition to its antifibrotic effects.[63] Reduction of TNF inflammatory cytokines with venlafaxine-103 has shown to reduce inflammation, steatosis, and cell death in alcoholic liver disease.[64]

Elafibranor (GTF-505), a PPAR α/δ modulator, has been shown in preclinical trials to decrease steatosis, inflammation, and display antifibrotic properties.[65] GFT505 has also been shown to significantly improve steatohepatitis, fibrosis, and inflammation in humans through the regulation of PPAR.[66] Honokiol exhibits an agonistic effect on PPARγ ligand-binding domains alleviating inflammation in mice.[67] It was shown that the regulation of insulin, gluconeogenesis, glycogenolysis, and triglycerides through the targeting of farnesoid X receptor by bile acids provides anti-inflammatory and antifibrotic benefits. Obeticholic acid has been shown to resolve NASH through many pathways, including inhibiting hepatic lipid synthesis and inducing lipid uptake by adipocytes.[68] Aramchol, a synthetic two component lipid molecule, has been shown to reduce hepatic fat levels considerably in animal studies.[69] Additionally, treatment with neutrophil elastase inhibitor ameliorated glucose tolerance and steatosis in mouse models.[70]

Recently, an anti-inflammatory antibody-drug conjugate composed of the synthetic GC dexamethasone linked to an antibody for macrophage receptor CD163 exhibited a reducing effect of cytokines in experimental tests on rats, thus preventing steatohepatitis without apparent serious systemic side effects.[71] With the shift from a two-hit hypothesis of NASH to a multivariable process, the therapeutic target of regulating the hepatic cholesterol metabolism became a key strategy in treating NASH. Regulating the *SREBP2* and *miR-33a* genes with natural antioxidants suppresses triglyceride infiltration and fibrosis in cellular and murine models.[72]

CONCLUSION

It has become clear over the past decades that hepatic macrophages are central to initiating and propagating hepatic inflammation. Targeting these macrophages seems to be a promising therapeutic approach to treating NASH. Overall, there are many therapeutic options being discovered and tested to treat NASH by targeting macrophages. In the USA, >20% of patients with NAFLD progress to NASH; as many as 25 million adults in the USA have some form of NASH. These studies have many implications in the lives of patients with NASH.

There are still challenges that need to be overcome in targeting human liver macrophages. Firstly, although there is substantial similarity between mouse models and humans, there are many differences too. Experimental conditions vary and are not the same as human diseases. Secondly, many of the therapies alleviate fibrosis to some extent, but not fully resolve NASH. Therefore, although the clinical implications of alleviating fibrosis are very beneficial, the need to find a more encompassing treatment remains. Additionally, the cellular and molecular mechanisms of the progression to hepatocellular carcinoma and its significance in patients with NASH is a topic that still has much to be discovered.

There remains many points in the pathways of NASH that can be studied and explored for their therapeutic potentials. Targeting NASH in its early pathogenic stages may be a superior method compared to trying to reverse the damage done at later stages of NASH and cirrhosis.

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