EMJ HEPATOLOGY

European Edition

Vol. 9.1 August 2021 emjreviews.com

ILC 2021

EDITOR'S PICK

Elevated Glucagon in a Patient with Necrolytic Acral Erythema: A Case Report and Review of the Literature

INTERVIEWS

Interviews with Philip Newsome, Thomas Berg, Stephen Ryder, and Kieron Lim

Contents

+ INTERVIEWS

+ ARTICLES

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.

EMJ also publishes 16 therapeutic area journals, which provide concise coverage of salient developments at the leading European congresses. These are published annually, approximately 6 weeks after the relevant congress. Further details can be found on our website: www.europeanmedical-journal.com

Editorial Expertise

EMJ is supported by various levels of expertise:

- Guidance from an Editorial Board consisting of leading authorities from a wide variety of disciplines.
- Invited contributors are recognised authorities from their respective fields.
- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
- An experienced team of editors and technical editors.

Peer Review

On submission, all articles are assessed by the editorial team to determine their suitability for the journal and appropriateness for peer review.

Editorial staff, following consultation with either a member of the Editorial Board or the author(s) if necessary, identify three appropriate reviewers, who are selected based on their specialist knowledge in the relevant area.

All peer review is double blind.

Following review, papers are either accepted without modification, returned to the author(s) to incorporate required changes, or rejected.

Editorial staff have final discretion over any proposed amendments.

Submissions

We welcome contributions from professionals, consultants, academics, and industry leaders on relevant and topical subjects.

We seek papers with the most current, interesting, and relevant information in each therapeutic area and accept original research, review articles, case reports, and features.

We are always keen to hear from healthcare professionals wishing to discuss potential submissions, please email: editorial.assistant@emjreviews.com

To submit a paper, use our online submission site: www.editorialmanager.com/e-m-j

Submission details can be found through our website: www.europeanmedical-journal.com/contributors/authors

Reprints

All articles included in EMJ are available as reprints (minimum order 1,000). Please contact hello@europeanmedical-journal.com if you would like to order reprints.

Distribution and Readership

EMJ is distributed through controlled circulation to healthcare professionals in the relevant fields across Europe.

Indexing and Availability

EMJ is indexed on DOAJ, the Royal Society of Medicine, and Google Scholar®; selected articles are indexed in PubMed Central®.

EMJ is available through the websites of our leading partners and collaborating societies.

EMJ journals are all available via our website: www.europeanmedical-journal.com

Open Access

Congress Notice

Staff members attend medical congresses as reporters when required.

This Publication

EMJ Hepatology is published once a year. For subscription details please visit: www.europeanmedical-journal.com

Front cover and contents photograph: Stockholm, Sweden, home of ILC 2021. © scanrail / 123rf.com

EMJ Hepatology 9.1

Welcome

Dear Readers,

Welcome to this year's issue of *EMJ Hepatology*. Here we share recent insights from innovative speakers, peer-reviewed authors, and exclusive interviews with leading experts in this field. *EMJ Hepatology* brings together the latest research and summaries of abstracts presented at the International Liver Congress (ILC), alongside interviews with pioneering professors and experts in the field. Due to COVID-19, ILC 2021 took place virtually for another year; however, this did not prevent the global hepatology community sharing in ground-breaking research in liver diseases such as epigenetic silencing and the development of new dialysis devices.

Speakers from this year's congress shared their research on the use of gene-editing scissors, CRISPR-CAS9, to effectively treat primary hyperoxaluria type 1 and other genetic liver diseases. Highlights from the congress cover exciting advancements, from using icosabutate to treat non-alcoholic steatohepatitis to infusion chemotherapy to treat liver cancer. We know that COVID-19 has impacted many therapy areas and that hepatology is not exempt; the impact of the pandemic is discussed in an interesting review that describes the rise of hospitalisation with alcohol-related liver disease during COVID-19.

This year's issue of *EMJ Hepatology* includes peer-reviewed articles that have been carefully selected from a pool of forward-thinking researchers to share with you the most exciting research in this therapy area. Nathwani et al. explore the potential use of rifaximin to treat cirrhosis. Another interesting study by Makar et al. aims to discover the impact of gastrointestinal and liver injury in hospitalised patients with COVID-19. A study by Biland et al. evaluates the most common genotypes of hepatitis C with reference to age and sex in a large cohort of patients.

We are thankful to the inspiring interviewees, Kieron Lim and Stephen Ryder, for sharing their insights on co-ordinating clinical trials, their personal career choices, and the future of hepatology research. Further to this, we were delighted to interview Philip Newsome and Thomas Berg, who helped shape ILC 2021.

Lastly, I would like to thank the Editorial Board, authors, and you, the readers, for your loyalty, as we continue to be the go-to place for healthcare professionals. We hope you enjoy reading this latest issue of *EMJ Hepatology*.

Spencer Gore

Chief Executive Officer, EMG-Health

Foreword

Dear Readers,

I take great pleasure in welcoming you to *EMJ Hepatology*, presenting the latest developments in the world of liver disease management. Despite the challenges presented by the COVID-19 pandemic, this issue continues to meet the standard set by previous publications, featuring a summary of the 56th International Liver Congress (ILC), high-quality peer-reviewed articles, and insightful interviews.

Hosted in Geneva, Switzerland, in June, the virtual format of the ILC 2021 delivered superb dissemination of information in the field of hepatology. It was an honour to participate and share in the sessions. The postgraduate course was particularly innovative this year. It was split into two main themes: the impact of lifestyle on liver disease, and the potential role of powerful -omics in enhancing our understanding of the mechanisms of liver disease pathophysiology; something which will hopefully result in development of truly personalised medicine for individuals with complex conditions such as non-alcoholic liver disease. This issue also features a summary of a stand-out session on genomic editing to treat hypercholesterolaemia and primary hyperoxaluria.

The congress review in this issue shares a selection of interesting abstract presentations delivered at the congress, summarised by the authors themselves. These include topics such as the use of microRNA sequencing in cholangiocarcinoma-affected and non-malignant livers to identify their roles in gene regulation and promotion of carcinogenesis. The issue also includes interviews conducted with the Secretary General and Vice-Secretary General of the European Association for the Study of Liver (EASL).

My Editor's Pick is the manuscript by Koblinski et al. 'Elevated Glucagon in a Patient with Necrolytic Acral Erythema: A Case Report and Review of the Literature,' which presents a review of the existing literature relating to necrolytic acral erythema, a relatively novel dermatologic disease associated with hepatitis C virus, and an associated case report. The authors highlight how necrolytic acral erythema is often misdiagnosed as cellulitis, leading to ineffective treatment and increased morbidity. It is fascinating to me that despite many years of diagnosing and treating hepatitis C virus, we are still learning about its extrahepatic manifestations.

I hope you find this latest eJournal informative and engaging, and that you enjoy reading the clinical research presented as well as the insights provided by international experts in the field.

Ahmed Elsharkawy

Consultant Hepatologist, University Hospitals Birmingham, UK

Congress Review

Review of International Liver Congress 2021

Location: ILC 2021

Date: 23rd–26th June 2021

Citation: EMJ Hepatol. 2021;9[1]:10-22. Congress Review.

PICTURESQUE Geneva, Switzerland, encircling Lac Léman with the famous Jet d'Eau fountain and overlooked by the Alps, would have been a vibrant setting to host the 56th annual International Liver Congress (ILC). Accustomed to meetings of this nature, flaunting three officially spoken languages, and usually a bustling economic hotspot, the Swiss financial centre is home to the highest number of international organisations in the world, including headquarters for Europe's United Nations and the Red Cross. Home to the CERN laboratory for nuclear research, containing the world's largest atom smasher, the city is familiar with revolutionary science. This drew attention to Geneva in 2014 when the monumental creation of antimatter and discovery of the elusive Higgs boson particle took place. Hoping to share in the metropolis' scientific legacy, attendees of ILC 2021 felt the underlying ethos of 'beating liver disease together' entwined in all the discussions that took place.

Woven together by the European Association for the Study of Liver (EASL), creating only their second fully virtual gathering in hepatology, this innovative online experience successfully overcame the digital barriers enforced by the COVID-19 pandemic to deliver a plethora of informative sessions, headed by EASL Secretary General Philip Newsome. He summarised the event's reach at close of congress: "I hope you have enjoyed our almost 150 sessions with over 500 presentations," also paying tribute to the diverse contributions made by young investigators, nurses, patients, and allied healthcare professionals. The success of the congress was illuminated in that over the 4 days, the ILC welcomed nearly 6,500 delegates from >100 countries, hosting 400 international faculties and close to 900 abstract presenters.

Content at ILC 2021 spanned several therapy areas; within broader hepatology fields, topics of focus included viral hepatitis, liver tumours, cirrhosis,

"The world of science and healthcare has been incredibly adaptable through an intensely digital time; we have missed the pure energy and dynamism of meeting in person."

immune-mediated and cholestatic disease, and finally metabolism, alcohol, and toxicity. During his farewell, Newsome highlighted the notable roundtable discussions that took place over the course of the congress, examining non-alcoholic fatty liver disease and hepatitis B. Other highlights from the event included the clinical studies conducted as a part of the EASL consortium on vascular liver disease, amidst studies relating to management of hepatitis B and C and liver cancer prevention, and emerging data suggesting we are nearing successful treatment for rare liver diseases like α-1-antitrypsin deficiency.

Conversations remained optimistic despite virtual barriers to discussion, mentioning the impressive 630,000 EUR awarded by EASL as fellowships and grants, some extended to aid research during the COVID-19 pandemic and to support future research leaders in hepatology. Sharing in this assistance are Teresa Brevini and Jan Masek, foundation EASL fellowship awardees, who appeared in a memorable presentation to share their research journeys. Newsome commended this session: "Really inspiring to hear their commitment to liver research, and it gave me reassurance that hepatology is going to be in safe hands as we go forwards."

Thomas Berg was congratulated as the incoming Secretary General during the welcome address and will guide the next phase of this international initiative, where he promises to "build on the decades of ILC tradition and improve it with new knowledge," especially at ILC 2022, due to take place in London, UK, and aiming to safely 'savour science together again'. Shedding light upon the success of another digital ILC, Berg commented: "The world of science and healthcare has been incredibly adaptable through an intensely digital time; we have missed the pure energy and dynamism of meeting in person".

ILC 2021 sparked a great deal of media attention during its course; on Day 1 alone there were >5 million impressions using the twitter hashtag #ILC2021, which would reach 21 million by Day 3. "We have managed to successfully bring together the hepatology community once again," stated Berg at the closing event. The highest engagement with any streamed content was seen in a 'lifestyle and the liver' presentation covering non-alcoholic fatty liver disease, viewed live by >2,000 attendees; this has undoubtedly reached more viewers since joining the hundreds of hours of content available on-demand on the EASL attendance platform.

Reflecting on the successes of EASL in adapting once more to an online format, the concluding remarks from the farewell handover looked ahead to continuing to provide opportunities for high engagement, inspiration, and creativity within hepatology research. Hopefully participants of ILC 2021 will expand on the content shared at this scientific epicentre to improve liver care worldwide, energised by a more successful, immersive online experience than ever before. ■

ILC 2021 REVIEWED →

Chronic Liver Disease, Alcohol Use Disorders, and the Burden of COVID-19 in France

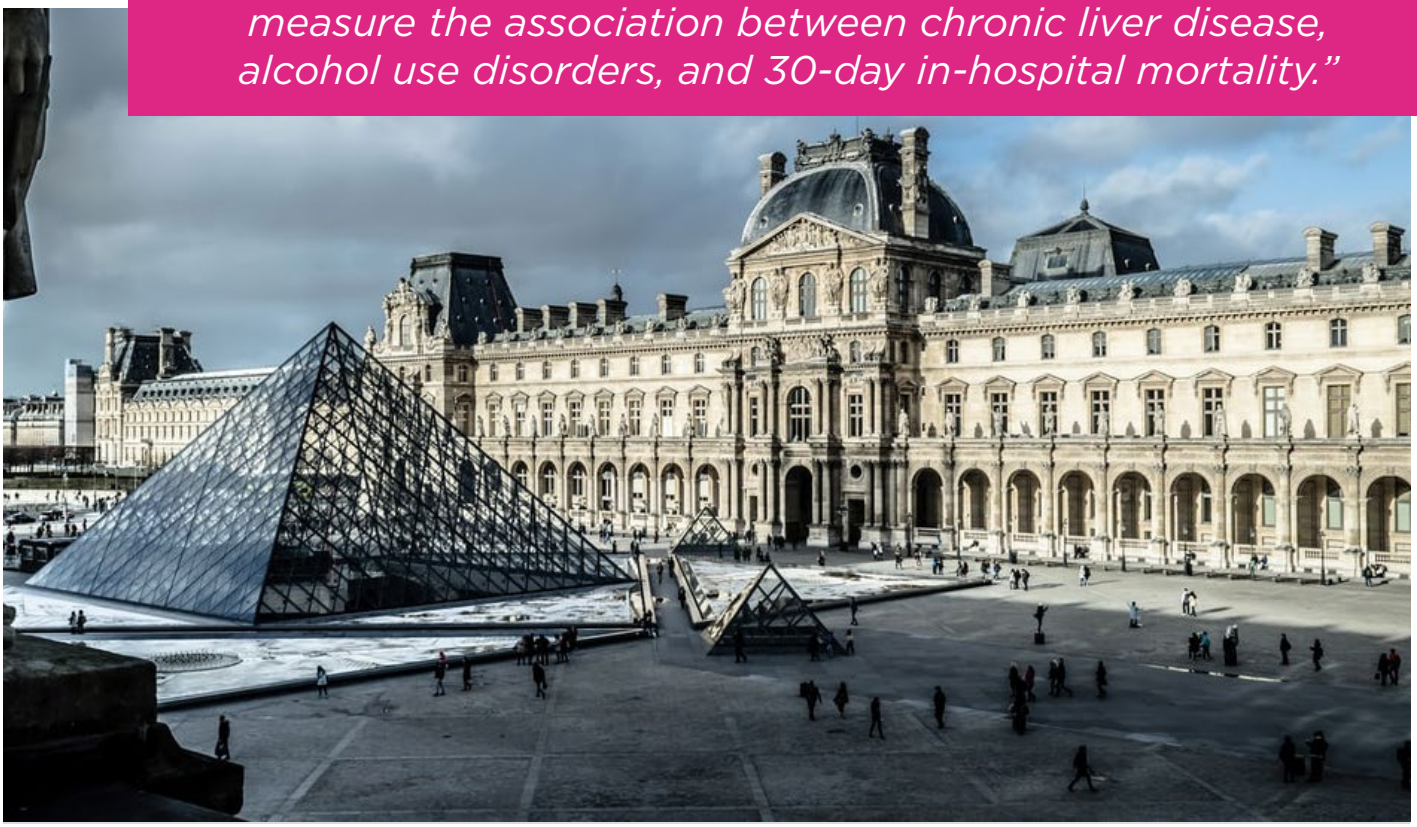
UNTIL recently, there was uncertainty surrounding the risk of death following severe acute respiratory syndrome coronavirus 2 infection in people with chronic liver disease. For this reason, a recent national study in France explored the outcomes of all adult patients (aged ≥18 years) who were discharged in 2020 with a diagnosis code for COVID-19. The researchers calculated adjusted odds ratios to measure the association between chronic liver disease, alcohol use disorders, and 30-day in-hospital mortality. The results of this investigation were presented by Vincent Mallet, Managing Senior Physician and Professor, Hepatology Unit, Cochin University Hospital, Paris, France, at EASL's ILC 2021.

The study used the French National Hospital Discharge database to select individuals (N=187,283) aged ≥18 years who were discharged in 2020 with a diagnosis code for COVID-19. The mean age of the group was 66 years (standard deviation: ±22 years), and 25% were male.

In total, 8.7% (n=16,338) of patients diagnosed with chronic liver disease were admitted for COVID-19 in France in 2020. Of these, 24.1% (n=3,943) died, including 63.9% (n=2,518) after a liver-related complication. The adjusted mortality hazard ratios for chronic liver disease and alcohol use disorders were 1.23 (95% confidence interval: 1.10–1.38; p<0.001) and 1.12 (95% confidence interval: 1.07–1.17; p<0.001), respectively. Finally, Mallet revealed that chronic liver disease increased the odds of in-hospital COVID-19 mortality by approximately 80%.

In summary, chronic liver disease and alcohol use disorders were an independent risk factor of COVID-19 mortality. In addition, Mallet noted that therapeutic effort limitation might have contributed to COVID-19 death of individuals with a liver-related complication or with alcohol use disorders. ■

"The researchers calculated adjusted odds ratios to measure the association between chronic liver disease, alcohol use disorders, and 30-day in-hospital mortality."



Advanced Hepatic Fibrosis Correlating with Weaker COVID Vaccine Response

FEATURING double-vaccinated patients with hepatic fibrosis, researchers have assessed the factors affecting coronavirus vaccine immune response. Rifaat Safadi, who headed the study, presented at ILC 2021, was able to conclude based on their findings that older age and advanced fibrosis with decreased steatosis were risk factors for a lower response to the Pfizer-BioNTech vaccine.

A lot of attention has been drawn to this topical study, as the ongoing COVID-19 pandemic warrants constant study in this field; this is particularly relevant in studies that bring forward evidence for at-risk populations like the present investigation. The research analysed 88 patients living with hepatic fibrosis, all having received both doses of the vaccine, and aimed to assess their immune response. This was achieved using histologic non-alcoholic fatty liver disease activity score (NAS) grading and clinical research network (CRN) fibrosis scoring, both of which presented significant changes.

The mean NAS score was 2.9±1.2 (p=0.045) in the excellent responders, compared with good responders; mainly because of significant steatosis changes of 1.6±0.9 against 1.2±0.7 (p=0.02). Alongside this, hepatocyte ballooning and lobular inflammation were found to be similar; advanced fibrosis scoring was noted in 23% versus 48% (p=0.05, respectively) of each group. Findings revealed that advanced fibrosis correlated with a weaker vaccine response, confirmed by significant changes in blood tests.

"Findings revealed that advanced fibrosis correlated with a weaker vaccine response, confirmed by significant changes in blood tests."

Despite bringing forward relevant and insightful findings, these results would be carried further into the spotlight if the sample size were to be expanded and include a larger and more diverse population. Safadi describes the implications of their research, adding that a third dose vaccine booster in populations with risk factors should be evaluated in future trials of this nature. ■

Infusion Chemotherapy with Sintilimab May Help Cure Liver Cancer

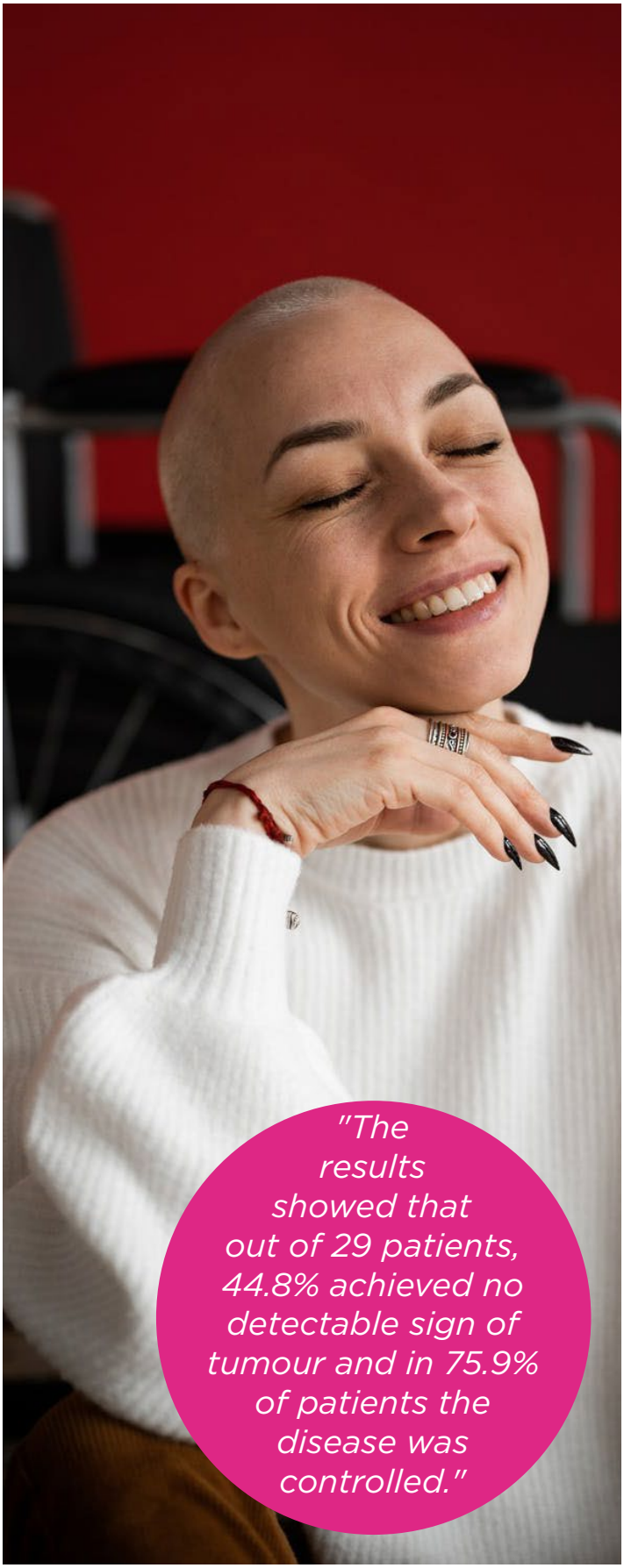
HEPATOCELLULAR carcinoma (HCC) is the most prevalent primary liver cancer in adults. Current treatment includes chemotherapy, surgery, and liver transplantation. When treating cancer, many successful therapies use a combination of drugs as this can help target different parts of the MAP-kinase pathway involved in the development of the cancer.

Li Xu from the Sun Yat-sen University Cancer Centre, Guangzhou, China, tested the safety and efficacy of this approach for resectable HCC by combining hepatic arterial infusion chemotherapy of modified FOLFOX (folinic acid, fluorouracil, and oxaliplatin; FOLFOX-HAIC) with sintilimab, a monoclonal antibody that inhibits programmed cell death, and shared the findings at ILC 2021. The scientists recruited 30 patients who had recently been diagnosed with HCC. The patients were mostly male and aged 34–70 years, with locally advanced disease.

The tumours of studied patients were regularly evaluated every 6–8 weeks, for a median treatment cycle of two years. Patients who had reduction in tumour size and were eligible to undergo surgery were referred for hepatectomy and continued to take sintilimab only.

The results showed that out of 29 patients, 44.8% achieved no detectable sign of tumour and in 75.9% of patients the disease was controlled. Overall, 14 patients studied remain tumour-free now. There were few serious adverse events (AE) in this study; most AEs were of the lowest grade (Grade 1), which included rash, itch, and fever. However, one patient experienced a reversible Grade 4 AE: immune-related liver dysfunction.

Overall, the combination of FOLFOX-HAIC with sintilimab demonstrated beneficial results with a good safety profile and high conversion rate. The researchers suggest that this combined approach to treating HCC could possibly cure this life-threatening liver cancer in the future. ■



Improved Prognosis with New Liver Dialysis Device

ENTERING the liver dialysis device market is the novel DIALIVE machine, addressing demand for effective treatment to combat acute-on-chronic liver failure (ACLF). Current disease-modifying practice options are limited to steroids; however, this holds an inherent risk of new infection, and large proportions of patients are non-responders and may require dialysis for kidney failure, pressors for raising low blood pressure, and support aimed at individual organs. Antibiotics are also used to treat infection, and liver transplant is an option, but is unavailable for many patients.

"DIALIVE treatment acts by replacing dysfunctional albumin as well as removing pathogens and damage-associated molecular patterns."

A study presented at ILC 2021 investigated 32 patients with ACLF with alcoholic cirrhosis, randomised to either receive standard care initiatives (outlined above) or DIALIVE treatment. DIALIVE treatment acts by replacing dysfunctional albumin as well as removing pathogens and damage-associated molecular patterns. Endpoints included safety, device performance, and the clinical and pathophysiologic effects, measured in patients requiring intensive care admission with acute flare-ups of chronic liver disease.

Banwari Agarwal, lead investigator, admitted their small sample size limits the strength of conclusions drawn from the described investigation. However, the study found that patients treated with DIALIVE were significantly more likely to recover from flare-ups and recover to pre-illness levels of liver and wider organ function. The device also exhibited good safety scores, significantly increased the proportion of patients resolving ACLF, and reduced time to resolution.

The study highlighted DIALIVE's simplicity of practice, emphasising ease of assembly, alongside good safety and efficacy scores. Future study is warranted to analyse the method's ability to correct impaired liver function, specifically its safety and usefulness against pathobiological mechanisms of declining albumin and systemic inflammatory response, on a large scale. ■

Food Insecurity, Poverty, and Mortality in NAFLD and Advanced Fibrosis

DISCOVERY of a significant interaction between food insecurity and poverty–income ratio among those with advanced fibrosis has been made in a USA study, shared at ILC 2021. Led by Ani Kardashian, University of Southern California, Los Angeles, California, USA, the researchers followed a large population for a median of 7.2 years, reporting that food insecurity was associated with greater mortality in adults with advanced fibrosis.

The investigation was of longitudinal design, including 34,134 participants and spanning nearly a decade in most cases. A sub-group of 4,816 had non-alcoholic fatty liver disease (NAFLD): 58% were male, with median age 51 years, and 14% below the poverty line. Meanwhile, 1,654 had advanced fibrosis: 55% male, with mean age 69 years, and 15% below the poverty line. Food insecurity was present in 28% and 21% of these groups, respectively. In the NAFLD participants, the all-cause age-adjusted mortality was 12 per 1,000 person-years, and 32 per 1,000 person-years for the advanced fibrosis cohort.

Food insecurity was independently associated with higher all-cause mortality in both sub-groups, (NAFLD: hazard ratio: 1.46, 95% confidence interval: 1.08–1.97; advanced fibrosis: hazard ratio: 1.37, 95% confidence interval: 1.01–1.86), observed in multivariate models adjusted for age, ethnicity, poverty–income ratio, education level, insurance status, HbA1c, BMI, and smoking. Kardashian and colleagues noted a significant interaction between food insecurity and poverty–income ratio in the population with advanced fibrosis and poverty (p=0.015). Based on this, Kardashian stated that, independent of other known causes, food insecurity was associated with greater all-cause mortality in adults with both advanced fibrosis and NAFLD, particularly when affected by poverty, but not among those without poverty.

Future studies to promote a better understanding of the relationship are needed. The sample size and duration are strengths of this study, putting forward strong evidence that intervention is necessary to address the food insecurity among adults with liver disease; in particular, prioritising at-risk categories to target improvement in their health outcomes. ■

"...the researchers followed a large population for a median of 7.2 years, reporting that food insecurity was associated with greater mortality in adults with advanced fibrosis."

Promising Gene Silencing Technique Aimed at Reducing Steatohepatitis

TARGETING the metabolism of hormones, fatty acids, and bile acids, HSD17B13 is a member of the hydroxysteroid dehydrogenase family; if manipulated correctly with ARO-HSD gene therapy, there is opportunity to provide strong protection against alcoholic and non-alcoholic steatohepatitis (NASH). Led by Rohit Loomba, University of California, San Diego, California, USA, this investigation shared at ILC 2021 the results of the first ongoing human clinical study tackling HSD17B13 expression in hepatocytes.

Conducted in healthy volunteers and patients with NASH or suspected NASH (both male and female, aged 19–52), ARO-HSD was administered by subcutaneous injection in a single-dose escalation design; doses of 25, 50, 100, and 200 mg were provided and followed to Day 113. Liver biopsy was conducted at baseline and Day 71, measuring change in hepatic HSD17B13 mRNA expression and protein level; safety assessment was also made in all subjects using laboratory measures of liver function. ARO-HSD was well-tolerated in all participants, without any treatment-related adverse events, drug discontinuations, or associated laboratory abnormalities. Five patients with suspected NASH were administered 100 mg ARO-HSD; they demonstrated reduction in hepatic HSD17B13 mRNA at Day 71 by an average of 84%, and decreased alanine aminotransferase by 46% at Day 85; two of the sub-group showed protein increases of 92% and 97%, respectively.

Lack of significant change to weight or lipid parameters, combined with the study results, helped the researchers to conclude that ARO-HSD is the first investigative RNA interference therapeutic to demonstrate robust inhibition of HSD17B13 mRNA. Advances in this sector, following studies with larger and longer design, will develop the insightful but limited results put forward by this pilot, conducted upon a small population. ■

"... helped the researchers to conclude that ARO-HSD is the first investigative RNA interference therapeutic to demonstrate robust inhibition of HSD17B13 mRNA."

Resmetirom for the Treatment of Patients with Non-alcoholic Steatohepatitis

ON DAY 3 of this year's EASL ILC, 23rd–26th June 2021, Stephen Harrison, Visiting Professor of Hepatology, University of Oxford, UK, presented results from a 52-week, double-blind, placebo-controlled registration trial designed to evaluate the efficacy and safety of resmetirom in >2,000 patients with non-alcoholic steatohepatitis (NASH).

In total, 169 patients were enrolled in the open-label arm: all completed 16 weeks and 64 had completed 52 weeks. The average age was 55.7 years (standard deviation [SD]: ±11.5 years) and 69% of the cohort were female, with a mean BMI of approximately 36. Forty-three percent of the patients had been diagnosed with diabetes, 62% with hypertension, and 70% with dyslipidaemia. In addition, the mean Atherosclerotic Cardiovascular Disease (ASCVD) score was 11.5%. The Fibroscan (7.7 kPa; SD: ±3.6 kPa) and mean MRI-based proton density fat fraction (18%; SD: ±7%) were both consistent with NASH and fibrosis stage F2.

Treatment with resmetirom (100 mg daily) was associated with a statistically significant ($p<0.0001$) 53% reduction in MRI-measured proton density fat fraction, as well as a 62% reduction in sex hormone binding globulin, after 52 weeks of treatment. Harrison also reported a 23% reduction in low-density lipoprotein cholesterol, a 22% reduction in apolipoprotein-B, and a 39% reduction in lipoprotein(a). As above, all reductions were statistically significant compared with baseline measurements. The levels of alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase were found to be significantly reduced (by 22, 12, and 25 IU, respectively; $p<0.0001$). Likewise, significant reductions in several inflammatory and fibrosis markers (e.g., high-sensitivity C-reactive protein, reverse T3, and M30) were observed. Finally, Harrison noted that no safety flags were identified.

Overall, these results support the use of non-invasive tests to monitor the response of individual patients with NASH to resmetirom treatment. ■

"Treatment with resmetirom (100 mg daily) was associated with a statistically significant (p<0.0001) 53% reduction in MRI-measured proton density fat fraction..."

Increase in Alcohol Use Disorder During COVID-19 Pandemic

"Patients with COVID-19 with accompanying AUD had a significantly longer hospital stay and died at a younger age..."

COVID-19 has shocked, isolated, and changed us all in some way or another. The global pandemic took the world by surprise and had people completely change their way of living. This has greatly impacted the mental health of individuals, especially those with underlying mental illnesses or behavioural problems. As a result, some people have turned to various forms of comfort such as alcohol. Hospitals have seen a sharp increase, by two-fold, in patients admitted with alcohol-related liver disease. A study by Subhani and colleagues from Nottingham University, UK, aimed to identify the main characteristics in patients with alcohol use disorder (AUD) and compare the changes in AUD admissions before and during COVID-19.

The study involved two large cohorts of patients from Nottingham University Hospital. The first cohort (n=27,356) included patients admitted pre-COVID-19 between 1st April and 31st October 2019. The second cohort (n=20,598) had patients who had been admitted during COVID-19 between 1st April and 31st October 2020. All patients from this study were given an alcohol assessment (AUDIT-C) to identify patients with AUD and those at high risk. Questions included how often a person drank alcohol, how many units of alcohol they consumed each week, and whether an injury has ever resulted after drinking. The higher the score, the more likely the patient is at risk of alcohol dependency. In this study, high-risk scores included: 5-7, 8-10, and 11-12. The latter score represented individuals that were completely dependent on alcohol. Factors such as age, sex, and ethnicity were considered when scores were derived. Finally, the researchers used multinominal logistic regression analysis to determine if primary and secondary outcomes influenced AUD.

The results showed that 18% of patients in both cohorts had AUD. Interestingly, more patients were alcohol-dependent in the COVID-19 cohort. Patients in the COVID-19 cohort also had a 16-fold increased risk of mental and behavioural disorders. Patients who were in all three alcohol risk groups were significantly younger than low-risk groups ($p<0.05$). Further to this, there was a higher occurrence of AUD in males and individuals of white ethnicity. Patients with COVID-19 with accompanying AUD had a significantly longer hospital stay and died at a younger age ($p<0.05$).

Subhani concluded that during the COVID-19 pandemic there were more patients with alcohol dependency compared to the pre-COVID-19 cohort. Some of these patients displayed mental health issues and behavioural problems. The results suggest that more individuals were drinking excessively during the pandemic and the effects of this were exacerbated in patients with COVID-19 as they died at a younger age. There is no doubt that COVID-19 has had a serious impact on alcohol-related disorders. Next steps such as providing targeted alcohol support services and closely monitoring patients with AUD might help decrease alcohol-related hospitalisations in the future. ■

Engineered Icosabutate Significantly Reduces Markers of NASH and Fibrosis

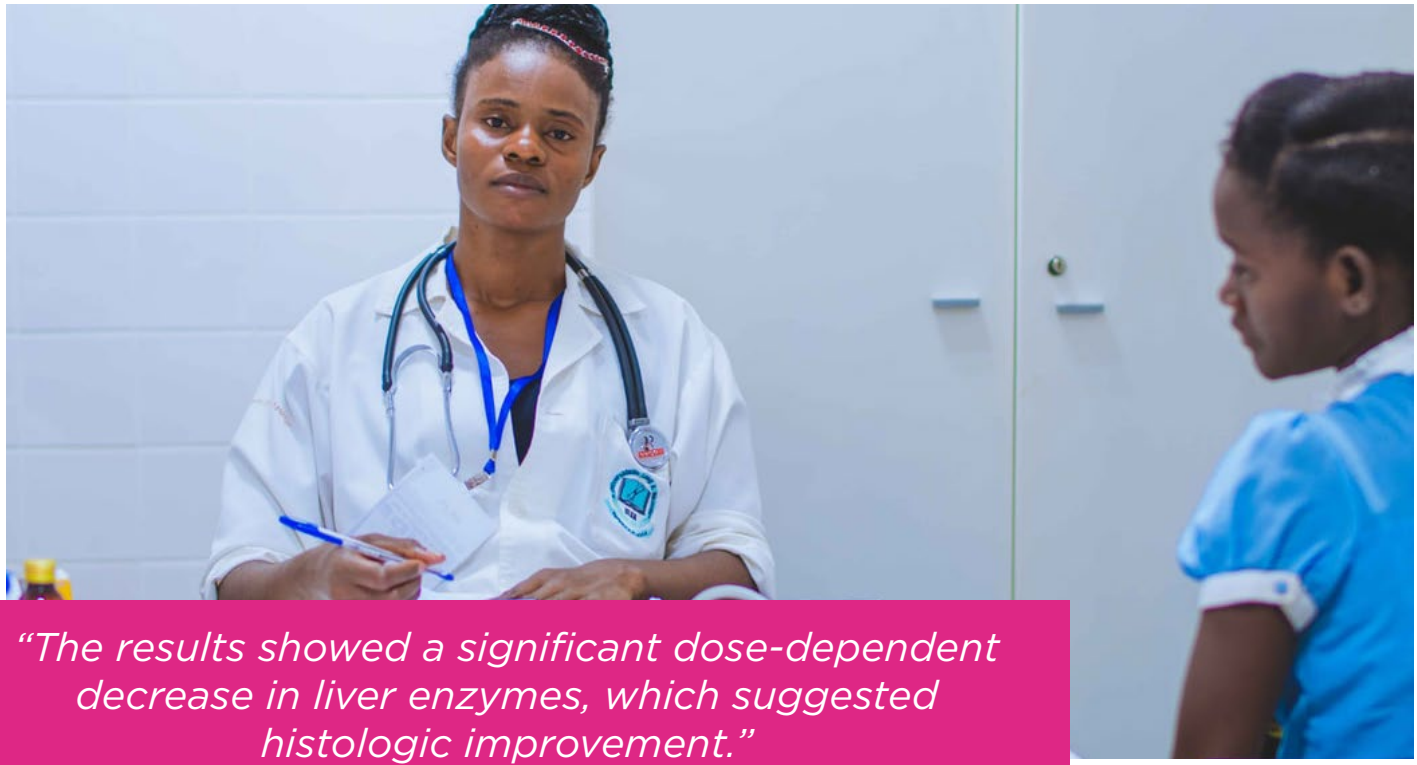
MANY individuals who have fat accumulating in the liver never even realise it. In the initial stages, a fatty liver is not necessarily worrisome. However, if the condition progresses to non-alcoholic liver disease and later non-alcoholic steatohepatitis (NASH), this can lead to serious health complications and, if left untreated, cirrhosis of the liver. Unfortunately, there is no cure for NASH, only non-specific medication and lifestyle changes to help manage symptoms.

An ongoing clinical trial, ICONA, shared at ILC 2021, studied the efficacy and safety profile of a novel oral drug engineered in a lab: a fatty acid called icosabutate. Icosabutate works by targeting the g-coupled protein receptor on the cell surface. The downstream effects of icosabutate result in decreased gene expression of fibrosis and pro-inflammatory genes.

The placebo-controlled trial included a total of 90 patients with confirmed NASH. Patients received either icosabutate 300 mg, icosabutate 600 mg, or a placebo in a randomised 1:1:1 ratio. The biomarkers relevant for NASH, fibrosis, and other closely related conditions were measured. The scientists conducted several plasma blood tests such as PRO-C3. PRO-C3 is a useful indicator of disease progression and the likelihood of fibrogenesis. Other tests conducted included enhanced liver fibrosis (ELF™) and high-sensitivity C-reactive protein to measure improvements in inflammation and fibrosis.

The results showed a significant dose-dependent decrease in liver enzymes, which suggested histologic improvement. The higher dose of icosabutate resulted in a significant decrease in PRO-C3 scores, therefore a lower chance of developing fibrogenesis. The ELF score and hsCRP were also significantly reduced; high-sensitivity C-reactive protein was decreased by 52%.

The tests show that there was an improvement in glycaemic control and key atherogenic lipoproteins, and no serious adverse events or safety concerns. The efficacy of icosabutate has shown some encouraging results, with low scores of PRO-C3 indicating improvement of fibrogenesis and possibly NASH. There were no serious adverse events or safety concerns. ■



"The results showed a significant dose-dependent decrease in liver enzymes, which suggested histologic improvement."

COVID-19 Restrictions Impacting Patients with Cirrhosis and Alcoholic Hepatitis

A COMPREHENSIVE Canadian study of hospital admissions has brought to light interesting information to do with the observed demographics and monthly admission rates over the course of the COVID-19 pandemic. This population study, shared at ILC 2021, identified 2,916 non-alcoholic cirrhosis hospitalisations: 2,318 for alcoholic cirrhosis and 1,408 with alcoholic hepatitis (AH), between 2018 and 2020.

Providing insight into the burden placed upon Canadian health infrastructure, and culminating in April 2020, researchers led by Abdel-Aziz Shaheen of Calgary University, Canada, noted significant increases in average monthly hospital admissions over the study period. Patients with AH admitted after implementation of COVID-19 restrictions had a younger median age of 43 years, compared with a prior median age of 47 years, but no significant difference was found in admission outcomes of the AH cohort. Monthly admissions were stable for both non-alcoholic and alcoholic cirrhosis; however, a 9% increase in AH admissions was observed per month between March and September. Average rate of AH hospitalisation compared to overall hospitalisation doubled from 11.6/10,000 to 22.1/10,000 in the same period.

These findings provoke further questioning to uncover why the average age of hospitalisation fell with the onset of social-distancing restrictions and AH admissions rose independently in the other two categories. These alarming statistics will prove useful to support other studies, but the data are restricted to the localised Albertan population of Canada. Expansion on the selection criteria to include a wider and more diverse population, and similar follow-up investigation conducted in the latter course of the pandemic to current-day, could improve the usefulness of these study insights. ■

"...the average age of hospitalisation fell with the onset of social-distancing restrictions and AH admissions rose independently in the other two categories."

Gene Editing to Treat Hypercholesterolaemia and Primary Hyperoxaluria

Heeral Patel
Editorial Assistant

Citation: EMJ Hepatol. 2021;9[1]:23-26.

THE INTERNATIONAL Liver Congress (ILC) 2021 of the The European Association for the Study of the Liver (EASL) invited three speakers to discuss their research on gene editing. The researchers discussed how to treat hypercholesterolaemia (HC) and primary hyperoxaluria Type 1 using the latest technology in gene editing.

Editing the genome was once believed to be a science-fiction scenario. However, in the past decade, developments in science and technology have led to innovative gene-editing techniques that have made repairing the function of faulty genes a reality. Pioneering scientists are continuously expanding the genetic toolbox, such as last year's Nobel prize winners, Emmanuelle Charpentier and Jennifer Doudna, who were recognised for their ground-breaking discovery: CRISPR-CAS9. These molecular scissors have the ability to accurately edit the genome. The development of CRISPR-CAS9 has now offered the exciting potential of a precise and effective solution for treating many genetic diseases in the future.

ILC 2021 invited three speakers to discuss their research in gene editing to treat liver diseases. The first of the speakers, Angelo Lombardo from HSR-TIGET, Italy, discussed current gene-editing techniques and new approaches such as epigenetic silencing. He explained that there are two main ways to edit genes: gene knock-out and gene insertion. Gene knock-out is the most common technique. This can have toxic effects as cells do not react well to an induced strand break. Therefore, scientists are researching new ways of editing genes; one of these ways is epigenetic silencing.

EPIGENETIC SILENCING TO TREAT HYPERCHOLESTEROLAEMIA

Epigenetic change is a natural process that allows genes to be switched on or off in cells without altering the genetic code. There are a few ways this can be done such as histone modification and DNA methylation. Unlike a gene knock-out, epigenetic changes are mostly transient, and the effects can be reversed.

Lombardo explained how epigenetic silencing can be used in the context of treating liver disease,

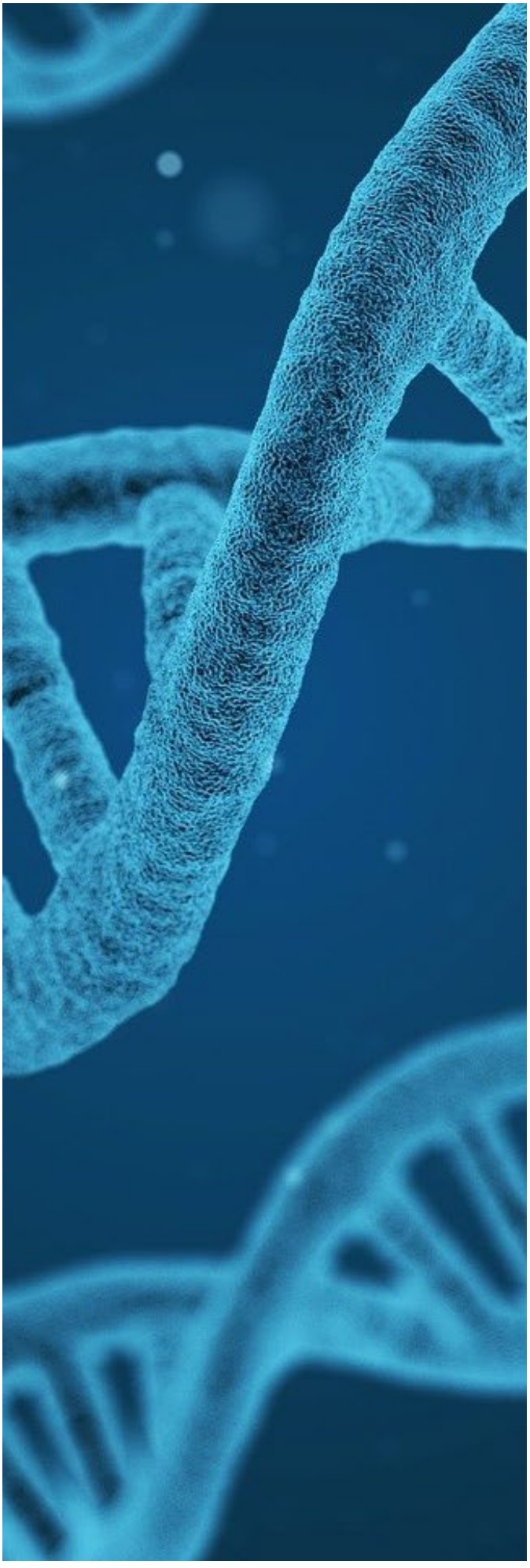
specifically HC. HC is characterised by high levels of low-density lipoprotein (LDL) in the blood. This leads to the formation of plaques in the arterial wall and the development of coronary heart disease. Patients need to adjust their lifestyle and rely on medications such as statins to improve their condition. At present there is no cure for HC, which is why there is a pressing need for novel treatment approaches.

HC can be caused by genetic mutations in the LDL receptor or the *PCSK9* gene, which codes a protein secreted by hepatocytes in the liver. This protein promotes clearance of the LDL receptor and removes the receptor from the cell membrane, thereby preventing the uptake of LDL into the cell. Normally, this is a balanced process; however, with a gain-of-function mutation in the *PCSK9* gene, more cholesterol accumulates in the blood, resulting in HC. On the contrary, loss of function of the *PCSK9* gene has been shown to protect individuals from coronary heart disease.

Ongoing clinical trials involve inhibiting the *PCSK9* gene via several techniques such as use of a monoclonal antibody. Although these trials are producing promising results, a downside to these techniques is that the therapies must be provided regularly, i.e., every 3 months. This signifies the importance of looking at alternative options. Lombardo believes gene editing is something to consider for treating HC as a 'one and done' approach.

Lombardo explained how scientists can target induced local lesions of the *PCSK9* promoter using guide RNA, specifically in the section rich in cytosine and guanines. The engineered transcriptional repressors recognise the promoter and can silence the gene. The data showed that some guide RNAs are very effective at influencing epigenetic silencing to the same degree of efficacy as gene editing. However, epigenetic silencing has the added benefit of being transient and not genotoxic. To determine the specificity of epigenetic silencing, Lombardo conducted RNA sequencing; the results showed that only the *PCSK9* gene is downregulated in gene silencing. Further to this, Lombardo noticed that there was a high level of DNA methylation at the *PCSK9* promoter, showing that epigenetic silencing is highly specific.

"The development of CRISPR-CAS9 has now offered the exciting potential of a precise and effective solution for treating many genetic diseases."



GENE EDITING TO TREAT PRIMARY HYPEROXALURIA TYPE 1

Gloria Gonzalez from the University of Navara, Spain, shared her exciting study in which she and her group used CRISPR-CAS9 to delete glycolate oxidase to treat primary hyperoxaluria Type 1. Hyperoxaluria is a serious metabolic condition that causes recurring kidney and bladder stones and, if left untreated, can lead to end-stage renal disease. Treatment involves a less than ideal liver–kidney transplant. The condition is an autosomal recessive disorder caused by a mutation in the enzyme mainly expressed in the liver: alanine-glyoxylate aminotransferase (AGT).

To understand how a mutation in AGT can lead to hyperoxaluria, Gonzalez explained the molecular biology of this metabolic pathway. In the liver cells, ethylene glycol is converted to glycolate, which in turn is converted to glyoxylate by the enzyme glycolate oxidase (GO). Finally, glyoxylate is converted to glycine by AGT and glycine is expelled through the urine. However, a mutation in the *AGT* gene prevents the final step from occurring and results in a build-up of the precursor molecule, glyoxylate. Glyoxylate cannot be secreted via the urine and is instead converted to oxalate. High levels of oxalate result in the formation of painful kidney stones.

One method to treat this condition is to inhibit GO, as this reduces the production of glyoxylate and consequently oxalate. Inhibiting GO leads to a build-up of glycolate; however, this is harmless as it can easily be removed through the urine. A successful clinical trial showed the efficacy and safety of using small interfering RNA bound to a linker to reversibly silence GO expression in hepatocytes. This inspired Gonzalez and her team to design a system to permanently reduce the expression of the GO protein using CRISPR-CAS9. The group designed two guide molecules to target the murine *hydroxyacid oxidase 1 (Hao1)* gene and used a viral vector to deliver the *Hao1* gene along with the *Staphylococcus aureus* Cas9 enzyme. Gonzalez treated primary hyperoxaluria mice with either a vector carrying guide 1 or a vector carrying guide

2. The team used saline and a Cas9 vector as a control. The mice were sacrificed, and the GO mRNA was analysed using reverse transcription polymerase chain reaction. There was a significant reduction in the expression of GO mRNA in the mice that had been given the guides.

Furthermore, primary hyperoxaluria mice were challenged with ethylene glycol, a molecule that increases oxalate production, for 7 days. The urine of the mice was analysed, and the results showed oxalate was significantly reduced in treated mice compared to wild-type and control mice. More importantly, the scientists discovered that only 1 out of 8 mice developed mild kidney stones, reinforcing the efficacy of mice treated using CRISPR-CAS9.

Finally, Gonzalez and colleagues conducted a molecular analysis of the CRISPR-CAS9 deletion and observed a high editing frequency in both guides. Analysing the pattern of the indel size, the researchers found that each guide had a different effect: Guide 1 resulted in the insertion of 1 nucleotide whereas guide 2 resulted in the deletion of 2 nucleotides. This difference prompted scientists to consider introducing both guides at the same time. The results demonstrated a clear decrease in GO when the two guides were given together with CRISPR-CAS9. Interestingly, at the genome level in >98% of cases, Gonzalez observed a precise deletion of 64 base pairs.

"...there was a high level of DNA methylation at the PCSK9 promoter, showing that epigenetic silencing is highly specific."

LIPID NANOPARTICLES TO DELIVER GENE-EDITING TOOLS

Gonzalez and Lombardo both highlighted how gene editing can be used to treat liver diseases in the future. It is equally important to consider how these genetic tools can be accurately and safely delivered to the correct cell. The final speaker, Roy Van Meel from Eindhoven University of Technology, the Netherlands, discussed recent research in this field and how lipid nanoparticle technology can be used to deliver the genetic cargo. Lipid nanoparticles (LNP) are composed of four molecules: phospholipid, cholesterol, polyethylene glycol (PEG)-lipid, and ionisable cationic lipid. These lipids create a protective barrier around the gene-editing tools. Van Meel explained the mechanism by which LNP is taken up into the hepatocytes. Upon injection, the PEG lipids dissociate, and endogenous apolipoprotein-E is recruited to the LNP. The LNP can then pass through the epithelium and bind to the LDL receptor, which allows uptake of the LNP. Endosomal escape is facilitated by the charged lipids, which release the gene-editing contents into the cell cytoplasm.

A study from 2021 evaluated the efficacy of LNPs in delivering CRISPR-based gene editing in primates. The results showed that LNPs allowed a precise introduction of *PCSK9* loss-of-function mutation, which resulted in a significant decrease in LDL cholesterol; thereby proving that LNPs are an effective delivery mechanism. Van Meel further showed how LNPs are versatile and can be used in all types of liver cells. He presented the results of another study that involved using Cre-mRNA inside LNPs and measured cell fluorescence. The authors showed that by increasing the amount of PEG-lipids, the LNPs reduced in size and the smaller particles passed through the endothelium more easily. However, excessively increasing the pegylation prevented uptake entirely. Van Meel concluded that LNPs are successful at delivering gene-editing tools and have a lot of versatility.

This session presented exciting and fresh ideas for treating liver diseases, such as epigenetic silencing. The research presented by Gonzalez provides hope for treating otherwise incurable primary hyperoxaluria using CRISPR-CAS9. This nicely tied in with the valuable insight from Van Meel into the use of LNPs for delivering gene therapy. One exciting prospect discussed was the possibility of using the LNP approach to deliver CRISPR-CAS9 for the treatment of primary hyperoxaluria. Overall, there is a great deal of potential for using gene editing to treat liver diseases, and scientists can learn from each other to optimise the approach. Hopefully, new therapies will come to fruition very soon. ■

Abstract Reviews

Sharing insights from abstracts presented at the International Liver Congress (ILC) 2021, global hepatologists and researchers have provided these enlightening summaries of their studies.

Prevalence and Prognostic Value of Cirrhotic Cardiomyopathy as Defined According with the Proposed New Classification

Authors: *Maurizio Cesari,[1] Anna Chiara Frigo,[2] Salvatore Piano,[1] Paolo Angeli[1]

1. Unit of Internal Medicine and Hepatology, Department of Medicine, University of Padua, Italy
2. Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Italy

*Correspondence to maurizio.cesari@unipd.it

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors would like to acknowledge Antonella Cecchetto for her precious contribution to the assessment of global longitudinal strain.

Keywords: Cardiomyopathy, cirrhosis, cirrhotic, diastolic dysfunction, echocardiography, strain, systolic function.

Citation: EMJ Hepatol. 2021;9[1]:28-29. Abstract Review No. AR1.

BACKGROUND AND AIMS

The prevalence and prognostic relevance of cirrhotic cardiomyopathy (CCM), as defined according with the new core criteria proposed in 2019[1] is still unknown. The authors investigated this relevant issue in a large cohort of cirrhotic patients in different stages of liver disease.

METHOD

The authors retrospectively interrogated a data set of 162 collected cirrhotic patients followed up for at least 6 years, which at baseline underwent standard Doppler echocardiography and were compared with 46 healthy subjects. Left ventricular (LV) geometry, systolic/diastolic function, global longitudinal strain and the main haemodynamic parameters were assessed according with current guidelines.

Table: Clinical, demographic, and biochemical features of cirrhotic patients with and without cirrhotic cardiomyopathy.

Variable	CCM present (n=24)	CCM absent (n=59)	P	Healthy subjects (n=46)
Age (years)	60±12	56±10	NS	55±10
Sex (Male %)	79	70	NS	48
Alcoholic aetiology (%)	50	43	NS	0
MELD	12±7	12±5	NS	0
Presence of ascites (%)	25	14	NS	0
Death after 6 y FW (%)	38	36	NS	0
Development of HRS, n (%)	1 (4%)	4 (7%)	NS	0
QTc	452±32	453±27	NS	410±21
MAP (mmHg)	93±11	96±13	NS	97±7
Heart rate (bpm)	71±11	69±10	NS	67±10
Creatinine (mmol/L)	89±37	96±80	NS	NA
Aldosterone (ng/dL)	59 (32–86)	66 (48–84)	NS	NA

The data are reported as mean±SD or median (and interquartile range) as appropriate.
HRS: hepatorenal syndrome; MAP: mean arterial pressure: NA, not available; NS: non-significant.

Systolic dysfunction was diagnosed if LV ejection fraction ≤50%, and/or GLS <18% or >22%. Advanced diastolic dysfunction was diagnosed if ≥3 of the following: left atrial volume index >34 mL/m^2, tricuspid regurgitation velocity >2.8 m/sec, early diastolic peak velocity of septal mitral annulus (septal e') velocity <7 cm/sec, early diastolic transmitral and myocardial velocity on tissue Doppler imaging ratio (E/e') >15, as proposed in the new criteria.[1]

RESULTS

Adequate echocardiographic images permitting speckle tracking analysis were available in 83 patients. No patient presented LV ejection fraction ≤50%; GLS <18% or >22% was evident in 25%; advanced diastolic dysfunction was evident in 10%. Overall the prevalence of CCM was 29%. Patients with and without CCM presented similar clinical, biochemical, haemodynamic and echocardiographic features at baseline, and similar incidence of death and/or hepatorenal syndrome after 6 years follow-up (see Table 1).

CONCLUSION

According with the new criteria CCM was detected in 29% of patients, mainly due to altered GLS at rest, but without prognostic relevance and therefore not optimal for the clinical management of cirrhotic patients. ■

References

1. Izzy M et al. Redefining cirrhotic cardiomyopathy for the modern era. 2020;71(1):334-45. Erratum in: Hepatology. 2020;72(3):1161.

Referral of Patients with Non-alcoholic Fatty Liver Disease with Significant Fibrosis from Primary Care to Secondary Care in Belgium

Authors: *Leen JM Heyens,[1-3] Dana Busschots,[1,3] Judith Wellens,[4] Marlies Devos,[5] Luc Present,[6] Birgitte Schoenmakers,[7] Kitty De Munck,[8] Eva Rubens,[8] Katrien Joris,[8] Roy Remmen,[1,9] Anouk Bongaerts,[10] Karen Breure,[10] Frederik Vanstraelen,[10] Valerie Vos,[10] Tom Bijnens,[10] Inge Houben,[11] Sanne Bertels,[11] Lisa Vanbrabant,[11] Yoni Groenendaels,[11] Liesbeth Vernijns,[10] Anneleen Robaeys,[11] Thomas De Somer,[12] Ger Koek,[2,13] Sven Francque,[14,15] Christophe Van Steenkiste,[12,15] Geert Robaeys[1,3,16]

1. Faculty of Life Sciences and Medicine, Hasselt University, Belgium
2. Faculty of Health, Medicine, and Life Sciences, Maastricht University, Netherlands
3. Gastroenterology and Hepatology, Ziekenhuis Oost-Limburg, Genk, Belgium
4. Translational Research in Gastrointestinal Disorders, KU Leuven, Belgium
5. Maatschappelijke Gezondheidszorg en Eerstelijnszorg, KU Leuven, Belgium
6. Huisartsenpraktijk Rendekens, Destelbergen, Belgium
7. Academisch Centrum voor Huisartsgeneeskunde, KU Leuven, Belgium
8. Faculty of Medicine, University of Antwerp, Antwerpen, Belgium
9. Maatschappelijke Gezondheidszorg en Eerstelijnszorg, University of Antwerp, Wilrijk, Belgium
10. Huisartsenbox, Genk, Belgium
11. Huisartsenpraktijk Termolen, Zonhoven, Belgium
12. Gastroenterology and Hepatology, AZ Maria Middelares, Gent, Belgium
13. Gastroenterology, MUMC+, Maastricht, Netherlands
14. Gastroenterology and Hepatology, University of Antwerp, Antwerpen, Belgium
15. Gastroenterology and Hepatology, Antwerp University Hospital, Edegem, Belgium
16. Gastroenterology, University Hospitals KU Leuven, Belgium

*Correspondence to leen.heyens@uhasselt.be

Disclosure: Heyens is funded by the Fonds Wetenschappelijk Onderzoek (FWO), Flanders. Busschots has received travel grants from AbbVie and Gilead Sciences; and has received research grants from Gilead. Robaeys has received research grants from AbbVie, Janssen Pharmaceuticals, and MSD; and has received consultancy agreements for AbbVie, BMS, Gilead Sciences, and MSD. All other co-authors have declared no conflicts of interest.

Acknowledgements: The authors would like to thank the general practitioners and nurses for their collaboration in patient recruitment.

Keywords: Belgium, non-alcoholic fatty liver disease, primary care, significant fibrosis.

Citation: EMJ Hepatol. 2021;9[1]:30-31. Abstract Review No. AR2.

BACKGROUND AND AIMS

Non-alcoholic fatty liver disease (NAFLD) is becoming the most frequent cause of chronic liver disease worldwide.[1] The stage of fibrosis has been identified as the most important predictor of prognosis. Patients with significant fibrosis have a higher risk of developing Type 2 diabetes mellitus, cardiovascular disease, and extra-hepatic malignancies.[2] However, the majority of fibrotic NAFLD patients are currently undetected. As the general practitioner's (GP) outpatient clinic is usually their first contact in the health care system, they can play an essential role in the identification of this group of patients. There are several non-invasive tests available that can be used in primary care. For example, the Fibrosis-4 (FIB-4) index has been proposed by Newsome et al. to predict fibrosis as it is based on the routine blood parameters aspartate aminotransferase, alanine aminotransferase, age, and thrombocytes.[3]

MATERIALS AND METHODS

In their current study, the authors aim to verify if the FIB-4 is a valuable tool to identify NAFLD patients with significant fibrosis or higher as compared to vibration-controlled transient elastography (VCTE) by FibroScan® (Echosens, France) as a reference method. In a prospective study in five Belgian GP practices, people were invited for VCTE measurement. Based on the most recent laboratory data from the electronic

patient file, the FIB-4 was calculated. To grade the VCTE measurements, the authors used the cut-off values as stated by the Belgian Association for the Study of the Liver (BASL).[4] The risk of significant fibrosis determined by the FIB-4 was based on a cut-off value ≥1.3 for people younger than 65 years and ≥2.0 for people older than 65 years.

RESULTS

A first analysis of the data revealed that of the 292 people who were screened, 248 (84.9%) had all the values available to calculate the FIB-4. The authors found that 64 (26.8%) of those people had a high FIB-4 index. However, when compared to the VCTE measurements, 52 (77.6%) people were graded F0–F1 (no or mild liver scarring), while the FIB-4 index indicated an increased risk for significant fibrosis. The calculated area under the receiving operating curve with VCTE as reference was only 0.632. As a good area under the receiving operating curve is usually considered to be higher than 0.700, the authors concluded that the usage of FIB-4 in primary care in Belgium is only able to detect NAFLD patients with significant fibrosis moderately.

CONCLUSION

The authors, therefore, suggest using other test strategies or a sequential combination. A disadvantage of the other available non-invasive tests is the usage of not readily available parameters. For example, the NAFLD Fibrosis Score (NFS) uses albumin. The addition of these parameters increases the costs and is therefore not preferred. Another possibility, as has been proposed by Shah et al., is to use different cut-off values for the FIB-4.[5] Nevertheless, the authors are still of the opinion that the FIB-4 is going to play a key in the referral from primary to secondary or tertiary care. ■

References

1. Tanaka N et al. Current status, problems, and perspectives of non-alcoholic fatty liver disease research. World J Gastroenterol. 2019;25(2):163-77.
2. Heyens LJM et al. Liver fibrosis in non-alcoholic fatty liver disease: from liver biopsy to non-invasive biomarkers in diagnosis and treatment. Front Med (Lausanne). 2021;8:615978.
3. Newsome PN et al. Guidelines on the management of abnormal liver blood tests. Gut. 2018;67(1):6-19.
4. Francque S et al. The Belgian Association for Study of the Liver guidance document on the management of adult and paediatric non-alcoholic fatty liver disease. Acta Gastroenterol Belg. 2018;81(1):55-81.
5. Shah S et al. FIB-4 cut-off of 1.3 may be inappropriate in a primary care referral pathway for patients with non-alcoholic fatty liver disease. J Hepatol. 2020;73(1):216-7.

MicroRNA-27a-3p Modulates FoxO1 to Induce Tumour-Like Phenotypes in Bile Ducts

Authors: Patricia Munoz-Garrido,[1†] Lea Duwe,[1†] Colm O'Rourke,[1] Letizia Satriano,[1] Monika Lewinska,[1] Juan LaFuente-Barquero,[1] Andrzej Taranta,[1] Awaisa Ghazal,[1] Dan Høgdall,[1,2] Jens Marquardt,[3] Matthias Matter,[4] Jesus Banales,[5] *Jesper Andersen[1]

1. Biotech Research & Innovation Centre (BRIC), Department of Health and Medical Sciences, University of Copenhagen, Denmark
2. Department of Oncology, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark
3. Department of Medicine I, UKSH-Campus Lübeck, Germany
4. Department of Pathology, Institute of Pathology and Medical Genetics, University Hospital of Basel, Switzerland
5. Biodonostia Health Research Institute, Department of Liver Diseases, San Sebastian, Spain

*Correspondence to jesper.andersen@bric.ku.dk

†Equal author contribution

Disclosure: The laboratory of Andersen is funded by a competitive grant from the Novo Nordisk Foundation (#14040). Munoz-Garrido, O'Rourke, and LaFuente-Barquero were awarded postdoctoral fellowships from the European Union (EU) Marie Curie programme (MiRCHOL, EpiTarget, and EpiCC, respectively). Munoz-Garrido was awarded a Sheila Sherlock postdoctoral fellowship from the European Association for the Study of the Liver (EASL) and Young Investigator Bursary for International Liver Congress (ILC) 2018. Taranta was awarded an individual postdoctoral fellowship from the Lundbeck Foundation. Duwe was awarded a PhD project grant from the Danish Cancer Research Foundation and Young Investigator Bursary for ILC 2021. This project was supported by the EU's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no. 801481.

Acknowledgements: The authors would like to thank the patients and their families for inclusion of samples and clinical data.

Keywords: Cholangiocarcinoma (CCA), epigenomics, FOXO1, microRNA (miR), transcription factor.

Citation: EMJ Hepatol. 2021;9[1]:32-33. Abstract Review No. AR.3.

BACKGROUND AND AIMS

Cholangiocarcinoma (CCA) is a clinically[1,2] and molecularly heterogeneous disease.[3,4] The ability of microRNAs (miRs) to target multiple signalling pathways links them to tumour heterogeneity.[5-7] Here, the authors analysed the miR landscape of CCA and non-malignant livers to define their roles in orchestrating gene regulation and promoting CCA development.

METHOD

The authors performed miR sequencing (miRseq) of 190 human samples (99 intrahepatic CCAs, 28 extrahepatic CCAs, and 63 matched adjacent livers), including matched transcriptome data from 184 samples, to study miRs and regulation of target genes in CCA. High-throughput screening (HTS) using a library of 2,700 miR mimics was performed to characterise their proliferative impact in three primary normal human cholangiocyte cultures. After miRseq-HTS data integration, the authors performed target prediction and pathway over-representation analyses. The main target was confirmed by luciferase reporter assay and characterised by quantitative PCR, immunoblotting, and *in situ* hybridisation in tissue microarrays. To identify the biological role, the authors generated miR-CRISPR knock-out lines and performed proliferation and wound-healing assays.

RESULTS

MiRseq revealed 398 differentially expressed miRs (388 upregulated, 10 downregulated) in CCA compared with non-malignant tissues. Unsupervised hierarchical clustering identified three tumour subgroups, differing significantly based on tumour location, overall survival, tumour microenvironment (i.e., macrophage, hepatic stellate, and endothelial cell content), and immune infiltrates (dendritic cells, CD4+, and CD8+ T cells).

In HTS, overexpression of 224 miRs increased the normal human cholangiocyte proliferation rate, of which 35 miRs were upregulated in CCA. Pathway over-representation analysis showed FoxO signaling as the major affected pathway.

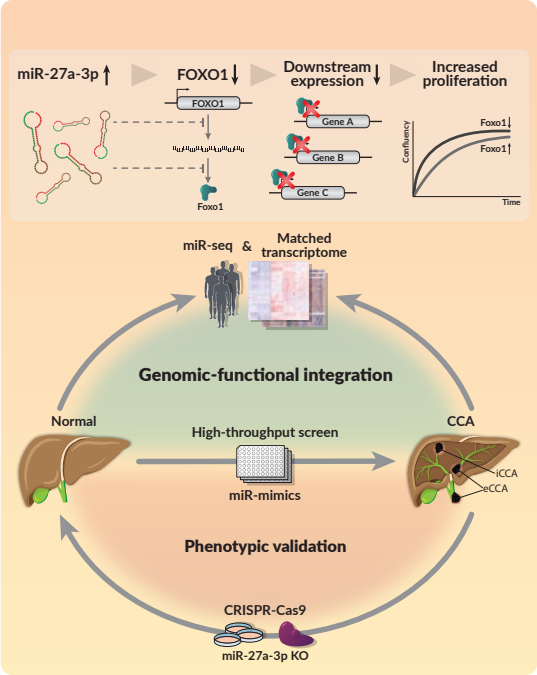


Figure 1: miR-27a-3p regulation of FOXO1 in cholangiocarcinoma.
CCA: cholangiocarcinoma; eCCA: extrahepatic cholangiocarcinoma; iCCA: intrahepatic cholangiocarcinoma; KO: knock out; miR-seq: microRNA sequencing.

Further, miR-27a-3p and FoxO1 interaction revealed a strong negative correlation in the CCA cohort, which was independently validated in an external cohort (TCGA-CHOL). This interaction was confirmed *in vitro* by luciferase reporter assay. *In situ* hybridisation showed that miR-27a-3p is highly expressed in tumour cells and vascular smooth muscle. MiR-27a-3p knock-out CCA cells showed decreased proliferation and migration, highlighting miR-27a-3p as an oncogenic dependency in CCA.

CONCLUSION

MiRs are highly deregulated in CCA. MiR-27a-3p targets FoxO1, contributing to altered FoxO signalling and tumour phenotypes (Figure 1). ■

References

1. Valle JW et al. Biliary tract cancer. Lancet. 2021;397(10272):428-44.
2. Banales JM et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol. 2020;17(9):557-88.
3. Nepal C et al. Genomic perturbations reveal distinct regulatory networks in intrahepatic cholangiocarcinoma. Hepatology. 2018;68(3):949-63.
4. Farshidfar F et al. Integrative genomic analysis of cholangiocarcinoma identifies distinct *IDH*-mutant molecular profiles. Cell Rep. 2017;19(13):2878-80.
5. Lozano E et al. Causes of hOCT1-dependent cholangiocarcinoma resistance to sorafenib and sensitization by tumor-selective gene therapy. Hepatology. 2019;70(4):1246-61.
6. Oishi N et al. Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. Hepatology. 2012;56(5):1792-803.
7. Lampis A et al. MIR21 drives resistance to heat shock protein 90 inhibition in cholangiocarcinoma. Gastroenterology. 2018;154(4):1066-79.e5.

A MET-Agonistic Antibody Mimicking Hepatocyte Growth Factor Accelerates Liver Regeneration and Improves Survival in Mice Undergoing Carbon Tetrachloride Exposure and Partial Hepatectomy

Authors: Kuai Ma,[1,2] Weitao Que,[1] Er-li Gu,[2] Wen-zhi Guo,[3] Liang Zhong,[4] *Paolo Michieli,[5,6] *Terumi Takahara,[7] *Xiao-Kang Li[1,3]

1. Division of Transplantation Immunology, National Research Institute for Child Health and Development, Tokyo, Japan
2. Department of Gastroenterology and Hepatology, Jing'an District Central Hospital, Jing'an Branch of Huashan Hospital, Fudan University, Shanghai, China
3. Department of Hepatobiliary and Pancreatic Surgery, The First Affiliated Hospital of Zhengzhou University, China
4. Department of Gastroenterology, Huashan Hospital, Fudan University, Shanghai, China
5. Molecular Biotechnology Center, University of Torino Medical School, Torino, Italy
6. AgomAb Therapeutics NV, Gent, Belgium
7. Third Department of Internal Medicine, University of Toyama, Japan

*Correspondence to ri-k@ncchd.go.jp, terutaka-tym@umin.ac.jp, and paolo.michieli@agomab.com

Disclosure: Michieli is a consultant and stockholder at AgomAb. The other authors have declared no conflicts of interest.

Acknowledgements: This study was supported by research grants from the Grants of Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grants-in-Aid 16K11064, 24/17H04277, 18K08558); grants from the National Center for Child Health and Development (29-09); Science and Technology Innovation Talents in Henan Universities (No.19HASTIT003).

Keywords: Agonistic monoclonal antibody, hepatocyte growth factor, liver regeneration, living donor liver transplantation, small-for-size syndrome.

Citation: EMJ Hepatol. 2021;9[1]:34-35. Abstract Review No. AR4.

BACKGROUND AND AIMS

Small-for-size syndrome (SFSS) is defined by prolonged cholestasis, coagulopathy, and ascites within the first week of liver transplantation caused by a partial liver graft that is inadequate to sustain metabolic demand in the recipient.[1-3] Due to persistent organ shortage, living donor liver transplantation is becoming the most viable option for patients with end-stage liver disease.[4] Thus, a major unmet medical need in hepatic medicine is to establish effective protocols allowing safe living donor liver transplantations using SFSS as the last hope for patients who would otherwise die.

METHOD

AgomAb has developed a series of agonistic monoclonal antibodies (agomAbs) that mimic hepatocyte growth factor (HGF) using arGEN-X SIMPLE Antibody™ platform.[5] HGF-mimetic agomAbs bind to MET at high affinity, promote receptor dimerisation and activation, and reproduce the full spectrum of biological activities that are characteristic of HGF. AgomAbs are cross-reactive over rodents, non-human primates, and humans. They all compete with HGF, some fully and some partially. In contrast to HGF, which has a half-life of a few minutes and a problematic tissue distribution, agomAbs are very stable *in vivo* and display ideal drug-like properties.

RESULTS

The therapeutic potential of 71D6, the non-humanised precursor of AGMB-101, was tested in a mouse model of partial hepatectomy on cirrhotic background. It significantly decreased mouse mortality consequent to insufficient regeneration of the cirrhotic liver. Analysis of liver specimens in satellite animals revealed that 71D6 promoted accelerated liver regeneration, characterised by increased liver-to-body weight, augmented mitotic index, and higher albumin levels. Remarkably, 71D6 resulted in powerful activation

of the Erk pathway, which is a key event in the spontaneous liver regeneration programme. Histological and immunohistochemical analysis of liver samples revealed that 71D6 significantly accelerated the resolution of hepatic fibrosis, as measured by Picro Sirius Red staining, and reduced the accumulation of activated stellate cells and myofibroblasts, as measured by Desmin and a-smooth muscle actin staining, respectively. Analysis of gene expression by real-time polymerase chain reaction confirmed that 71D6 administration suppressed the expression of key pro-fibrotic genes, including *PDGF*, *TIMP3*, and *TGF*-b. Liver samples were also analysed by immunohistochemistry for the presence of infiltrating immune cells, which typically invade the parenchyma of fibrotic livers and have an adverse impact on graft survival. This analysis revealed that 71D6 strongly reduced liver infiltration by macrophages, as measured by both CD-68 staining and F4/80 staining. Gene expression analysis confirmed that 71D6 administration suppressed the expression of key pro-inflammatory genes, including *TNF*-α, *IL-1b*, *CCL-3*, and *CCL-5*. Periodic acid-Schiff staining of liver sections revealed that 71D6 promotes the synthesis and storage of glycogen. This finding is consistent with the notion that HGF stimulates glucose uptake by hepatocytes. Together with the observation that antibody-treated animals have higher levels of albumin, these data suggest that 71D6 accelerates the recovery of liver function following hepatectomy.

CONCLUSION

In summary, the results obtained in this study provide evidence that 71D6 significantly improves the ability of cirrhotic livers to regenerate following partial hepatectomy, resulting in accelerated recovery of liver function and extended mouse survival. Importantly, they also demonstrate that 71D6 not only accelerates liver regrowth, but also resolves hepatic fibrosis and suppresses inflammation, two important risk factors that increase the occurrence of SFSS. Overall, these results suggest that activating the MET pathway via a HGF-mimetic antibody may be beneficial in patients with SFSS and possibly other types of acute and chronic liver disorders. ■

References

1. Ikegami T et al. Small-for-size graft, small-for-size syndrome and inflow modulation in living donor liver transplantation. J Hepatobiliary Pancreat Sci. 2020;27(11):799-809.
2. Masuda Y et al. Small-for-size syndrome in liver transplantation: definition, pathophysiology and management. Hepatobiliary Pancreat Dis Int. 2020;19(4):334-41.
3. Riddiough GE et al. A systematic review of small for size syndrome after major hepatectomy and liver transplantation. HPB (Oxford). 2020;22(4):487-96.
4. Greenbaum LE et al. Clinical translation of liver regeneration therapies: a conceptual road map. Biochem Pharmacol. 2020;175:113847.
5. Michieli P. Anti-Met antibodies and uses thereof. European patent application EP 3475302 A1. 2019. Available at: https://www.lens.org/lens/patent/062-815-545-734-095. Last accessed: 13 July 2021.

VIEW MORE ABSTRACTS ONLINE ←

Congress Interviews

Philip Newsome and Thomas Berg spoke to EMJ about their respective roles at the European Association for the Study of the Liver (EASL) and what inspired them to follow careers in hepatology.

Featuring: Philip Newsome and Thomas Berg.

Philip Newsome

Secretary General at the European Association for the Study of the Liver (EASL) 2021; Director of the Centre for Liver and Gastrointestinal Research; Professor of Hepatology, University of Birmingham; Consultant Hepatologist at the Liver Unit, Queen Elizabeth Hospital, Birmingham, UK

Q1 With your years of experience as a consultant hepatologist, what initially sparked your interest to pursue a career in this field and motivates you to continue researching?

It was a clinical attachment when I was a third-year medical student in Edinburgh, UK. I then did a summer project in the Liver Laboratory in Edinburgh, which motivated me to pursue a career in liver disease with research as a principal focus.

Q2 What are the main goals and hopes for the European Association for the Study of the Liver (EASL) congress?

I suppose it is to be able to bring the liver community together, albeit in an online form, so we can get that interaction to share new data, new knowledge, and facilitate discussions between different patients, doctors, and researchers.

Q3 How much of an impact do you believe EASL has, both directly on hepatologists and indirectly on patients?

Directly, it has a major impact because it provides a forum, whether it be physical or virtual, to access information, disseminate information, and have a discussion. Then, indirectly, that translates into great research activity, more clinical trials, and improved patient care.

Q4 Could you talk a bit about the underlying ethos of the International Liver Congress (ILC) and how your work relates to this?

I think the underlying ethos of the ILC is that we want to bring together people who have an interest in improving the outcome of patients

with liver disease, and also improving the understanding of the factors that drive liver diseases. My work as the Secretary General is to try and bring together as many people as possible to that forum.

Q5 What are the most significant changes you have seen in the field of hepatology during your time working within the field?

I believe that the most significant change has been the discovery of a new condition, hepatitis C, and the development of effective treatment strategies in a 10–15 year time period. This is a demonstration of what innovation can do in terms of defining a condition then identifying and developing treatments for it.

Q6 What changes have you brought into effect whilst serving as Secretary General for EASL?

I have really supported the delivery of EASL Campus, which is the online platform that curates a lot of the activities we do. I have also brought in the first equality, diversity, and inclusion policy to try and make sure that, as an organisation, we are much more conscious of embedding diversity, in terms of the representational committee, the faculty, and at conferences. I think we have really tried to push the focus to being on liver disease rather than on the hepatologist, trying to focus on a patient-centric view of the work.

Q7 Since your appointment to Director of the Centre for Liver and Gastrointestinal Research and Professor of Hepatology, at the University of Birmingham, UK, what has been your proudest achievement?

My proudest achievement is probably delivering, alongside a colleague at the University of Edinburgh, Stuart Forbes, the landmark trial testing stem cells in patients with liver cirrhosis.

Q8 As an educator, where can we expect to see your focus lie in the coming years?

I think the increased focus has been providing education to patients of the public. I have done quite a lot of work on liver disease and therapies for liver disease, which I think is important because you become able to understand patient perspectives and they can understand what any new therapies might look like. ■

"I think the underlying ethos of the ILC is that we want to bring together people who have an interest in improving the outcome of patients with liver disease, and also improving the understanding of the factors that drive liver diseases."

Thomas Berg

Vice-Secretary General at the European Association for the Study of the Liver (EASL) 2021; Professor of Medicine, University of Leipzig; Head of the Division of Hepatology, Department of Medicine II, Leipzig University Medical Center, Germany

Q1 Was there a particular event or person that encouraged you to pursue a career in hepatology?

The truth is my interest and fascination for liver and hepatology was very much influenced by my father, who was a pathologist and immunologist working on autoimmune liver diseases. Perhaps, working in hepatology is genetic or epigenetic for me. He was fascinated early on by the fact the liver is an organ that mediates tolerance, and I have to say, I have always felt very much at home in the scientific hepatology community because of its tolerance. It is a funny way of thinking, but you can wonder if there is a connection between the properties of an organ and the people who are studying it.

I started my career at the Charité, a joint-medical faculty of the Humboldt University and University of Berlin, and learnt a lot from my mentor there, Prof Uwe Hopf, who was a great clinician. Also from this establishment, Peter Neuhaus who is a well-known and innovative transplant surgeon.

Q2 The University in Leipzig is thought of as one of the oldest and most prestigious in Germany. What do you think other university hospitals could learn from the approach taken there?

It is true the University has a very long tradition, not only in biomedical science but also in music. Looking back at when the Berlin Wall divided Germany, setbacks in research arose due to the dependency of exchange across borders, which was very limited. What was and is still special and unique about Leipzig, is how they were able to rebuild the university structures from scratch after the Berlin Wall came down, this is still a very exciting process. Therefore, the challenges we often face, like the current COVID-19 pandemic, help us to rethink our position and sometimes even accelerate breakthroughs.

Q3 Since your appointment as Head of the Department of Hepatology at the Leipzig University Medical Centre, Germany, what has been your proudest achievement?

The motto at Leipzig University Medical Centre is 'teamwork builds success', and perhaps one of the most important achievements is the formation of various interdisciplinary interest groups around patients diagnosed with different forms of liver disease. We are now able to concentrate expertise around the patient, so they do not have to go to different places to get specialised care. We have also developed several interdisciplinary centres.

With regards to personal research, I could mention studies carried out on the individualisation of hepatitis C therapy to induce a cure, and innovations in hepatitis B including diagnostics and therapy. We have recently introduced new biomarkers to study treatment success. More recently, studies on liver regeneration for patients with acute or chronic liver failure.

Q4 Can you talk about the ways in which the International Liver Congress (ILC) 2021 will be living up to its aims to empower patients and create a dialogue aimed at optimal healthcare benefits?

This annual meeting is like an inventory, acknowledging where we stand currently in terms of knowledge and understanding of the underlying mechanisms of liver disease. There is a special focus on issues related to how we optimise the diagnostics and treatments of liver disease.

"Therefore, the challenges we often face, like the current COVID-19 pandemic, help us rethink our position and sometimes even accelerate breakthroughs."

The highlights and innovations presented during the ILC may change the way we treat patients with liver disease. These are selected with help from patient groups and are available in the form of webinars or even podcasts etc., which can all be seen on our website and shared with the patients. Then we have patient symposia during or after the ILC, used as ways to disseminate information whilst bringing liver experts and patients together.

We also have our first guidelines written for patients diagnosed with common fatty liver disease, which will be presented during the ILC. Additionally, the relationship with the media is an equally important way of disseminating the information on recent innovations.

Q5 You are currently serving as Vice-Secretary General for European Association for the Study of the Liver (EASL). Could you please explain what this position entails and how it contributes to the success of the ILC?

The Secretary General and Vice-Secretary work together with a governing board for a period of 2 years, after this the Vice-Secretary becomes the Secretary General to mark a total term of 4 years. The reasoning for this is allow continuation of the achievements and innovations of your predecessor and begin early work for the following year.

Concerning ILC, as Vice-Secretary I am closely involved and jointly responsible for the final programme including lecture selection, session composition, and implementation of live studio sessions. These sessions will play a large part of this year's ILC, with three to four a day, discussing with delegates and international experts. Although there will not be a face-to-face meeting, we will try to have the highest level of interactivity in the digital meetings and create a platform where people can really connect. I am very much behind this philosophy.

Q6 You currently have more than 200 international publications to your name, with research spanning chronic viral hepatitis, liver transplantation, liver

disease, and liver failure, to name a few. What do you believe to be the current gaps in literature and what topics merit greater attention?

I think the increase in knowledge and the number of publications, especially in pathology, is remarkable. This is reflected in the growth of our EASL journal in just a few years, and this shows where the interest lies in your research.

In my opinion, the research should be on two things. Firstly, the management of patients diagnosed with liver cancer and how to preserve the liver function during cancer treatment. Most research is currently focussed on finding better drugs to treat the cancer. This is a relevant issue because a significant percentage of patients diagnosed with liver cancer do not die from cancer progression, but from deterioration of liver function until failure. I think we need to do more studies on how to preserve liver function in these patients.

The second point relates to the palliative care of patients diagnosed with cirrhosis, and the stigma associated with liver disease, especially if it is alcohol induced cirrhosis. Decompensated cirrhosis is not curable, especially if a patient does not have the chance to get a transplant as this is available for very few patients. We need to study more how to improve the quality of life for these patients and find ways to increase liver regeneration, in turn bringing a better palliative care concept to patients. In comparison, we are doing much more at current for patients diagnosed with advanced and curable end stage cancer disease.

Q7 Are there any innovations on the horizon in the field of liver research that you think are particularly noteworthy?

In recent years there has been a rise in the awareness of liver cancer importance and new systemic therapy, particularly immune checkpoint inhibitor-based combination therapy. This is a very important step forward in the management of patients diagnosed with cancer. We also have an increasingly better understanding of which diseases lead to liver cancer and how early treatment of these conditions could prevent it, clearly shown through the cure of hepatitis C helping to prevent cancer. There are fantastic long-term studies showing that with the invention of curative therapies, the number of decompensated cirrhosis transplantations and cancers due to hepatitis C have declined rapidly. There are large multicentre trials starting to evaluate novel treatment approaches in patients diagnosed with chronic liver failure.

Q8 What advice would you give new trainees just beginning their careers in hepatology?

Well, depending on the individual's personality, there are different approaches, but my personal view is get to work with real enthusiasm and stick to it, stay curious, and always ask questions if unsure. Work on how to close knowledge gaps, even if they are only small as they are still significant. There will always be drawbacks in your career, and it is easy to compare yourself with your peers; you might see some fellow researchers achieving their goals faster than yourself. But in my view, serious continued scientific endeavours will always be recognised by the scientific community. ■

Interviews

Stephen Ryder and Kieron Lim spoke to EMJ about the influences and inspirations that led them to work in the field of hepatology and the effects the COVID-19 pandemic has had on liver disease.

Featuring: Stephen Ryder and Kieron Lim.

Stephen Ryder

Clinical Director of Research and Innovation, Nottingham University Hospitals NHS Trust; Chair of the Clinical Advisory Board, British Liver Trust; hepatology Vice President of the British Society of Gastroenterology

Q1

What inspired you to study medicine? Was there a particular aspect of your medical education that influenced your career path?

A lot of it is serendipity: you happen to be in a particular place at a particular time. And an awful lot of it is down to the people that you meet. While I was a trainee in medicine, there were two or three very influential people who shaped my future. One of them was a gastroenterologist in in the West Midlands, Hugh Bradby, who persuaded me down the path of gastroenterology rather than cardiology, which is where I had started looking. After that, it was the coming together of a few things: the practical aspects of gastroenterology and the machines to work with, as well as the people. And then finally, it was working with two eminent hepatologists, Howard Thomas at St Mary's and Roger Williams at King's, that really sparked a major interest in the liver for me. From an academic viewpoint, it was working with Jon Rhodes in Liverpool, who was my MD supervisor for a research project surrounding cancer and the gastrointestinal tract; working with John was a very pivotal moment for me in terms of research, and he has been a very close friend ever since.

Q2

The hepatitis C virus was isolated for the first time in 1988, just a few years after you qualified as a doctor. Could you please tell me about this time in your career and how hepatitis C virus became your major clinical focus?

"A whole new disease was discovered in that early phase of my gastroenterology career; just being there at the start of something and seeing it build was very impressive"

Hepatitis C virus (HCV) was discovered when I was a trainee and was a huge scientific breakthrough. A whole new disease was discovered in that early phase of my gastroenterology career; just being there at the start of something and seeing it build was very impressive. I remember the first studies published following the discovery of HCV and the discovery that a significant number of people around the world were infected with the virus. At that stage nobody knew what it meant to have HCV. Previous studies had all suggested HCV to be a fairly benign disease, a chronic persistent hepatitis. But over the first few years, the magnitude of the disease became very obvious and set the scene for me when I arrived in Nottingham in 1994; here, I was very fortunate to have a colleague, Will Irving, working in virology and who has been very instrumental in HCV research. When I arrived, I received a call from Will asking me if I was interested in working with HCV and my answer was yes, and that is how it all started.

Q3 You have played a large role in co-ordinating clinical trials for HCV treatment and ensuring patient access to these trials. What are the main barriers to clinical trial access in the UK? And what is your opinion on the future of decentralised clinical trials?

We are very fortunate in the UK to have very good clinical trial infrastructure. We have seen it with the COVID-19, for example: the UK led the world in introducing therapies for a new disease. I think that the UK is a very good place to be a patient in terms of trial access. The main challenge we have is that there are still inequalities with access to trials. Individuals still rely on their local hospital and team, where there needs to be an interest for having the right infrastructure in place to make trials work. There are areas of the country that have relatively little trial activity in particular specialties.

There is no doubt that the way in which trials are being conducted is changing and, due to digital, the way in which they are delivered is also changing. I think we will see a lot more trials being delivered in primary and community care. This was seen with the COVID-19 trials, which were performed in nursing homes and community settings. There will also be more use of real-world data, data that has been collected for other reasons, as part of clinical trial outcomes; this will make trials much more accessible and deal with some of the access inequalities. I can see the trials from the hospital setting, where I am based, becoming more interventional and being performed earlier. We conduct more trials in sick inpatients and there are more trials of new drugs being administered to the very first human subjects. That is going to be the biggest change, I believe: seeing those trials developing and concentrating in big hospitals and seeing a lot more trials developing out of primary and community care.

Q4 The highest rates of HCV are in developing and resource-limited countries, where the path towards HCV management and

eradication may be different to high-income countries. What measures need to be in place to ensure patients access to treatment and progress toward the management or eradication of HCV?

There are a set of things that would need to be in place in any country to make it work: you have to be able to find people who have the disease, you have to be able to afford to get treatment to those people, and the people have to want to take the treatment. At its most basic, those are the three areas and if you look at each of them in turn, some resource-poor countries actually have really good public health infrastructures because they recognise that the best way, economically speaking, of improving the health of the population is to know what the local public health problems are, and to manage it locally. Using the infrastructures already in place is absolutely critical and we have seen a number of low- to middle-income countries do really well in terms of HCV treatment and access; however, I think that good public health infrastructure is one of the defining factors of the countries that do. In terms of access to medicines at an affordable price, this is clearly still an issue. Although, I think the manufacturers have been significantly altruistic in their approach in terms of allowing manufacturing at much lower costs in various parts of the world. This needs to continue to make sure that people have access to affordable treatment. There is still a large need globally to educate and for people to recognise HCV as a problem because unless most people want treatment, we are not going to get there. There is still an ongoing role for the World Health Organization (WHO) and governments to keep pressure on the elimination target so that governments sit up and take it seriously and use the resources they have to sensibly reach that target for their population.

Q5

The COVID-19 pandemic has seen innovation in vaccine development and technologies and has influenced the development of other vaccines (e.g., mRNA vaccines against HIV from both Moderna and Oxford University's Jenner Institute). Has the pandemic innovated or advanced the possibility for an HCV vaccine?

The technology changes that happened with the development of COVID-19 vaccines were very rapid; we saw RNA vaccines really appear for the first time and I think that there will be many new technologies applied to other vaccines now. It is a very exciting time to see how everything will progress. Something else that the pandemic has done is made governments realise how important vaccines are and that there needs to be an infrastructure in place that can both develop and deliver vaccines, while being robust. I think we will see a lot more investment in that capacity. It is an incredible opportunity: both the new technologies and the political drive that will come out of seeing the impact an infectious

disease can have and what vaccines can achieve. I am very optimistic that it will translate.

Q6 You recently raised your concerns about the effect the COVID-19 pandemic has had on the incidence of liver disease in the UK. What measures or initiatives are needed to support those individuals at risk?

It has certainly been one of the major downsides of lockdown, particularly because people are less active and drink more, which is unquestionably not good for the liver. Certainly, from a local perspective in Nottingham, I have seen more people admitted and more people who are dependent on alcohol and have more mental health problems related to alcohol. There is a big agenda here and a big need for public health messages and more support for these individuals. The government needs to take action to destigmatise issues surrounding alcohol. It is a very common problem and alcohol support services and treatment services must be invested in, so that people can get the help they need when they need it.

This is also an opportunity for the Chancellor. This is perhaps not so popular to say, but the supermarkets, although they did a very good job at keeping us supplied during lockdown, made a lot of money and sold an awful lot of alcohol. There is an opportunity here to increase alcohol prices and tax in the supermarket and to reduce the cost and tax of pub and restaurant meal and beverages. As we come out of lockdown, encouraging people to be more active and watch their weight will be really important. The same applies to food and weight as it does with alcohol; unless people have access to weight management services, weight is difficult to control because food is cheap and all around us. If you combine that with inactivity, then you are going to end up with health problems, including problems for your liver later on in life. It needs to be a concerted approach, which focuses on including behaviour change as part of the infrastructure for individuals as we come out of lockdown.

Q7 Could you discuss the impact your work as Chair of the Clinical Advisory Board, British Liver Trust, has for research and society?

I have been involved with the British Liver Trust (BLT) for a long time now, and it is partly due to the fact that the patient voice has needed strengthening because of all those issues I mentioned around stigmatisation. Individuals have found it difficult to come forward and talk about their problems and to publicise them. Therefore, I was very keen to support the BLT, prior to becoming their medical adviser. The Clinical Advisory Board is to ensure that everything is connected: to bring together clinicians who are interested in liver disorders and connect them with patient groups to ensure a single voice and access to patient views both locally and nationally, and help push the whole agenda for patients forward. I have been hugely impressed with people's willingness and dedication of time to support the themes of the BLT.

From a research perspective it has also had a benefit; when designing a clinical trial, you have to find someone to fund that trial and having patient involvement is absolutely critical. It is not simply a group of doctors dictating what is important; you need patients to tell you what is important. The BLT having links with many patient groups around the country has been very helpful in pushing that agenda forward too.

Q8 Finally, if you could write a letter to yourself in 1985 when you had just qualified, what advice would you give to yourself?

It is an interesting thought to look back to a time and situation that is so very different from now. One of the most striking reflections is how different healthcare is now. HCV had not even been discovered and now we are near a stage of eliminating it as a human disease, which is extraordinary. I think firstly it would be about medicine as a career. It is still hugely rewarding, and I still love coming to work every day. Secondly, I would say that you can make a difference and be there for patients at an individual level and have that one-on-one interaction with someone and try to solve a problem. And then you are able to scale the impact you can make at the level of a clinical trial, which will change the future of medical practice and certain health policies. Looking back to 1985, I would never have thought any of what I mentioned was achievable; but looking back over the course of my career, they absolutely have been. ■

Kieron Lim

Senior Consultant, Gastroenterology & Hepatology; Director, Mount Elizabeth Hospital Liver Transplant Program, Mount Elizabeth Hospital, Singapore; Visiting Consultant, National University Hospital Singapore

Q1

Was there a defining moment, or series of moments, during your medical degree at St Bart's in London, that influenced your decision to pursue a medical career within the field of hepatology and gastroenterology?

Without a doubt – it was Prof Parveen Kumar. We had a lecture during our pre-clinical undergraduate years on gastrointestinal bleeding from Prof Parveen Kumar. She brought in a bucket of 'melena' and asked for volunteers who would take a look and a 'whiff'. I thought that was a very impactful way of introducing the topic to us! I was fortunate to have her as my consultant when I was a medical student and again as a House Officer. Her passion for gastroenterology and teaching inspired me and piqued my own interest in gastroenterology. Our paths were destined to cross again years later in Singapore during the postgraduate MRCP exams where Kumar was the external examiner. At our first meeting I was a trainee registrar helping to organise the exams. At our second meeting I had 'evolved' to become the Head of Gastroenterology at the National University Hospital in Singapore and was a fellow co-examiner. She had the same energy and passion when she spoke, commanding deep respect and admiration from her audience. I never fail to ask her what was really in the bucket that she brought into the lecture theatre... and she never fails to answer with just a cheeky laugh!

Q2

Since you started your medical career, how has the approach and attitude changed to interdisciplinary care in hepatology?

It has evolved tremendously to the benefit of our patients. Doctors and health professionals from different specialties are now more willing to discuss and proactively co-manage these complex liver patients. This is best seen in the context of multidisciplinary team (MDT) meetings for tumour boards and liver transplant listing meetings. Each member of the MDT can provide their input and share important perspectives about our patients' care. Indeed, there are published literature to show that hepatocellular carcinoma (HCC) patients have superior outcomes if an MDT approach is adopted.

Q3

How has research progressed in recent years to improve the prognosis of HCC?

Research in the field of HCC has enabled us to better understand the molecular signatures, tumour biology, and hence treatments that translate to patient outcome and survival. It further drives home the fact that HCC is a complex disease: having to treat both the cancer and in most cases the underlying liver cirrhosis and the associated complications. It is worth mentioning that the proliferation of data and approved therapies for advanced HCC has also provided clinicians with more treatment options to offer our patients.

Q4

How has your research and work in the field of liver transplantation contributed to the advancements and recent innovations we see today?

I have been fortunate to have been involved in a few clinical trials and studies looking at various aspects of liver transplantation and immunosuppression. The results achieved and experience gained have helped to deepen the understanding in: firstly, immunosuppression and post-transplant metabolic complications; secondly, the role of mTORs in liver recipients; and thirdly, hepatitis E in transplant recipients of Asian heritage.

Q5

What do you think the future holds for non-alcoholic fatty liver disease (NAFLD)

treatment, and what particular treatment advances would you like to see?

I think treatments for NAFLD/non-alcoholic steatohepatitis (NASH) will be as game-changing as the direct-acting antiviral agents were for hepatitis C virus (HCV). In fact, I think drug development for NAFLD/NASH will be bigger than for HCV, from the sheer number of individuals around the world with NAFLD/NASH. The data has established that NAFLD/NASH affects 20–40% of the world's population and is the fasting rising indication for liver transplantation. I hope that we will soon have effective therapies that can not only prevent the progression of NAFLD/NASH to cirrhosis but effectively result in regression to a normal and healthy liver.

Q6

You recently published a paper on the management of patients with liver disease during the COVID-19 pandemic; what are the main considerations and your recommendations to doctors treating these patients in the pandemic?

COVID-19 has impacted everyone around the world. The situation is fluid as evidenced by recurrent waves and emerging variant strains. Despite the availability of multiple vaccines, the pandemic rages on in various parts of the world. Liver patients, especially those with advanced disease, liver transplant recipients, and those with hepatobiliary cancer, are at increased risk for a severe disease course of COVID-19. As advised by recommendations from multiple professional bodies and societies, the benefits of the COVID-19 vaccination greatly outweigh the risks. My recommendations to both healthcare colleagues and patients are to get vaccinated when the opportunity arises, continue to take the necessary precautions even after vaccination, and seek medical attention early if you feel unwell. Indeed, no one is safe until the whole world is safe.

Q7

You have worked in the UK, USA, and Singapore; can you discuss how the working environments differ? What have you learnt from the different cultures in regard to treatment innovation and patient care that you apply to your work today?

This is a tricky question to answer as all three countries have different healthcare systems. However, I have had the privilege of working in tertiary centres with 'giants' in the field of hepatology as mentors and colleagues. The opportunity to learn from these mentors in their respective institutions has shaped my outlook and attitude in clinical and academic medicine. I usually never name-drop, but I will take great pride in doing so here: Profs Geoffrey Dusheiko (Royal Free London), Scott Friedman and Thomas Schiano (Mount Sinai New York), and Lim Seng Gee (National University Hospital Singapore) all imparted knowledge, experience, and an ethos that are truly invaluable. Their vision and leadership are evident in their academic and clinical achievements. They have taught me to ask the right clinical and research questions and to be a strong advocate for our liver patients. I try not to lose sight of those pearls when the going gets tough!

Q8

You are actively involved with postgraduate education and the training of future specialists; if you could give one piece of advice to your younger self as an aspiring medical student, what would it be and why?

I would have two pearls of wisdom are enjoy medical school as life will get tougher as you progress through postgraduate training; and learn to manage your time well as we cannot bring back those missed moments in life. ■

Elevated Glucagon in a Patient with Necrolytic Acral Erythema: A Case Report and Review of the Literature

EDITOR'S PICK

This paper provides an interesting and thorough description of a specific case of hepatitis C infection with necrolytic acral erythema, contributing novel information to the limited existing knowledge about this condition. With high originality and significance, the investigation gives a well-structured account of the improvement seen in the patient following oral zinc replacement therapy, alongside a review of currently available necrolytic acral erythema case literature.

Authors: *Jenna E Koblinski,[1] Blake W Traube,[1] Margaret Kessler,[2] Brenda Shinar[1,2]

1. The University of Arizona College of Medicine – Phoenix, Arizona, USA
2. Banner University Medical Center – Phoenix, Arizona, USA

*Correspondence to jennakoblinski@email.arizona.edu

Disclosure: The authors have declared no conflicts of interest.

Received: 05.02.21

Accepted: 30.03.21

Keywords: Dermatology, gastroenterology, glucagon, hepatitis, hepatology, necrolytic acral erythema (NAE).

Citation: EMJ Hepatol. 2021;9[1]:47-56.

Abstract

Necrolytic acral erythema (NAE) is a relatively newly described dermatologic disease that is often associated with hepatitis C virus (HCV). Oral zinc therapy is a successful treatment; however, therapy is often delayed due to misdiagnosis. There are limited reports of NAE in the literature. This paper presents a case of NAE in a 68-year-old male with untreated HCV, whose NAE was diagnosed and treated as recurrent cellulitis for 12 years. He had low serum zinc and elevated serum glucagon levels. Elevated glucagon is not often reported in NAE, but the patient's CT abdomen was negative, ruling out glucagonoma and necrolytic migratory erythema. He improved with oral zinc replacement and was referred to the hepatology department for HCV treatment. This paper additionally presents a review of the literature for NAE cases.

INTRODUCTION

Necrolytic acral erythema (NAE) was first described in 1996 by el Darouti and Abu el Ela in Egypt.[1] It is most often associated with hepatitis C virus (HCV) infection and is similar in morphology to other necrolytic erythemas.[2] Classically, NAE presents as well-demarcated, dusky erythematous plaques over the dorsum of the feet and toes, with thickening of the

plaques over time;[3] however, NAE rarely develops in other areas such as the hands and elbows as well.[2] While there have been more reports of NAE since its original designation, there is still a paucity of data regarding this entity. A case of NAE associated with elevated glucagon levels in a 68-year-old male with untreated HCV is presented here. His NAE was diagnosed and treated as recurrent cellulitis for 12 years.

CASE REPORT

The patient was a 68-year-old white, non-Hispanic male with a past medical history of untreated HCV, ex-intravenous (IV) drug abuse, chronic venous insufficiency, hypertension, tinea pedis, and recurrent left foot cellulitis, who was admitted to the hospital for progressive worsening of a painful left foot plaque. He reported that the lesion had started 3–4 weeks prior, but that he had experienced intermittent episodes of the same rash for the past 12 years, which had resolved with oral antibiotics, topical ketoconazole, and topical clotrimazole–betamethasone. The current plaque began localised to the left third digit and was associated with a burning pain. Prior to his current admission, he had been seen by multiple providers and had been unsuccessfully treated with oral cephalexin and topical mupirocin, doxycycline, and topical clotrimazole, and finally, IV vancomycin. Due to progression of his disease to his remaining left digits and proximal left foot, and new-onset redness and pain in his right foot, he was evaluated by podiatry who recommended he come to the emergency department for evaluation. He denied recent trauma or burns to his feet. His review of systems was not significant.

His past medical history was significant for untreated-HCV, ex-IV drug abuse, chronic venous insufficiency, hypertension, history of tinea pedis, and recurrent left foot cellulitis. He was diagnosed with HCV approximately 40 years prior and had never received treatment due to transportation and insurance barriers. His only medication was lisinopril.

On initial physical examination (Figure 1 and Figure 2) by the internal medicine team he had a well-demarcated, erythematous, ulcerated plaque extending to the dorsal surface with increased oedema on his left foot. There were macerations, fissuring, and sloughing of the epidermis at the plantar surfaces near the digits on both feet. Thickened nail plates with yellow discolouration and subungual debris were seen on multiple toenails, bilaterally. His leukocyte count and creatinine were elevated, and his serum albumin was low. His HCV antibody screen was >11 index (normal: ≤0.79) and his HCV RNA was 13,800,000 international units/mL (normal: <15 international units/mL). The patient was started on IV vancomycin and piperacillin/tazobactam, as well as oral terbinafine and topical clotrimazole–betamethasone for presumed left foot infection and right hallux infection. An X-ray was ordered, demonstrating some concern for osteomyelitis; a follow-up MRI was negative.

Due to failure to respond to antimicrobial therapy, dermatology was consulted on hospital Day 5. His physical examination at that time demonstrated dark erythema with superficial desquamation of bilateral dorsal and plantar feet and toes. Multiple toenails were thickened, with yellow discolouration and subungual debris. In addition, his bilateral hands, elbows, and heels had keratotic erythematous plaques with additional fissures and desquamation of his hands. He had fingernail clubbing. The remainder of his skin was clear. In addition to NAE, the differential diagnosis included lichen planus, psoriasis, atopic dermatitis, necrolytic migratory erythema (NME), niacin deficiency, acrodermatitis enteropathica, eczematous dermatoses, and tinea pedis.

The diagnosis of NAE was suspected based on clinical presentation of the hyperkeratotic plaques affecting his acral surfaces, as well as with his history of HCV.[2] This diagnosis also remained the most likely when working through the differential diagnoses. While lichen planus does have a similar distribution, it has a characteristic violaceous colour and polygonal shape not seen in the patient.[4] Psoriasis was not strongly considered as the patient's rash did not have the typical silvery scale of psoriasis, but rather a very superficial desquamation.[4] NME affects the perioral and peri-genital regions as well as the extremities (discussed below).[5]

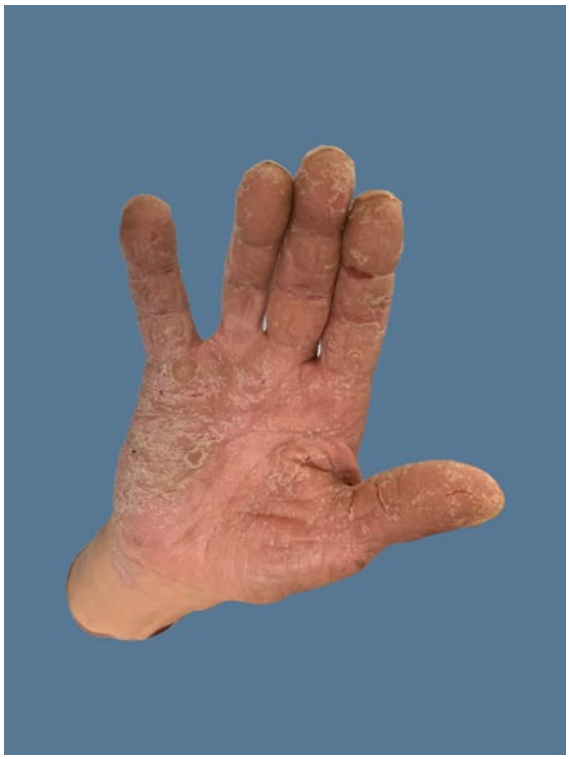


Figure 1: Necrolytic acral erythema affecting the right hand.

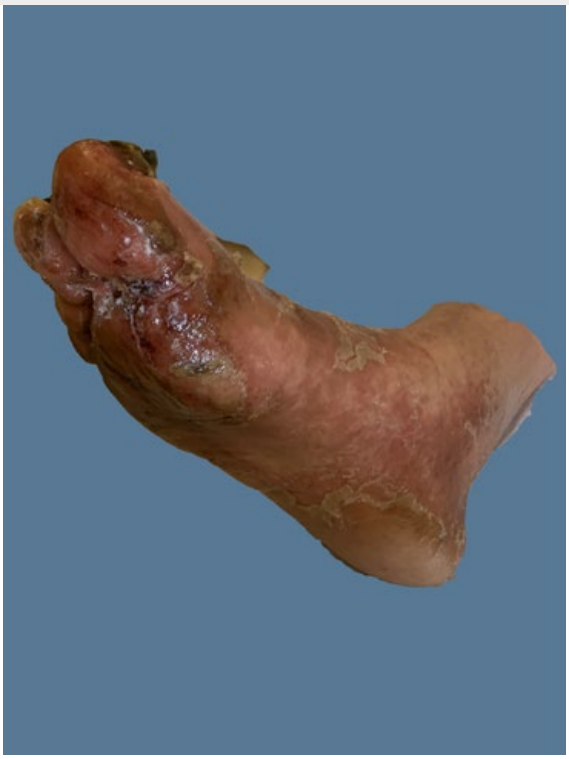


Figure 2: Necrolytic acral erythema affecting the right foot.

Acrodermatitis enteropathica has a periorificial and peri-acral distribution and also often presents as a triad with diarrhoea and alopecia.[4] Niacin deficiency can result in pellagra, which is characterised by the triad of dermatitis (typically affecting the neck or chest and hands), diarrhoea, and dementia.[4] Simple eczema should also be included, but the rash was not blistering or oedematous.[4] Tinea pedis was also considered; however, tinea does tend to be more inflammatory, with intense pruritus, brighter erythema, and finer scale (rather than the broad sheets of scale the patient had on his feet).[4] He also had repeatedly failed treatment for tinea. While NAE was suspected, a biopsy was performed for more definitive diagnosis and laboratory tests were ordered for further evaluation.

The pathology report showed psoriasiform dermatitis with slight pallor in the superficial epidermis with foci of parakeratosis and serum, which was compatible with NAE given the corresponding clinical features. His glucagon level was elevated at 96 pg/mL (normal: 8–57 pg/mL) and his zinc level was low at 41 mcg/dL (normal: 60–130 mcg/dL). His cryoglobulin screen was negative and his rheumatoid factor, C3, and C4 levels were within normal limits. The clotrimazole–betamethasone, antibiotics, and terbinafine were discontinued and he was started on a zinc supplement and topical clobetasol. The patient improved to the point of being able to ambulate and there was no longer visible skin sloughing. He was discharged and scheduled for hepatology follow-up and a CT abdomen to definitively rule out glucagonoma due to his elevated glucagon level. The CT abdomen was negative for pancreatic lesions, which again verified the diagnosis of NAE as opposed to NME; the CT was also negative for hepatic cirrhosis and hepatocellular carcinoma. The patient is currently being seen by the hepatology service, with plans to start treatment for his HCV.

DISCUSSION

NAE is a relatively newly described pathology and is often associated with HCV.[1] While it was once believed to be pathognomonic for HCV infection, it has since been reported in seronegative patients as well.[3,6] While the exact incidence and prevalence of NAE is unknown, the majority of cases have been reported from Egypt, which has the highest prevalence of HCV in the world.[7] Since its initial description in Egyptian patients, it has been reported in other countries, including the USA,[3] India,[6] Pakistan,[8] and Canada, among others.[9]

On 13th January 2021, a literature search of the PubMed database was conducted using the search term 'Necrolytic Acral Erythema'; all cases written in English were included (Table 1).[1,3,6,9-47] There were 42 reports found, with a total of 63 cases of NAE described. Of these cases, 16 patients were black, two were white, three were Asian, and 42 did not explicitly list the patient's race or ethnicity. The presented patient is white, non-Hispanic, which contributes to the epidemiology of NAE as it has mainly been described in the black population, although there is a paucity of data on race in the reports. Fifteen of the 16 cases reported in the black population were reports from the USA; this is likely because in the USA, HCV is more prevalent in African Americans than any other racial group.[48] For the patients with an HCV diagnosis, it is unclear how long they had had the infection. At 68 years old, the patient is the same age as one patient and older than all but two of the reported cases; he had untreated HCV for approximately 40 years. While the exact pathogenesis of NAE is not yet known, it is hypothesised to be multifactorial, with contributions from zinc deficiency, hypo/hyperglucagonaemia, hepatic dysfunction, and hypoalbuminaemia, among others.[2] The patient's extensive history of untreated HCV, paired with his recurrent NAE, may suggest an association between infection duration and NAE. More studies need to be completed to evaluate for risk factors in NAE aetiopathogenesis.

As aforementioned, NAE can be associated with an array of laboratory abnormalities. The patient had hypoalbuminaemia, zinc deficiency, and hyperglucagonaemia. Interestingly, NAE has been demonstrated to be responsive to zinc therapy in patients with both normal zinc levels[10,11] and zinc deficiency.[13,28] The patient was found to be zinc deficient and improved with zinc supplementation, contributing to the proposed link between zinc deficiency and NAE. Zinc levels should be checked in all patients with suspected NAE.

Table 1: PubMed-indexed cases of necrolytic acral erythema.

Study	Patient	Age (years); sex	Ethnicity	HCV status	Serum zinc level	Serum glucagon level	Treatment	Other diseases in patient
el Darouti et al.,[1] 1996	1	55; F	N/A	Positive	Normal	N/A	Zinc and amino acids	N/A
	2	12; F	N/A	Positive	Normal	N/A	Zinc and amino acids	N/A
	3	45; M	N/A	Positive	Normal	N/A	Zinc and amino acids	N/A
	4	38; M	N/A	Positive	N/A	N/A	IFN-α and zinc	N/A
	5	50; M	N/A	Positive	N/A	N/A	Liver supplements	N/A
	6	40; F	N/A	Positive	Normal	N/A	Zinc and amino acids	N/A
	7	35; F	N/A	Positive	N/A	N/A	IFN-α	DM
Khanna et al.,[10] 2000	1	43; F	Black	Positive	Normal	Normal	IFN and zinc	Atopic dermatitis, asthma, allergic rhinitis
Hivnor et al.,[11] 2004	1	11; F	Black	Positive	Normal	Normal	IFN and ribavirin	HCV-associated nephrotic syndrome
Nofal et al.[12] 2004	1	48; F	N/A	Positive	Low	High	Zinc and amino acids	Four of the five had DM, not specified which. No other diseases discussed.
	2	24; F	N/A	Positive	Normal	Normal	Zinc and amino acids	
	3	43; F	N/A	Positive	Normal	High	Zinc and amino acids	
	4	35; F	N/A	Positive	Low	Normal	Zinc and amino acids	
	5	51; F	N/A	Positive	Normal	Normal	Zinc and amino acids	HIV, hypothyroidism, HTN
Abdallah et al.,[3] 2005	1	46; F	Black	Positive	Normal	N/A	Zinc	SLE, prior HBV infection, liver cirrhosis, HTN, DM, CAD
Najarian et al.,[13] 2006	1	48; F	N/A	Positive	Low	N/A	Zinc	HIV-1
Bentley et al.,[14] 2008	1	46; M	Black	Positive	Normal	Normal	Zinc	Haemophilia
Fielder et al.,[15] 2008	1	42; F	N/A	Positive	N/A	N/A	Zinc	HIV, hypothyroidism, HTN
Liu et al.,[16] 2008	1	56; F	White	Negative	Normal	Normal	N/A	Crohn's disease, psoriasis/ psoriatic arthritis, hypothyroidism, fibromyalgia, HLD
Najarian et al.,[17] 2008	1	65; F	Black	Positive	Low	N/A	Zinc	HTN
De Carvalho Fantini et al.,[18] 2008	1	24; F	White	Positive	Low	N/A	Zinc	Liver cirrhosis

Table 1 continued.

Study	Patient	Age (years); Sex	Ethnicity	HCV Status	Serum zinc level	Serum glucagon level	Treatment	Other diseases in patient
Manzur et al.,[19] 2008	1	15; M	Asian (Pakistani)	Positive	N/A	N/A	Topical tacrolimus	N/A
Wu et al.,[20] 2009	1	32; F	Asian (Taiwanese)	Negative	Low	Normal	Zinc and steroid cessation	SLE, lupus nephritis
Nikam[21] 2009	1	38; M	N/A	Negative	Normal	N/A	Zinc	Down's Syndrome
	2	25; F	N/A	Negative	N/A	N/A	Zinc	N/A
Zeller et al.,[22] 2009	1	55; M	N/A	Positive	Low	N/A	Zinc	N/A
Halpern et al.,[23] 2009	1	50; M	N/A	Positive	High	Low	IFN, ribavirin, zinc	None
Tabibian et al.,[24] 2010	1	19; F	Black	Positive	Low	N/A	Zinc	None
	2	58; F	Black	Positive	Low	Normal	Zinc	Anaemia, HTN, fibromyalgia, s/p cholecystectomy
Panda et al.,[25] 2010	1	68; M	N/A	Negative	Normal	N/A	Zinc, topical clobetasol propionate	DM, hypertriglyceridaemia
Patel et al.,[26] 2010	1	53; F	N/A	Positive	Normal	N/A	Zinc	Dermatomyositis, iron-deficiency anaemia
Kapoor et al.,[27] 2011	1	44; M	N/A	Positive	Low	N/A	Zinc	Lead toxicity, syphilis, narcotic and alcohol addiction
Ridder et al.,[28] 2011	1	17; F	Black	Positive	Low	N/A	Zinc	N/A
Grauel et al.,[29] 2012	1	59; M	Black	Positive	N/A	N/A	Zinc	Liver cirrhosis
Raphael et al.,[30] 2012	1	69; M	Black	Positive	N/A	N/A	Clobetasol and hand/foot psoralen plus UVA therapy	HIV, DM
	2	69; M	Black	Positive	N/A	N/A	Topical triamcinolone	None
	3	52; M	Black	Positive	N/A	N/A	Liver transplantation	HIV
	4	40; M	Black	Positive	N/A	N/A	Narrowband UVB therapy	None
	5	55; M	Black	Positive	N/A	N/A	Initially: narrowband UVB therapy. After relapse and hepatocellular carcinoma diagnosis: topical triamcinolone, IFN, and ribavirin	DM

Table 1 continued.

Study	Patient	Age (years); Sex	Ethnicity	HCV Status	Serum zinc level	Serum glucagon level	Treatment	Other diseases in patient
Yost et al.,[31] 2013	1	56; F	N/A	Positive	Low	N/A	N/A	Dermatomyositis
Pernet et al.,[32] 2014	1	50; M	Black (Cameroonian)	Negative	Normal	Normal	None	None
Iyengar et al.,[33] 2014	1	61; M	N/A	Positive	Low	N/A	Zinc and HCV treatment recommended	Intravenous drug abuse
Jakubovic et al.,[9] 2015	1	34; F	N/A	Negative	Low	N/A	Total parenteral nutrition	NAFLD, morbid obesity, prior Roux-en-Y gastric bypass
Das et al.,[34] 2016	1	30; F	N/A	Negative	N/A	N/A	Zinc	None
Hou et al.,[35] 2016	1	60; M	N/A	Positive	Low	N/A	Zinc	Psoriasis/psoriatic arthritis, HTN, DM
Pandit et al.,[6] 2016	1	24; M	N/A	Negative	Low	N/A	Zinc	None
	2	40; M	N/A	Negative	Low	N/A	Zinc	None
Botelho et al.,[36] 2016	1	31; F	N/A	Positive	Low	N/A	Zinc	Atopy, drug-induced hepatitis from OCP
Srisuwanwattana et al.,[37] 2017	1	64; F	N/A	Negative	Normal	N/A	Zinc, doxepin, combined 0.05% betamethasone dipropionate/3% salicylic acid ointment	None
Dabas et al.,[38] 2018	1	52; F	N/A	Positive	Low	N/A	Zinc, sofosbuvir, daclatasvir	N/A
Xiaoling et al.,[39] 2018	1	36; F	N/A	Negative	Low	N/A	N/A	NAFLD, obesity, DM
Xue et al.,[40] 2019	1	35; M	Asian (Chinese)	Positive	N/A	N/A	Zinc treatment recommended	Chronic hepatitis B
Oikonomou et al.,[41] 2019	1	66; F	N/A	Positive	Low	N/A	Prednisolone and zinc	HIV, liver cirrhosis, COPD
Pathania et al.,[42] 2019	1	36; M	N/A	Negative	N/A	N/A	Zinc and topical corticosteroid	PE on rivaroxaban, rivaroxaban-induced NAE
Fukushima et al.,[43] 2020	1	55; F	N/A	Negative	Low	N/A	Zinc	Metastatic ovarian cancer
Kumar et al.,[44] 2020	1	40; F	N/A	Negative	Normal	N/A	B12 supplementation and beclomethasone (0.1%) + salicylic acid (3%)	Vitamin B12 deficiency

Table 1 continued.

Study	Patient	Age (years); Sex	Ethnicity	HCV Status	Serum zinc level	Serum glucagon level	Treatment	Other diseases in patient
Parihar et al.,[45] 2020	1	41; M	N/A	Negative	Low	N/A	Zinc and levothyroxine	Hypothyroidism following radioactive iodine ablation for Graves' disease
	2	27; F	N/A	Negative	Low	N/A	Zinc and levothyroxine	Hypothyroidism following radioactive iodine ablation for Graves' disease
	3	35; F	N/A	Negative	Low	N/A	Zinc and levothyroxine	Hypothyroidism following radioactive iodine ablation for Graves' disease
	4	48; F	N/A	Negative	Low	N/A	Zinc and levothyroxine	Hypothyroidism following total thyroidectomy for papillary carcinoma
	5	33; F	N/A	Negative	Low	N/A	Zinc and levothyroxine	Primary hypothyroidism
Shah et al.,[46] 2020	1	51; M	Black	Negative	Low	Normal	Zinc	Sarcoidosis, seronegative chronic hepatitis likely secondary to sarcoidosis, DM
Davis et al.,[47] 2020	1	58; F	N/A	Positive	N/A	N/A	Clobetasol, zinc, and HCV treatment recommended	N/A
Koblinski* et al., 2021	1	68; M	White	Positive	Low	High	Clobetasol, zinc, and HCV treatment recommended	Chronic venous insufficiency, HTN, tinea pedis, intravenous drug abuse

*Case from this paper.

CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; F: female; HBV: hepatitis B virus; HCV: hepatitis C virus; HLD: hyperlipidaemia; HTN: hypertension; IFN: interferon; M: male; N/A: not available; NAE: necrolytic acral erythema; NAFLD: non-alcoholic fatty liver disease; OCP: oral contraceptive pill; PE: pulmonary embolism; SLE: systemic lupus erythematosus; UVA: ultraviolet A; UVB: ultraviolet B.

The link between NAE and disruption in glucagon levels is often discussed, especially due to the similar entity NME. NME is the dermatosis associated with glucagonoma syndrome and presents as painful erythematous plaques that coalesce into bullous lesions; it affects the perioral and peri-genital regions as well as the extremities.[5] While NAE and NME are similar in morphology, they have different body distributions.[2] In addition, the two have similar findings under histopathological evaluation (as well as overlapping features with pellagra and acrodermatitis enteropathica), with neither having pathognomonic features.[2,49,50] Both conditions can reveal parakeratosis, acanthosis, psoriasiform hyperplasia, and superficial epidermal necrosis depending on stage of disease and biopsy site.[2,49,50] These similarities

demonstrate how clinical correlation as well as laboratory tests and imaging are necessary for a more definitive diagnosis. For further distinction between the two, patients with NME typically have glucagon levels >500 pg/mL,[5] while patients with NAE can have normal,[10] low,[16] or, less often reported, high levels of glucagon.[12] The patient was found to have an elevated glucagon of 96 pg/mL (normal: 8–57 pg/mL).

While this raised suspicion for NME, the patient's dermatologic findings were in a distribution consistent with NAE and he had a negative CT abdomen. Of the PubMed-indexed cases, only two cases reported by Nofal et al.[12] had high glucagon levels, so this is an infrequently reported (or potentially under-reported) finding. For those two cases, the actual values of their elevated glucagon were not listed, but both did have a negative CT scan of the pancreas as well as HCV.[12] There is debate about whether NAE and NME are distinct entities or if NAE is truly a variant of NME, especially due to the similar histopathologic findings,[12] and the mildly elevated glucagon level in the patient may suggest the latter. Whether the two pathologies are different or on a spectrum, glucagon levels should be checked when either is suspected. An elevated glucagon level warrants imaging to rule out glucagonoma. It has been hypothesised that in NAE, elevated serum glucagon induces inflammation through high levels of arachidonic acid and its metabolism.[28]

The patient's diagnosis of NAE had been missed for 12 years resulting in significant morbidity. It was affecting his quality of life and he had been exposed to unnecessary and costly treatment throughout this time. As the incidence and prevalence are unknown, it is unclear how many other patients with NAE may be misdiagnosed as well. It is important for providers to be able to recognise this entity, especially as it is treatable with zinc replacement and, if indicated, HCV therapy.[2] This will help to avoid unnecessary morbidity for patients. Patients with HCV especially should be counselled about the cutaneous manifestations of HCV, including NAE, and told to report any concerns to their provider. As more cases are reported, the understanding of the epidemiology of NAE will increase and potentially lead to better recognition and care.

CONCLUSION

NAE is important to have on the differential diagnoses for patients with well-demarcated, erythematous acral plaques, especially in a patient with HCV. Misdiagnosis of NAE can lead to ineffective and unnecessary treatment and increased patient morbidity. If NAE is suspected, laboratory tests for HCV status as well as serum zinc and glucagon levels are warranted, and this case highlights elevated glucagon levels can be found in patients with NAE. Oral zinc is often a successful treatment and, if indicated, referrals to hepatology for HCV therapy are necessary.

References

1. el Darouti M, Abu el Ela M. Necrolytic acral erythema: a cutaneous marker of viral hepatitis C. Int J Dermatol. 1996;35(4):252-6.
2. Inamadar AC et al. Necrolytic acral erythema: current insights. Clin Cosmet Investig Dermatol. 2020;13:275-81.
3. Abdallah MA et al. Necrolytic acral erythema: a patient from the United States successfully treated with oral zinc. Arch Dermatol. 2005;141(1):85-7.
4. Bolognia J et al., Dermatology essentials, (2014) 2nd edition, Saint Louis, Missouri: Saunders.
5. Sandhu S et al., Glucagonoma Syndrome, (2020) Treasure Island, Florida: StatPearls Publishing.
6. Pandit VS et al. Seronegative necrolytic acral erythema: a report of two cases and literature review. Indian Dermatol Online J. 2016;7(4):304-7.
7. Gomaa A et al. Hepatitis C infection in Egypt: prevalence, impact and management strategies. Hepat Med. 2017;9:17-25.
8. Ilyas S et al. Necrolytic acral erythema: a rare entity. J Pak Assoc Dermatol. 2016;26(4):395-8.
9. Jakubovic BD et al. Zinc deficiency presenting with necrolytic acral erythema and coma. Am J Med. 2015;128(8):e3-4.
10. Khanna VJ et al. Necrolytic acral erythema associated with hepatitis C: effective treatment with interferon alfa and zinc. Arch Dermatol. 2000;136(6):755-7.
11. Hivnor CM et al. Necrolytic acral erythema: response to combination therapy with interferon and ribavirin. J Am Acad Dermatol. 2004;50(5 Suppl):S121-4.
12. Nofal AA et al. Necrolytic acral erythema: a variant of necrolytic migratory erythema or a distinct entity? Int J Dermatol. 2005;44(11):916-21.
13. Najarian DJ et al. Zinc deficiency associated with necrolytic acral erythema. J Am Acad Dermatol. 2006;55(5 Suppl):S108-10.
14. Bentley D et al. Lack of classic histology should not prevent diagnosis of necrolytic acral erythema. J Am Acad Dermatol. 2009;60(3):504-7.

15. Fielder LM et al. Necrolytic acral erythema: case report and review of the literature. Cutis. 2008;81(4):355-60.
16. Liu A et al. Necrolytic acral erythema: a case not associated with hepatitis C infection. Dermatol Online J. 2008;14(6):10.
17. Najarian DJ et al. Hypozincemia and hyperzincuria associated with necrolytic acral erythema. Int J Dermatol. 2008;47(7):709-11.
18. de Carvalho Fantini B et al. Necrolytic acral erythema successfully treated with oral zinc. Int J Dermatol. 2008;47(8):872-3.
19. Manzur A and Siddiqui AH. Necrolytic acral erythema: successful treatment with topical tacrolimus ointment. Int J Dermatol. 2008;47(10):1073-5.
20. Wu YH et al. Necrolytic acral erythema without hepatitis C infection. J Cutan Pathol. 2009;36(3):355-8.
21. Nikam BP. Necrolytic acral erythema seronegative for hepatitis C virus--two cases from India treated with oral zinc. Int J Dermatol. 2009;48(10):1096-9.
22. Zeller S et al. Necrolytic acral erythema associated with hepatitis C and serum zinc deficiency. J Am Acad Dermatol. 2009;60(3):AB52.
23. Halpern AV et al. Necrolytic acral erythema: an expanding spectrum. Cutis. 2009;84(6):301-4.
24. Tabibian J et al. Necrolytic acral erythema as a cutaneous marker of hepatitis C: report of two cases and review. Dig Dis Sci. 2010;55:2735-43.
25. Panda S, Lahiri K. Seronegative necrolytic acral erythema: a distinct clinical subset? Indian J Dermatol. 2010;55(3):259-61.
26. Patel U et al. Necrolytic acral erythema. Dermatol Online J. 2010;16(11):15.
27. Kapoor R, Johnson RA. Necrolytic acral erythema. N Engl J Med. 2011;364(15):1479-80.
28. Ridder K et al. Necrolytic acral erythema in an adolescent. Pediatr Dermatol. 2011;28(6):701-6.
29. Grauel E et al. Necrolytic acral erythema. J Drugs Dermatol. 2012;11(11):1370-1.
30. Raphael BA et al. Low prevalence of necrolytic acral erythema in patients with chronic hepatitis C virus infection. J Am Acad Dermatol. 2012;67(5):962-8.
31. Yost JM et al. Necrolytic acral erythema. Dermatol Online J. 2013;19(12):20709.
32. Pernet C et al. Necrolytic acral erythema following hepatitis B vaccination. Br J Dermatol. 2014;171(5):1255-6.
33. Iyengar S et al. Necrolytic acral erythema masquerading as cellulitis. Dermatol Online J. 2014;20(11):15.
34. Das A et al. Necrolytic acral erythema in the absence of hepatitis C virus infection. Indian J Dermatol. 2016;61(1):96-9.
35. Hou YC, Wu CY. Zinc-responsive necrolytic acral erythema in a patient with psoriasis: a rare case. Int J Low Extrem Wounds. 2016;15(3):260-2.
36. Botelho LF et al. Necrolytic acral erythema: a rare skin disease associated with hepatitis C virus infection. An Bras Dermatol. 2016;91(5):649-51.
37. Srisuwanwattana P, Vachiramon V. Necrolytic acral erythema in seronegative hepatitis C. Case Rep Dermatol. 2017;9(1):69-73.
38. Dabas G et al. Necrolytic acral erythema leading to diagnosis of chronic hepatitis C. Dig Liver Dis. 2018;50(8):854.
39. Xiaoling Y et al. Image gallery: seronegative necrolytic acral erythema. Br J Dermatol. 2018;179(2):e88.
40. Xue R et al. Necrolytic acral erythema in a Chinese patient with hepatitis C and hepatitis B virus coinfection. An Bras Dermatol. 2019;94(4):446-8.
41. Oikonomou KG et al. Necrolytic acral erythema in a human immunodeficiency virus/hepatitis C virus coinfected patient: a case report. World J Hepatol. 2019;11(2):226-33.
42. Pathania YS, Budania A. Rivaroxaban induced necrolytic acral erythema. Postgrad Med J. 2019;95(1128):563.
43. Fukushima H et al. Zinc-responsive necrolytic acral erythema in ovarian cancer. J Dermatol. 2020;47(7):e266-7.
44. Kumar R et al. Necrolytic acral erythema in seronegative hepatitis C patient with vitamin B12 deficiency. Indian Dermatol Online J. 2020;11(2):278-9.
45. Parihar AS et al. Necrolytic acral erythema in association with hypothyroidism. JAMA Dermatol. 2020;156(11):1268-70.
46. Shah P et al. Necrolytic acral erythema in a patient with sarcoidosis. JAAD Case Rep. 2020;6(11):1162-4.
47. Davis S, Creditt A. Bilateral foot skin eruption in a hepatitis C patient. Clin Pract Cases Emerg Med. 2020;4(3):491-2.
48. Sims OT et al. Racial disparities in hepatitis C treatment eligibility. Ann Hepatol. 2017;16(4):530-7.
49. Plaza JA, Prieto VG, "Inflammatory skin conditions," Weidner N et al (eds.), Modern Surgical Pathology, (2009) 2nd edition, Philadelphia: Saunders.
50. Foss MG, Ferrer-Bruker SJ, Necrolytic migratory erythema, (2020) Treasure Island, Florida: StatPearls Publishing.

Gastrointestinal Manifestations and Liver Injury: Correlation with Mortality and Clinical Outcomes in Patients with COVID-19

Authors: Michael Makar,[1] Carlos D. Minacapelli,[2] Kapil Gupta,[2] Abhishek Bhurwal,[2] You Li,[2] Carolyn Catalano,[2] Romy Bareket,[3] Samuel Jo,[1] Abhishek A. Chouthai,[3] *Vinod Rustgi[2,4]

1. Department of Internal Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA
2. Department of Medicine, Division of Gastroenterology and Hepatology, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA
3. Rutgers Robert Wood Johnson Medical School, New Jersey, USA
4. Center for Liver Diseases and Masses, Rutgers Robert Wood Johnson School of Medicine, New Brunswick New Jersey, USA

*Correspondence to vinod.rustgi@rutgers.edu

Disclosure: The authors have declared no conflicts of interest. The study was reviewed and approved for publication by the authors' Institutional Reviewer.

Acknowledgements: All authors contributed to all four criteria, concept and design, data acquisition, statistical analysis, and drafting of manuscript. The authors thank data collection assistance by Vinit Palayekar (MD candidate), Daniel Kats (MD candidate), and Robert Gaffey (MD).

Received: 19.09.20

Accepted: 24.11.20

Keywords: Coronavirus disease (COVID-19), gastrointestinal (GI) manifestations, intensive care unit (ICU), liver Injury (LI), mortality.

Citation: EMJ Hepatol. 2021;DOI/10.33590/emjhepatol/20-00233.

Abstract

Background and aims: Reports indicate that patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection present with gastrointestinal (GI) manifestations and abnormal liver function; however, the impact on clinical findings is unclear. The aim of this study is to report the impact of gastrointestinal and liver injury (LI) associated with coronavirus disease (COVID-19).

Material and methods: The authors retrospectively evaluated patients who presented to the emergency department and were diagnosed with COVID-19 by PCR nasopharyngeal swab. Primary outcomes were the impact of GI findings and LI on in-hospital mortality. Secondary outcomes were length of stay in hospital and need for intensive care unit (ICU) level care.

Results: Of the entire cohort, 64.0% had LI during hospitalisation. LI was present in 88.7% of those who died. In multivariate analysis, GI manifestations were associated with decreased risk of mortality (odds ratio [OR]: 0.46; 95% confidence interval [CI]: 0.24–0.90; p=0.002). GI symptoms did not impact length of stay, 5.06 days for patients with GI symptoms versus 4.74 in patients without (OR: 1.01; 95% CI: 0.96–1.07; p=0.43), or need for ICU (OR: 0.97; 95% CI: 0.60–1.58; p=0.9076) (Table 1). In multivariate analysis, LI was associated with increased mortality (OR: 8.60; 95% CI: 3.49–21.15;

p<0.0001), need for ICU (OR: 10.94; 95% CI: 4.07–29.45; p<0.0001), and length of stay 5.87 days versus 3.01 days (OR: 1.23; 95% CI: 1.14–1.32; p<0.0001).

Conclusion: The results of the data analysis show that GI symptoms may inversely correlate with mortality, while LI is associated with increased mortality, length of stay, and ICU admission.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) arose in December 2019 in Wuhan, China, and was noted to cause a severe respiratory distress syndrome termed coronavirus disease (COVID-19).[1] In March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic and it has subsequently become a global public health and economic threat.[2] As of September 2020, there were 30,055,710 confirmed cases and 943,433 confirmed deaths across 216 countries and territories globally.[3] The full genome sequencing and protein sequence analysis of the COVID-19 virus revealed that it shares 79.5% sequence identity with SARS-CoV and is classified as a SARS-related coronavirus (CoV).[4] In the past 20 years, specifically two epidemics caused by CoV have occurred: SARS-CoV in China 2002–2003 (8,000 cases, 800 deaths, fatality rate of 9.6%) and the 2012 Middle East respiratory syndrome (MERS)-CoV in Saudi Arabia (2,500 cases, 800 deaths, fatality rate of 35.0%).[1]

SARS-CoV-2 is a positive-stranded, enveloped, RNA virus with a nucleocapsid, which belongs to the *Coronaviridae* family. SARS-CoV-2 is able to produce nonstructural proteins (nsps; nsp 1–16) and structural proteins: envelopes, nucleocapsid proteins, and spikes.[1,5] The virulence of SARS-CoV-2 links to its nsp and structural proteins. The nsps have been hypothesised to play a role in halting innate immunological response, while the structural proteins may play a role in the pathogenicity of SARS-CoV-2 by mediating viral assembly and release.[1] The structural spike glycoproteins (subunits S1 and subunit S2) have a receptor binding domain, which acts as a binding site for the human angiotensin converting enzyme 2 receptor (ACE2R). The receptor binding domain spike thereby can bind to ACE2R in expressing tissue.[1]

The ACE2R is part of a circulating hormonal system, the renin–angiotensin system (RAS), which plays a significant role in blood pressure and fluid balance regulation homeostasis.[6] Organ-specific RAS activity promotes tissue growth, tissue differentiation, and local inflammation.[7] ACE2R specifically has been found to be expressed in the epithelial tissue of the oral mucosa, lung (Type II alveolar cells), oesophagus (oesophageal epithelial cells), ileum (absorptive enterocytes), colon (absorbtive enterocytes), kidney (proximal tubule cells), and bladder (urothelial bladder cells), and the pancreas.[8] SARS-CoV-2 enters the lung parenchyma by utilising the Type II alveolar cells and mediates entry by binding to the ACE2R and is subsequently cell free and/or macrophage-phagocytosed, and can thereby spread to other organs that may also express the ACE2R, yielding multiorgan injury.[4] Coronaviruses can cause a broad spectrum of disease, including but not limited to respiratory, enteric, hepatic, and neurological disease.[1,4,8,9] Clinically, COVID-19 is characterised as either asymptomatic, uncomplicated mild illness (symptoms of upper respiratory infection, malaise, headache, myalgia, anosmia, nausea, vomiting, diarrhoea), moderate-to-severe pneumonia. On the other end of the spectrum, severe COVID-19 can result in respiratory failure and acute respiratory distress syndrome requiring mechanical ventilation, to multiorgan and systemic disease, including sepsis, septic shock, and multiple organ dysunction syndrome.[1]

Currently, literature reviewing COVID-19 demonstrates the multiorgan impact of COVID-19. Gastrointestinal (GI) manifestations and liver injury (LI) are increasingly recognised findings in patients with COVID-19. Initial studies have reported digestive symptoms to be present in approximately 40–50% of patients.[10,11] In a meta-analysis of patients with COVID-19, GI symptoms had a prevalence rate of 9.8% in adults and 9.6% in children;[12] this included diarrhoea, nausea, vomiting, abdominal discomfort, and abdominal pain. SARS-CoV-2 RNA was detected in the faeces of 30.3% of patients positive for COVID-19.[12] Diarrhoea is the most common GI symptom and has been reported in as many as

36% of patients.[13] However, there is a significant variation in studies regarding the prevalence and impact of GI symptoms in COVID-19.[14] The impact of GI symptoms on clinical outcomes is also unclear. Studies have shown an association between abnormal liver enzymes and the severity of COVID-19.[9] In addition, LI has been associated with adverse clinical outcomes and mortality.[15]

At present, studies are needed to identify the prevalence and clinical impact of GI and LI findings in patients with COVID-19. Therefore, this study reports the prevalence and clinical impact of GI and LI associated with COVID-19 in the highly diverse patient population seen in the USA.

MATERIALS AND METHODS

This is an institutional review board-approved retrospective cohort study performed at Rutgers Robert Wood Johnson University Hospital in New Brunswick, New Jersey, USA. All adult patients (aged ≥18 years) who presented to the emergency department between January 1, 2020 and April 30, 2020 and had a confirmed COVID-19 diagnosis by PCR nasopharyngeal swab testing were included. Patients who did not have a positive test in the hospital were excluded from the analysis. Data were extracted from patients' medical records using a standardised abstraction format. GI manifestations were defined as nausea, vomiting, abdominal pain, or diarrhoea. LI was defined as an elevation in liver enzymes (aspartate transaminase [AST] >45, alanine aminotransferase [ALT] >46, alkaline phosphatase >120, or total bilirubin >0.8).[16] Patients who had GI manifestaitons or liver abnormalities on admission were included in the analysis. The primary clinical outcome was the impact of GI findings and LI on in-hospital mortality. Secondary outcomes were length of stay in hospital and need for intensive care unit (ICU) level care.

Statistical Analysis

Statistical analysis was conducted using the SAS 9.4 programme. The categorical variables were presented as frequency with percentages, and continuous variables as means with standard deviations. Categorical and continuous variables to compare patients with GI symptoms versus patients without GI symptoms were made by using chi-square and student t-test. All odds ratios (OR) with 95% confidence intervals (CI) were calculated by using logistic regression models adjusting for demographics including age, sex, race, clinical characteristics, and medical comorbidities according to the charlson comorbidity index. A p value <0.05 was considered statistically significant.

RESULTS

A total of 800 patients' records were reviewed manually, of which 539 patients had presence of SARS-CoV-2 confirmed in the authors' hospital and were included in the study. Demographics of the entire cohort are described in Table 1. The mean age was 60.48 years, average BMI was 28.79, and 56.2% of patients were male; 34.2% of patients were Caucasian, 12.6% African American, and 24.9% Hispanic.

Overall mortality was 97/539 (18%). At least one digestive complaint on admission was seen in 31% of patients. At presentation, 17.6% had diarrhoea, 14.4% had nausea, 10.3% had vomiting, and 5.9% had abdominal pain. Of the entire cohort, 64.0% had LI during hospitalisation, and LI was present in 88.7% of those who died (Table 1).

In multivariate analysis, GI manifestations were associated with decreased risk of mortality (OR: 0.46; 95% CI: 0.24–0.90; p=0.0020). GI symptoms did not impact length of stay, 5.06 days for patients with GI symptoms versus 4.74 in patients without (OR: 1.01; 95% CI: 0.96–1.07; p=0.43), or need for ICU (OR: 0.97; 95% CI: 0.60–1.58; p=0.9076) (Table 2). In multivariate analysis, LI was associated with increased mortality (OR: 8.60; 95% CI: 3.49–21.15; p<0.0001), need for ICU (OR: 10.94; 95% CI: 4.07–29.45; p<0.0001), and length of stay 5.87 days versus 3.01 days (OR: 1.23; 95% CI: 1.14–1.32; p<0.0001).

The impact of COVID-19 on LI and trend of liver function tests are documented in Table 3. AST and ALT were elevated on admission, with mean values of 68.8 IU/L and 50.2 IU/L, respectively. On admission, 44.0% had abnormal AST and 27.1% had abnormal ALT. At peak values during hospitalisation, 55.5% had abnormal AST and 39.9% had abnormal ALT.

Table 1: Demographics and clinical characteristics.

Patient characteristics	Patients n=539
Age, mean (SD)	60.48 (18.7)
BMI, mean (SD)	28.79 (7.1)
Sex, n (%)	
Male	303 (56.2)
Female	236 (43.8)
Race/ethnicity, n (%)	
Caucasian	185 (34.2)
African American	68 (12.6)
Hispanic	134 (24.9)
South Asian	30 (5.6)
East Asian	38 (7.1)
Other	84 (15.6)
Type of stay, n (%)	
Triage	45 (8.4)
Observation	72 (13.4)
Inpatient	422 (78.3)
Gastrointestinal symptoms, n (%)	
Nausea	78 (14.5)
Vomiting	56 (10.4)
Diarrhoea	95 (17.6)
Abdominal pain	32 (5.9)

Table 2: Impact of gastrointestinal manifestations and liver injury on mortality and clinical outcomes.

Outcomes	GI symptoms (n=165)	No GI symptoms (n=374)	OR (95% CI)	p value
Mortality, n (%)	17 (10.3)	80 (21.4)	0.46 (0.24–0.90)	0.0020
Length of hospital stay, mean (SD)	5..06 (4.6)	4.74 (4.2)	1.01 (0.96–1.07)	0.4302
ICU, n (%)	28 (17)	65 (17.4)	0.90 (0.51–1.59)	0.9076
Outcomes	**Liver injury (n=345)**	**No liver injury (n=194)**	**OR (95% CI)**	**p value**
Mortality, n (%)	86 (24.9)	11 (5.7)	8.60 (3.49–21.15)	<0.0001
Length of hospital stay, mean (SD)	5.87 (4.5)	3.01 (3.3)	1.23 (1.14–1.32)	<0.0001
ICU, n (%)	86 (24.9)	7 (3.6)	10.94 (4.07–29.45)	<0.0001

CI: confidence interval; GI: gastrointesinal; ICU: intensive care unit; OR: odds ratio; SD: standard deviation.

Table 3: Trend of liver laboratory test.

	Mean initial (SD)	Abnormal initial*, n (%)	Mean peak† (SD)	Abnormal peak†, n (%)	Mean trough† (SD)	Abnormal trough†, n (%)
AST (IU/L)	68.8 (163.2)	237 (44)	160.4 (590.3)	299 (55.5)	N/A	N/A
ALT (IU/L)	50.2 (96.8)	146 (27.1)	110.96 (399.1)	215 (39.9)	N/A	N/A
ALP (IU/L)	83.0 (51.9)	54 (10.0)	97.28 (64.2)	101 (18.7)	N/A	N/A
Albumin (g/dL)	3.6 (1.3)	245 (45.5)	N/A	N/A	3.1 (0.8)	411 (76.3)
Bilirubin (mg/dL)	0.5 (0.3)	61 (11.3)	N/A	N/A	N/A	N/A

*initial = on admission.

†= peak/trough during hospitalisation

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate transaminase; SD: standard deviation.

DISCUSSION

Gastrointestinal Manifestations

The results of this study indicate that GI symptoms are inversely associated with the risk of mortality. In total, 30.6% of patients in the cohort had GI symptoms on admission. In the patients who died, 10.3% had GI symptoms, whereas GI symptoms were present in 21.4% of those who survived, for an OR of 0.46.

Published literature has provided mixed results. A study published in China reported that digestive symptoms were more pronounced as the severity of COVID-19 increased.[13] A meta-analysis confirmed similar results in that patients with severe or critical disease were more likely to have GI symptoms. However, the risk of severe disease was not associated with GI symptomology.[17] No differences in mortality or length of stay were reported between the two groups. Hajifathalian et al.[15] showed that the presence of GI symptoms on initial presentation had no significant effect on mortality in a cohort of patients in the USA.[15] In addition, these patients had lower rates of death and ICU admissions. Further, Nobel et al.[18] demonstrated that GI symptoms were associated with longer duration of illness and a trend toward lower ICU admission rates and lower mortality after a median 8-day follow-up in the outpatient setting. These findings may suggest that patients with digestive symptoms are less likely to have respiratory symptoms. Conversely, in a recent systematic review and meta analysis of COVID-19 patients by Tariq et al.,[19] 21 studies, totalling 4,982 patients, reported mortality as an outcome in patients with GI symptomology. Tariq et al.[19] found that one in five patients report GI symptomology and found that patients with GI manifestations had similar overall mortality to those who did not. Furthermore, in a systematic review by Gul et al.,[20] revealed that patients with GI symptoms of COVID-19 did not have an increased risk of mortality but may be at higher risk to develop acute respiratory distress syndrome. Cao et al.,[21] demonstrated that GI symptomology are not associated with severe disease or worse outcomes, but cautioned the presence of SARS-CoV-2 RNA in faeces after attenuation of respiratory symptoms. In addition, previous studies have also demonstrated no further increased risk of COVID-19-associated mortality in patients with inflammatory bowel disease.[22,23]

SARS-CoV-2 RNA has previously been identified by several studies in the stool samples of patients with COVID-19. Wu et al.[9] demonstrated the presence of SARS-CoV-2 RNA in stool samples

after respiratory samples tested negative and respiratory illness subsided. This could indicate the viability of COVID-19 in the GI tract. It remains to be demonstrated whether pulmonary manifestations and higher resultant mortality rates are 'attenuated' by viral shedding via the GI tract. It should be recongised that symptoms may be under-reported in earlier studies due to incomplete questionaires and may be higher prevalence today due to increasing awareness of GI manifestations of COVID-19 and more comprehensive review of systems in recent hospitalisations. Initial data were collected through subjective reports of GI symptomology while specific GI testing to date has occurred in limited studies, i.e., labarotory testing, imaging, endoscopic evaluation, and histopathology.

Liver Injury

This study indicates that LI was associated with a significantly increased risk of mortality, need for ICU admission, and length of stay. Of the patients who died, 88.7% had LI, whereas only 64.0% of patients had abnormal liver enzymes during hospitalisation. Further, patients had increasing LI during their hospitalisation as they had rising mean elevations in liver enzymes and increased LI (Table 3). Interestingly, mean AST results were higher than ALT values at initial presentation as well as at peak values.

Thirteen studies reported the association between LI and severity of the disease but only reported univariate analysis.[14] Hajifathalian et al.[15] performed a multivariate analysis that reported an association between LI on presentation and a significantly higher risk of ICU admission and death.[15] Previous studies alongside these findings suggest that liver enzyme abnormalities may portend worse outcomes and increased mortality. In a multicentre retrospective cohort study by Lei et al.,[24] elevated LI, particularly AST, was strongly associated with increased mortality risk and was associtated with decreased lymphocyte count, neutrophilia, and male sex. In a systematic review by Ghoda et al.,[25] LI correlated with severity of COVID-19 illness.

Liver damage may be ascribed to direct viral infection of hepatocytes and cholangiocytes, mitochondrial injury, drug hepatotoxicity (drug-induced LI), immune-mediated inflammation, cytokine-mediated injury, sepsis, and liver failure in patients who are critically ill.[26] Microthrombi, decreased perfusion, and pneumonia-induced hypoxaemia may also contribute to LI.[27] Autopsy results from patients with the 2002–2003 SARS-CoV demonstrated that SARS-CoV was directly able to induce cytopathic damage to hepatocytes rather than damage elicited by cytokine damage secondary to sepsis. A recent study by Alqahtani and Schattenberg that overviewed pathological findings of liver biopsy specimens of patients with COVID-19 demonstrated inflammation in the lobular and portal regions of the liver and microvesicular steatosis, both of which are nonspecific injuries to the liver and may be secondary to sepsis or drug-induced LI.[28] Caveat to elevations in transaminases, specifically AST, is the possibility of muscle injury.[29]

Literature demonstrated higher presence of ACE2R expression in cholangiocytes in comparison with hepatocytes, and as a result, cholangiocytes may be responsible for susceptability and injury of the hepatobiliary system secondary to COVID-19.[30] This may include interrupting liver physiology including, but not limited to, adaptive immune response mechanism, regeneration, and basal function, i.e., dysregulation of formation and transport of bile.[28] Three studies that analysed a cholestatic-specific marker, gamma glutamyl transferase, support hepatobiliary disruption mediated by COVID-19.[31-33] As current COVID-19 data are limited in breadth, future studies and prospective cohorts should incorporate specific markers, for example gamma glutamyl transferase, to determine and confirm specific ramifications of the viral infection in the hepatobiliary system.

LI data from COVID-19 do not take into account pre-existing liver disease or follow-up of LI after acute hospitalisation. Further analaysis of patients with pre-existing disease and disease prognosis and outcomes subsequently after COVID-19 infection must be evaluated in future research. Limitations of this study include that it's a retrospective study and single-centre analysis. This analysis included a relatively small cohort of patients but included a diverse population.

CONCLUSION

GI findings and LI are common in hospitalised patients with COVID-19. GI symptoms may

inversely correlate with mortality, while LI is associated with increased mortality, length of stay, and ICU admission. Further study is needed on the mechanisms of these findings along with prospective studies to determine the long-term implications of COVID-19 in the GI and hepatobiliary systems.

References

1. Cascella M et al. Features, evaluation, and treatment of coronavirus. In: StatPearls [Internet]. 2020. Treasure Island (FL): StatPearls Publishing.
2. Balkhair AA. COVID-19 pandemic: a new chapter in the history of infectious diseases. Oman Med J. 2020;35(2):e123.
3. World Health Organization (WHO). Coronavirus disease (COVID-19) pan-demic. 2020. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Last accessed: 26 Jan 2021.
4. Ni W et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care. 2020;24(1):422.
5. Lei J et al. Nsp3 of coronaviruses: structures and functions of a large multi-domain protein. Antiviral Res. 2018;149:58-74.
6. Cole-Jeffrey CT et al. ACE2 and microbiota: emerging targets for cardio-pulmonary disease therapy. J Cardiovasc Pharmacol. 2015;66(6):540-50.
7. Fliser D et al.; EUropean Trial on Olmesartan and Pravastatin in Inflamma-tion and Atherosclerosis (EUTOPIA) Investigators. Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. Circulation. 2004;110(9):1103-7.
8. Xu J et al. Digestive symptoms of COVID-19 and expression of ACE2 in digestive tract organs. Cell Death Discov. 2020;6(1):1-8.
9. Wang D et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-9.
10. Pan L et al. Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. Am J Gastroenterol. 2020;115(5):766-73.
11. Villapol S. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. Transl Res. 2020;226:57-69.
12. Rokkas T. Gastrointestinal involvement in COVID-19: a systematic review and meta-analysis. Ann Gastroenterol. 2020;33(4):355-65.
13. Cheung KS et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from a Hong Kong cohort: systematic re-view and meta-analysis. Gastroenterology. 2020;159(1):81-95.
14. Sultan S et al. AGA institute rapid review of the gastrointestinal and liver manifestations of COVID-19, meta-analysis of international data, and recommendations for the consultative management of patients with COVID-19. Gastroenterology. 2020;159(1):320-34.e27.
15. Hajifathalian K et al. Gastrointestinal and hepatic manifestations of 2019 novel coronavirus disease in a large cohort of infected patients from New York: clinical implications. Gastroenterology. 2020;159(3):1137-40.e2.
16. Malakouti M et al. Elevated liver enzymes in asymptomatic patients – what should i do? J Clin Transl Hepatol. 2017;5(4):394-403.
17. Mao R et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2020;5(7):667-78.
18. Nobel YR et al. Gastrointestinal symptoms and coronavirus disease 2019: a case-control study from the United States. Gastroenterology. 2020;159(1):373-5.e2.
19. Tariq R et al. Prevalence and mortality of COVID-19 patients with gastrointestinal symptoms: a systematic review and meta-analysis. Mayo Clin Proc. 2020;95(8):1632-48.
20. Gul F et al. Meta-analysis of outcomes of patients with COVID-19 infection with versus without gastrointestinal symptoms. Proc (Bayl Univ Med Cent). 2020;33(3):366-9.
21. Cao C et al. Clinical features and outcomes of COVID-19 patients with gastrointestinal symptoms. Crit Care. 2020;DOI:10.1186/s13054-020-03034-x. [Epub ahead of print].
22. Taxonera C et al. 2019 novel coronavirus disease (COVID-19) in patients with inflammatory bowel diseases. Aliment Pharmacol Ther. 2020;52(2):276-83.
23. Bezzio C et al. Outcomes of COVID-19 in 79 patients with IBD in Italy: an IG-IBD study. Gut. 2020;69(7):1213-7.
24. Lei F et al. Longitudinal association between markers of liver injury and mortality in COVID-19 in China. Hepatology. 2020;72(2):389-98.
25. Ghoda A, Ghoda M. Liver injury in COVID-19 infection: a systematic review. Cureus. 2020;12(7):e9487.
26. Li J, Fan J-G. Characteristics and mechanism of liver injury in 2019 corona-virus disease. J Clin Transl Hepatol. 2020;8(1):13-7.
27. Pawlotsky J-M. COVID-19 and the liver-related deaths to come. Nat Rev Gastroenterol Hepatol. 2020;17(9):1-3.
28. Alqahtani SA, Schattenberg JM. Liver injury in COVID-19: the current evi-dence. United European Gastroenterol J. 2020;8(5):509-19.
29. Jin M, Tong Q. Rhabdomyolysis as potential late complication associated with COVID-19 Emerg Infect Dis. 2020;26(7):1618-20.
30. Chai X et al. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. bioRxiv. 2020;DOI:10.1101/2020.02.03.931766. [Preprint].
31. Fan Z et al. Clinical features of COVID-19-related liver functional abnormality. Clin Gastroenterol Hepatol. 2020;18(7):1561-6.
32. Zhang C et al. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5(5):428-30.
33. Xu L et al. Liver injury during highly pathogenic human coronavirus infections. Liver Int Off J Int Assoc Study Liver. 2020;40(5):998-1004.

Difficult Biliary Cannulation in Endoscopic Retrograde Cholangiopancreatography: Definitions, Risk Factors, and Implications

Authors: Brian M. Fung,[1,2] Teodor C. Pitea,[2] *James H. Tabibian[3,4]

1. Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Arizona College of Medicine – Phoenix, Arizona, USA
2. Banner – University Medical Center Phoenix, Arizona, USA
3. Division of Gastroenterology, Department of Medicine, Olive View-UCLA Medical Center, Sylmar, California, USA
4. Vatche and Tamar Manoukian Division of Digestive Diseases, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

*Correspondence to jtabibian@dhs.lacounty.gov

Disclosure: The authors have declared no conflicts of interest.

Author contributions: Fung reviewed the literature for relevant original studies and other content, designed the figures, and drafted the manuscript. Pitea and Tabibian critically reviewed the manuscript. All authors provided critical input and approved the final version of the manuscript.

Acknowledgements: This work was supported in part through the United States National Institutes of Health (NIH) grant UL1 TR000135.

Received: 09.01.21

Accepted: 29.03.21

Keywords: Adverse effects, endoscopic retrograde cholangiopancreatography (ERCP), guidewire-assisted cannulation, post-ERCP pancreatitis, selective biliary cannulation.

Citation: EMJ Hepatol. 2021;9[1]:64-72.

Abstract

Over the past 50 years, endoscopic retrograde cholangiopancreatography (ERCP) has become the preferred minimally invasive method of treating a vast array of pancreatobiliary diseases. An initial *sine qua non* for ERCP success is selective ductal cannulation. Despite significant progress in the optimisation of ERCP methods and accessories, selective biliary cannulation using conventional techniques remains unsuccessful in approximately 15% of native papilla cases. Furthermore, difficult biliary cannulation has been associated with an increased risk of post-ERCP pancreatitis, among other adverse events. Here, in the first of a two-part series, the authors provide a primer on standard biliary cannulation techniques and discuss the definition, risk factors, and implications of difficult biliary cannulation. The second part of the series will provide an overview of the existing advanced techniques used in cases of difficult biliary cannulation as well as the approach to their selection.

INTRODUCTION

In 1968, McCune et al. from George Washington University reported the first cohort of successful endoscopic biliary cannulation using an Eder fibreoptic duodenoscope with a forward and side lens.[1] Although this initial technique was only successful in <50% of attempts, it is considered by many to be the first description of endoscopic retrograde cholangiopancreatography

(ERCP). A year later, a side-viewing fibreoptic duodenoscope was developed in Japan by Oi et al. and was able to improve cannulation success rates to 77%.[2] Over the next few years, multiple endoscopists, including Classen, Cotton, Demling, Geenen, Safrany, Siegla, Silvis, and Vennes, experimented on and improved this new technique, and by 1974, the first successful cases of endoscopic biliary sphincterotomy were reported, further paving the foundation for ERCP.[3-5] These innovations, along with many other technical advances, have since transformed ERCP from a primarily diagnostic modality into an effective therapeutic technique that is now the preferred minimally invasive treatment for many pancreaticobiliary diseases. However, despite significant technological advances, ERCP is still considered a challenging procedure for many gastrointestinal endoscopists, in large part because of the failure of biliary cannulation in 5–15% of cases as well as the risk of post-ERCP pancreatitis (PEP) and other adverse events (AE).[6,7]

Here, in the first of a two-part series, the authors provide an overview of standard biliary cannulation techniques in patients with normal as well as altered anatomy and discuss the definition, predictors, and implications of difficult biliary cannulation. The second part of the series will provide an overview of the existing advanced techniques used in cases of difficult biliary cannulation as well as the approach to their selection.

STANDARD TECHNIQUE FOR SELECTIVE BILIARY CANNULATION

In the standard approach to selective biliary cannulation, a side-viewing endoscope (i.e., duodenoscope) is first advanced to the second part of the duodenum, wherein the major duodenal papilla is usually located in the middle third (but sometimes more proximally or distally), along the medial aspect. The duodenoscope is then positioned so that the lens and working channel are located below the major papilla. Finding the major papilla can sometimes be challenging, e.g., in the case of an intradiverticular papilla (discussed later), severe duodenal inflammation, or portal hypertensive duodenopathy, and what might initially appear to be the major papilla may not always be so (instead can be a prominent minor papilla, subepithelial lesion, or other). In general, the major papilla can be distinguished from the minor papilla based on its characteristic mucosal folds, larger size, and location approximately 2 cm distal to the minor papilla.

Once the major papilla is confirmed, the duodenoscope is appropriately positioned for cannulation in the vector (i.e., x, y, z co-ordinates) of the ampulla and distal bile duct. A variety of manoeuvres are employed to align the sphincterotome for proper insertion; these can be conceptually organised into direct manipulation of the sphincterotome (e.g., bowing the tip of the sphincterotome via traction, using the elevator lever to lift the sphincterotome); manipulation of the endoscope (i.e., up/down dial, left/right dial, torquing the scope, advancing or pulling back the scope); and patient-level adjustments (e.g., changes in patient position, variable insufflation of the gastrointestinal lumen, external abdominal pressure). Once aligned, a sphincterotome or other cannulation catheter is then engaged into the orifice of the major duodenal papilla, aiming usually towards the 11 o'clock position (as compared to 2 o'clock for pancreatic duct cannulation). Historically, a cannulation catheter was the first choice for cannulation given its high flexibility and tip shape compared with the sphincterotome; however, most endoscopists now choose the sphincterotome because of its ability to bow the catheter tip by pulling or relaxing the cutting wire, facilitating alignment with the biliary duct, as well as the ability to perform sphincterotomy. Furthermore, there are data suggesting higher cannulation rates with the use of a sphincterotome over standard catheter cannulation (84–97% versus 62–75%).[8-10]

After initial engagement of (or 'seating' into) the orifice of the major papilla, the sphincterotome is subsequently advanced into the biliary duct with the assistance of either contrast or guidewire. In contrast-assisted cannulation, contrast is injected under fluoroscopy after the tip of the sphincterotome or catheter is inserted into the papillary orifice. This helps to visualise the biliary tree and provides feedback regarding catheter position. However, this technique frequently requires repetitive probing and multiple injections of contrast, factors that have been associated with higher rates of PEP.[11] Thus, guidewire-assisted cannulation has become a

preferred technique for most, as described in the following subsection.

Guidewire-Assisted Cannulation

Guidewire-assisted cannulation is generally the first-line method for selective biliary cannulation.[12] This is largely because of several studies suggesting a lower risk of AEs, particularly PEP, with the use of a guidewire compared to contrast injection-assisted cannulation.[13-16] A systematic review and meta-analysis of 12 randomised controlled trials (RCT) comprising 3,450 participants found that the risk of PEP was significantly reduced when a guidewire was used for cannulation rather than contrast injection (3.4% versus 6.7%; relative risk [RR]: 0.51; 95% confidence interval [CI]: 0.32–0.82).[17] Guidewire-assisted cannulation was also associated with greater primary cannulation success rates (RR: 1.07; 95% CI: 1.00–1.15) and less precut sphincterotomy (RR: 0.75; 95% CI: 0.60–0.95).[17] The reduction in rate of PEP is thought to be in part because of less papillary trauma and the avoidance of contrast, which has been hypothesised to increase hydrostatic pressure within the duct, induce a chemical inflammatory response, as well as introduce bacteria into the pancreatic duct.[18]

In addition to the traditional technique of first inserting and 'seating' the catheter into the papillary orifice and then advancing the wire ('touch' technique), there is also the option of a 'no touch' technique, wherein the guidewire is first advanced a few millimetres out of the sphincterotome and then cannulation is attempted. However, a recent multicentre RCT found that the primary cannulation rate was higher and the mean number of cannulation attempts was lower when the touch technique was used rather than the no-touch technique (88% versus 54%; p<0.001, and 4.6 versus 5.5 attempts; p=0.006, respectively).[19] The rate of AEs did not differ between the two techniques. Despite evidence supporting the use of the touch technique, the no-touch technique may still be useful for small or stenotic papillary orifices.[19] There are also many variations of these two techniques, frequently chosen based on papillary characteristics (e.g., size, position, mobility) and physician comfort.

The most commonly used guidewire is a 0.035 inch diameter, hydrophilic-tipped guidewire.[20] The use of a hydrophilic tip allows for reduced friction during advancement of the guidewire, and a 0.035 inch diameter is thought to allow for easier visualisation during fluoroscopy, among other advantages, including better tactile feel and pushability.[21] However, the use of other guidewire diameters ranging from 0.018 to 0.035 inches may also be used, with two studies finding no difference in cannulation rate between the use of a 0.035 inch and 0.025 inch guidewire.[22,23] Guidewires also come with various tip options, e.g., different shapes. One RCT found no difference in biliary cannulation rate between the use of a straight or angled-tip guidewire, though cannulation time appeared to be shorter with the use of an angled guidewire (20 versus 63 sec; p=0.01).[24] More recently, a study using *in vitro* bile duct models suggested that an angled-tip guidewire had a higher cannulation success rate compared to other types of guidewires.[25] J-tip and loop-tip guidewires have also been evaluated and do not appear to have significantly different biliary cannulation rates than more conventional guidewires.[26,27] Overall, differences in cannulation outcomes attributable to tip shape alone are likely minimal among experienced endoscopists but may be important in early endoscopists and in select scenarios (e.g., variant anatomy).

Despite the widespread use of guidewire-assisted cannulation, it is important to note that not all studies have found a decrease in incidence of PEP with this technique.[28-30] The use of an assistant to manipulate the guidewire may also be associated with a higher rate of complications.[31] Furthermore, the use of a guidewire has its own risks, including the risk of the creation of a false tract, pancreatic duct injury, and perforation.[32] Thus, the potential drawbacks of this technique should be appreciated in order to reduce AE and increase success rates.

DIFFICULT BILIARY CANNULATION: MYRIAD CONSIDERATIONS

Selective biliary cannulation fails in 5–15% of cases, even in the hands of experienced endoscopists.[33] In such scenarios, collectively referred to as 'difficult biliary cannulation', the endoscopist must decide whether to continue with standard

cannulation techniques, switch to more advanced techniques, consult a more senior colleague (if available), or abort the procedure and consider re-attempting at a later time. The urgency of the procedure, duration of the procedure, availability of requisite accessories, logistical and cost factors, and likelihood of increased AE risk are all important considerations in this decision.[11,34,35]

DEFINITION OF DIFFICULT BILIARY CANNULATION

How difficult biliary cannulation is defined varies considerably in the published literature.[36] Most studies define difficult cannulation based on the number of cannulation attempts (typically between five and 15) and/or the time spent on cannulation (typically greater than 5–30 min).[13,29,35] The number of unintentional passages or contrast injections into the pancreatic duct should also be considered, as these correlate with cannulation difficulty and increase the risk of PEP.[37,38] A prospective study found that after five attempts, the risk of PEP increased from 6.1% to 11.9%.[39] The European Society of Gastrointestinal Endoscopy (ESGE) has defined difficult biliary cannulation as the presence of more than five contacts with the papilla while attempting to cannulate, more than 5 min spent attempting to cannulate, or more than one unintended passage or contrast injection into the pancreatic duct.[20,39] This definition was validated in a recent study by Ismail et al.[40] A recent international consensus guideline has suggested a slightly different definition, defining difficult biliary access as the inability to achieve selective biliary cannulation by standard ERCP techniques within 10 min or after up to five cannulation attempts, or the inability to access the major papilla (e.g., because of gastric outlet obstruction or Roux-en-Y anatomy).[41]

In addition to variations in the definition of difficult biliary cannulation, there is also no standardised definition for a cannulation attempt. In a study by Friedland et al., a cannulation attempt was described to be any repositioning or wedging of the cannulation device while attempting biliary cannulation.[42] However, others have defined cannulation attempt as an intentional continuous contact with the papilla; Bailey et al. defined an attempt as sustained contact for at least 5 sec.[11,39] Similarly, and perhaps for this reason, quantification of the number of cannulation attempts may be subjective. A prospective study by Tian et al. found that inter-observer variability in the assessment of cannulation attempts was high, and that cannulation time appeared to be a more objective and accurate tool for assessing cannulation difficulty.[43]

RISK FACTORS FOR DIFFICULT BILIARY CANNULATION

The likelihood of difficult biliary cannulation is based on both operator and patient factors. In the following subsections, the authors describe some of the factors which may impact the risk of difficult biliary cannulation.

Endoscopist Factors

As one may expect, increased experience with ERCP is associated with higher cannulation success rates.[44] A success rate of 80% has been suggested as the goal during ERCP training.[45] This success rate appears to require a minimum of 200 ERCP examinations.[46] One study found that cannulation rate increased from 43% to ≥80% after 350–400 supervised procedures, with success rate continuing to improve to >96% post-training.[47] Routine performance of ERCP also appears to be required to maintain ERCP proficiency, as lower provider volume has been associated with a higher failure rate and a greater need for post-procedure hospitalisation.[48-50] One study showed that a second-attempt ERCP at an ERCP referral centre had a success rate of >95% after having an initial failed attempt at a lower volume hospital.[51]

Patient Factors

The success of selective biliary cannulation fundamentally depends on careful alignment of the sphincterotome (or other cannulating catheter) with the vector of the papillary orifice, hepatopancreatic ampulla, and distal common bile duct (CBD). Thus, variations in these structures or factors that affect visualisation, positioning, and manoeuvrability in this regard can increase cannulation difficulty. A normal papilla has four main anatomical parts: orifice, frenulum, hood, and infundibulum (Figure 1), each of which can vary significantly (Figure 2).[52]

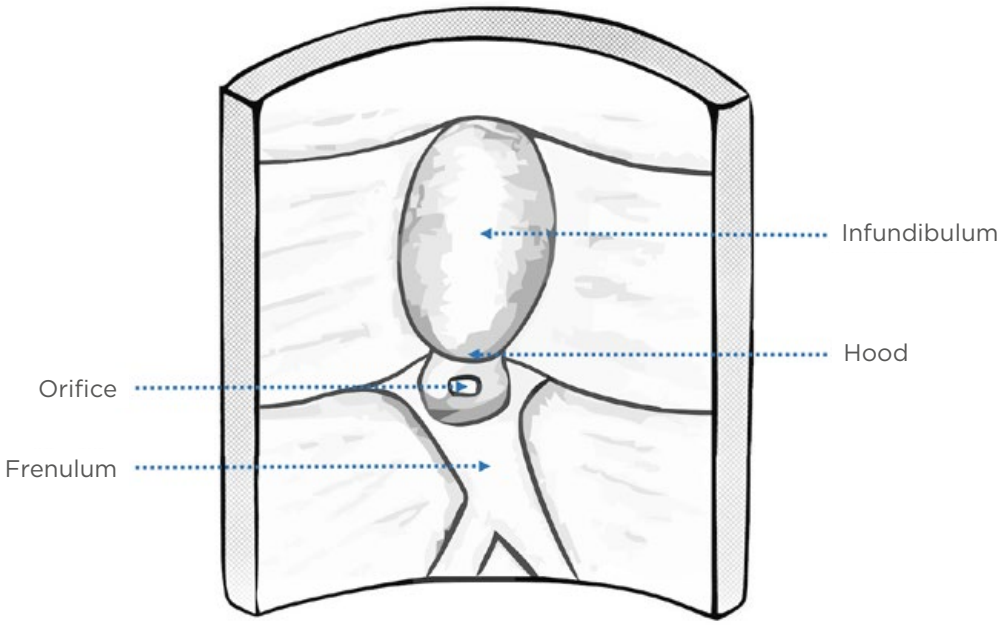


Figure 1: Diagram of normal papillary anatomy showing the four main anatomical features.

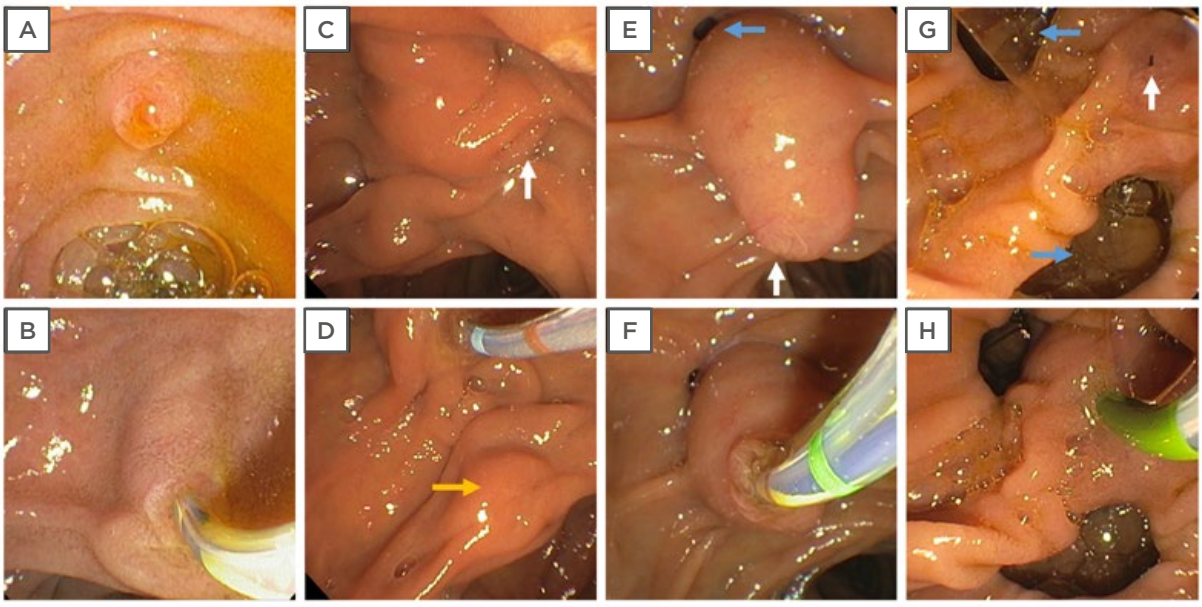


Figure 2: Examples of anatomical variations in the major duodenal papilla.

A) Normal native papilla. **B)** Biliary cannulation is achieved with conventional techniques using a tapered-tip (3.9 Fr outer diameter) sphincterotome. **C)** Papilla with tortuous distal portion of the infundibulum and drooping hood, the latter limiting visualisation of the papillary orifice (white arrow), which is oriented to the right. **D)** Cannulation is achieved using a sphincterotome (4.4 Fr distal tip outer diameter). A submucosal lesion (yellow arrow) is incidentally seen just distal to the papilla and was initially mistaken for the papilla. **E)** Large, protuberant papilla with its orifice oriented downwards and to the left. The papillary appearance is suggestive of an impacted ampullary stone, and a juxtapapillary diverticulum (blue arrow) is also incidentally noted just proximal to the papilla. **F)** Cannulation is readily achieved using a sphincterotome; had this not been successful, a precut suprapapillary fistulotomy could have been performed to release the suspected stone. **G)** A diminutive, flat, interdiverticular papilla, with duodenal diverticula (blue arrows) immediately proximal and distal to it. **H)** Cannulation is achieved using a tapered-tip sphincterotome.

In the following sub-section, the authors describe the papillary characteristics, as well as location, peripapillary findings (e.g., diverticulum, lipoma, duplication cyst), and surgically altered anatomies that can make selective biliary cannulation difficult.

Papillae come in many sizes and morphologies, and a classification system has been previously developed to describe these characteristics and their implications on biliary cannulation.[53] Small papillae are often more difficult to identify (discussed further below), especially when there is significant duodenal oedema, inflammation, or redundant mucosal folds, and thereafter cannulate.[54] A small papilla increases the odds of the sphincterotome coming in contact with the septum instead of insertion into the bile duct, especially if highly compressible, and may make it more difficult to make adjustments to the position of the sphincterotome without losing contact with the papilla. On the other hand, a large papilla may be more relaxed and floppy, providing less stability during cannulation.[55] Protuberant papillae also appear to have higher rates of difficult cannulation, be this because of an impacted stone or otherwise.[55] Advanced age and sex have not been found to be associated with increased rates of difficult biliary cannulation.[56,57]

Although the hepatopancreatic ampulla (and hence the major papilla) usually enters the duodenum in the second portion, it is occasionally located at the superior duodenal angle or in the third portion of the duodenum, making the papilla more difficult to identify and/or reach.[58] The papilla can also be hidden behind overlying folds; lifting the folds gently with the tip of a catheter can sometimes reveal the papilla. In some cases, the papilla can be difficult to locate because of tumour infiltration into the papilla/duodenum or pancreatitis causing oedema of the duodenum.[59] Increased tissue friability and bleeding in cases of malignancy can decrease visibility of the ampulla. Extra-papillary pathology can also decrease access to the papilla. Benign or malignant narrowing of the gastric outlet, duodenal bulb, or second part of the duodenum proximal to the papilla can prevent adequate access and positioning for biliary cannulation.[60] In some cases, endoscopic dilation with or without the placement of a self-expanding metallic stent is first required.[61]

It is known that intradiverticular and peridiverticular papillae can increase cannulation difficulty.[62] One report found the success rate for cannulation of peridiverticular papillae to be 62.4% versus 92.7% when there is no diverticulum.[63] These herniations of the mucosa or submucosa are found in up to 15% of patients undergoing ERCP.[64] When the papilla is found on the edge of or within a diverticulum, it can obscure the papilla or distort its orientation, to the extent where the pancreatic and biliary orifice locations can be reversed. A variety of approaches, including the 'no-touch' technique,[65] have been reported to facilitate cannulation in such cases, depending on the specific nature of the diverticula and the anatomic relation of the major papilla to them.

The level of sedation may also impact cannulation success rate. A case-control study of 31,001 ERCP procedures found that the use of propofol was associated with higher cannulation success rate (89% versus 86.7%; $p<0.0001$) and fewer intra-procedural complications (2.9% versus 3.7%; $p<0.0001$) compared with the use of moderate sedation (midazolam in combination with opioids).[66]

Surgically Altered Anatomy

Patients with surgically altered anatomy such as post Billroth II gastrectomy or post-Roux-en-Y gastric bypass present with a unique set of challenges,[67,68] and ERCP in these patients is generally performed at referral centres by endoscopists who have experience with these types of anatomical alterations.[51,69] In patients who are post Billroth II, a standard duodenoscope can be used because of the short distance to the papilla through the afferent loop. However, determining the afferent from the efferent limb can be challenging, and approaching the papilla from the opposite direction can be disorienting. On the other hand, ERCP in patients who have had a Roux-en-Y requires the use of a longer length endoscope (or a direct transgastric approach) because of the need to transverse a large length of small bowel prior to reaching the descending duodenum. This requires the use of a colonoscope or enteroscope, both of which are forward-viewing instruments that are not designed for ERCP. Similar to Billroth II cases, recognising the proper intestinal lumen and selective cannulation from the opposite direction can be challenging.

IMPLICATIONS OF DIFFICULT BILIARY CANNULATION

PEP is the most common complication of ERCP, resulting in significant cost and morbidity.[6,70] Although minor variations are found in the literature, the ESGE currently defines PEP as new or worsened abdominal pain combined with >3 times the normal value of amylase or lipase at more than 24 hours after ERCP and admission or prolongation of a planned admission.[12] It is thought to occur because of a number of contributing factors, including increased hydrostatic pressure within the duct with contrast, mechanical trauma, thermal injury from electrosurgical current during sphincterotomy, and chemical inflammation from contrast-injection.[18] In meta-analyses, the incidence of PEP varies from 3.5–9.7%, with the majority of cases being mild and a mortality rate of only 0.1–0.7%.[6,70] However, in cases of difficult biliary cannulation, the incidence of PEP is higher (odds ratio: 1.76–14.9),[12] likely because of increased manipulation of the papilla leading to tissue oedema and higher frequency of inadvertent cannulation of the pancreatic duct.

Various measures have been found to help reduce the risk of PEP aside from cannulation technique, such as rectal indomethacin, aggressive intravenous fluid hydration (preferably with lactated Ringer's solution), and pancreatic duct stenting; an overview of these methods can be found in the American Society for Gastrointestinal Endoscopy (ASGE) and ESGE guidelines.[12,71] Of note, pancreatic duct stent placement can also help facilitate bile duct access in cases of difficult biliary cannulation, in addition to reducing the risk of PEP.

Difficult biliary cannulation is also associated with increased risk of bleeding and perforation.[12,72] A case-control study examining ERCP-related perforations found that difficult biliary cannulation was an independent risk factor for perforation.[72] Specifically, periampullary perforation resulting from biliary or pancreatic sphincterotomy or precut techniques (Stapfer Type II perforations) and perforations of the bile duct or pancreatic duct caused by instrumentation or stenting (Stapfer Type III perforations) can occur.[71,73] Thus, difficult biliary cannulation is negatively impactful on a variety of clinical levels.

CONCLUSION

Over the past several decades, ERCP has become an indispensable tool in the diagnosis and treatment of many pancreaticobiliary diseases. However, despite technological advances, it remains a challenging procedure, with selective biliary cannulation being one of the key rate-limiting steps during ERCP. Difficult biliary cannulation, generally defined by the number of cannulation attempts and/or the time spent on cannulation, is more likely to occur in cases where variations in native anatomical structures or surgically altered anatomy affect visualisation, positioning, and manoeuvrability during ERCP. Endoscopist-dependent factors may also affect the risk of difficult biliary cannulation. Despite advances in cannulation techniques, difficult biliary cannulation is often associated with an increased rate of complications, particularly PEP, because of increased manipulation of the papilla and pancreatic duct. The second part of this series will provide an update and overview of the existing advanced techniques used in cases of difficult biliary cannulation as well as the approach to their selection.

References

1. McCune WS et al. Endoscopic cannulation of the ampulla of vater: a preliminary report. Ann Surg. 1968;167(5):752-6.
2. Oi I. Fiberduodenoscopy and endoscopic pancreatocholangiography. Gastrointest Endosc. 1970;17(2):59-62.
3. Classen M, Demling L. Endoskopische sphinkterotomie der papilla Vateri und steinextraktion aus dem ductus choledochus. Dtsch Med Wochenschr. 1974;99(11):496-7. (In German).
4. Kawai K et al. Endoscopic sphincterotomy of the ampulla of Vater. Gastrointest Endosc. 1974;20(4):148-51.
5. McHenry L, Lehman G, "Approaching 50 Years," Kozarek RA, Baron TH (eds.), The Future of ERCP (2017) 3rd edition, Philadelphia, PA: Saunders-Elsevier, pp.1-6.e1.
6. Andriulli A et al. Incidence rates of post-ERCP complications: a systematic survey of prospective studies. Am J Gastroenterol. 2007;102(8):1781-8.

7. Harewood GC, Baron TH. An assessment of the learning curve for precut biliary sphincterotomy. Am J Gastroenterol. 2002;97(7):1708-12.
8. Schwacha H et al. A sphincterotome-based technique for selective transpapillary common bile duct cannulation. Gastrointest Endosc. 2000;52(3):387-91.
9. Cortas GA et al. Selective cannulation of the common bile duct: a prospective randomized trial comparing standard catheters with sphincterotomes. Gastrointest Endosc. 1999;50(6):775-9.
10. Laasch HU et al. Comparison of standard and steerable catheters for bile duct cannulation in ERCP. Endoscopy. 2003;35(8):669-74.
11. Bailey AA et al. Needle-knife sphincterotomy: factors predicting its use and the relationship with post-ERCP pancreatitis (with video). Gastrointest Endosc. 2010;71(2):266-71.
12. Dumonceau JM et al. ERCP-related adverse events: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2020;52(2):127-49.
13. Artifon ELA et al. Guidewire cannulation reduces risk of post-ERCP pancreatitis and facilitates bile duct cannulation. Am J Gastroenterol. 2007;102(10):2147-53.
14. Bailey AA et al. A prospective randomized trial of cannulation technique in ERCP: effects on technical success and post-ERCP pancreatitis. Endoscopy. 2008;40(4):296-301.
15. Lee TH et al. Can wire-guided cannulation prevent post-ERCP pancreatitis? A prospective randomized trial. Gastrointest Endosc. 2009;69(3 Pt 1):444-9.
16. Cheung J et al. Guidewire versus conventional contrast cannulation of the common bile duct for the prevention of post-ERCP pancreatitis: a systematic review and meta-analysis. Gastrointest Endosc. 2009;70(6):1211-9.
17. Tse F et al. Guidewire-assisted cannulation of the common bile duct for the prevention of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis. Cochrane Database Syst Rev. 2012;12:CD009662.
18. Tryliskyy Y, Bryce G. Post-ERCP pancreatitis: pathophysiology, early identification and risk stratification. Adv Clin Exp Med. 2018;27(1):149-54.
19. Bassi M et al. A multicenter randomized trial comparing the use of touch versus no-touch guidewire technique for deep biliary cannulation: the TNT study. Gastrointest Endosc. 2018;87(1):196-201.
20. Testoni PA et al. Papillary cannulation and sphincterotomy techniques at ERCP: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. Endoscopy. 2016;48(7):657-83.
21. McCarthy JH et al. Cannulation of the biliary tree, cystic duct and gallbladder using a hydrophilic polymer-coated steerable guide wire. Gastrointest Endosc. 1990;36(4):386-9.
22. Kitamura K et al. 0.025-inch vs 0.035-inch guide wires for wire-guided cannulation during endoscopic retrograde cholangiopancreatography: A randomized study. World J Gastroenterol. 2015;21(30):9182-8.
23. Bassan MS et al. The impact of wire caliber on ERCP outcomes: a multicenter randomized controlled trial of 0.025-inch and 0.035-inch guidewires. Gastrointest Endosc. 2018;87(6):1454-60.
24. Vihervaara H et al. Angled- or straight-tipped hydrophilic guidewire in biliary cannulation: a prospective, randomized, controlled trial. Surg Endosc. 2013;27(4):1281-6.
25. Kwon CI et al. Technical reports of endoscopic retrograde cholangiopancreatography guidewires on the basis of physical properties. Clin Endosc. 2020;53(1):65-72.
26. Tsuchiya T et al. Effectiveness of the J-tip guidewire for selective biliary cannulation compared to conventional guidewires (The JANGLE Study). Dig Dis Sci. 2015;60(8):2502-8.
27. Hwang JC et al. A prospective randomized study of loop-tip versus straight-tip guidewire in wire-guided biliary cannulation. Surg Endosc. 2018;32(4):1708-13.
28. Kawakami H et al. A multicenter, prospective, randomized study of selective bile duct cannulation performed by multiple endoscopists: the BIDMEN study. Gastrointest Endosc. 2012;75(2):362-72, 372.e1.
29. Mariani A et al. Guidewire biliary cannulation does not reduce post-ERCP pancreatitis compared with the contrast injection technique in low-risk and high-risk patients. Gastrointest Endosc. 2012;75(2):339-46.
30. Kobayashi G et al. Wire-guided biliary cannulation technique does not reduce the risk of post-ERCP pancreatitis: Multicenter randomized controlled trial. Dig Endosc. 2013;25(3):295-302.
31. Buxbaum J et al. Randomized trial of endoscopist-controlled vs. assistant-controlled wire-guided cannulation of the bile duct. Am J Gastroenterol. 2016;111(12):1841-7.
32. Takano Y et al. Perforation of the papilla of vater in wire-guided cannulation. Can J Gastroenterol Hepatol. 2016; DOI: 10.1155/2016/5825230.
33. Cennamo V et al. Can early precut implementation reduce endoscopic retrograde cholangiopancreatography-related complication risk? Meta-analysis of randomized controlled trials. Endoscopy. 2010;42(5):381-8.
34. Katsinelos P et al. Risk factors for therapeutic ERCP-related complications: an analysis of 2,715 cases performed by a single endoscopist. Ann Gastroenterol. 2014;27(1):65-72.
35. Testoni PA et al. Risk factors for post-ERCP pancreatitis in high- and low-volume centers and among expert and non-expert operators: a prospective multicenter study. Am J Gastroenterol. 2010;105(8):1753-61.
36. Tabibian JH, Leung JW. Training in ERCP: a multifaceted enterprise now more than ever. Endosc Int Open. 2018;6(1):E90.
37. Lee YS et al. Difficult biliary cannulation from the perspective of post-endoscopic retrograde cholangiopancreatography pancreatitis: identifying the optimal timing for the rescue cannulation technique. Gut Liver. 201;15(3):459-65.
38. Dumonceau J-M et al. Prophylaxis of post-ERCP pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Guideline – Updated June 2014. Endoscopy. 2014;46(9):799-815.
39. Halttunen J et al. Difficult cannulation as defined by a prospective study of the Scandinavian Association for Digestive Endoscopy (SADE) in 907 ERCPs. Scand J Gastroenterol. 2014;49(6):752-8.
40. Ismail S et al. Criteria for difficult biliary cannulation: start to count. Eur J Gastroenterol Hepatol. 2019;31(10):1200-5.
41. Liao WC et al. International consensus recommendations for difficult biliary access. Gastrointest Endosc. 2017;85(2):295-304.
42. Friedland S et al. Bedside scoring system to predict the risk of developing pancreatitis following ERCP. Endoscopy. 2002;34(6):483-8.
43. Tian C et al. Cannulation time is a more accurate measure of cannulation difficulty in endoscopic retrograde cholangiopancreatography than the number of attempts. Gastroenterol Rep. 2013;1(3):193-7.
44. Guda N, Freeman M. Are you safe for your patients – how many ERCPs should you be doing? Endoscopy. 2008;40(8):675-6.
45. Wicks ACB et al. Structured training

and assessment in ERCP has become essential for the Calman era. Gut. 1999;45(1):154-6.

46. Wani S et al. Setting minimum standards for training in EUS and ERCP: results from a prospective multicenter study evaluating learning curves and competence among advanced endoscopy trainees. Gastrointest Endosc. 2019;89(6):1160-8.e9.

47. Verma D et al. Establishing a true assessment of endoscopic competence in ERCP during training and beyond: a single-operator learning curve for deep biliary cannulation in patients with native papillary anatomy. Gastrointest Endosc. 2007;65(3):394-400.

48. Coté GA et al. Lower provider volume is associated with higher failure rates for endoscopic retrograde cholangiopancreatography. Med Care. 2013;51(12):1040-7.

49. Freeman ML et al. Complications of endoscopic biliary sphincterotomy. N Engl J Med. 1996;335(13):909-18.

50. Loperfido S et al. Major early complications from diagnostic and therapeutic ERCP: a prospective multicenter study. Gastrointest Endosc. 1998;48(1):1-10.

51. Choudari CP et al. Success of ERCP at a referral center after a previously unsuccessful attempt. Gastrointest Endosc. 2000;52(4):478-83.

52. Balan GG et al. Anatomy of major duodenal papilla influences ERCP outcomes and complication rates: a single center prospective study. J Clin Med. 2020;9(6):1637.

53. Haraldsson E et al. Macroscopic appearance of the major duodenal papilla influences bile duct cannulation: a prospective multicenter study by the Scandinavian Association for Digestive Endoscopy Study Group for ERCP. Gastrointest Endosc. 2019;90(6):957-63.

54. Chen PH et al. Duodenal major papilla morphology can affect biliary cannulation and complications during ERCP, an observational study. BMC Gastroenterol. 2020;20(1):310.

55. Watanabe M et al. Transpapillary biliary cannulation is difficult in cases with large oral protrusion of the duodenal papilla. Dig Dis Sci. 2019;64(8):2291-99.

56. Tabak F et al. Endoscopic retrograde cholangiopancreatography in elderly patients: Difficult cannulation and adverse events. World J Clin Cases. 2020;8(14):2988-99.

57. Vihervaara H et al. Female gender and post-ERCP pancreatitis: is the association caused by difficult cannulation? Scand J Gastroenterol. 2011;46(12):1498-502.

58. Baillie J. Difficult biliary access for ERCP. Curr Gastroenterol Rep. 2012;14(6):542-7.

59. Anderloni AA et al. Tu1437 difficult biliary cannulation in patients with distal malignant biliary obstruction: a restrospective analysis of a single center experience. Gastrointest Endosc. 2018;87(6):AB598.

60. Nabi Z, Reddy DN. Endoscopic management of combined biliary and duodenal obstruction. Clin Endosc. 2019;52(1):40-6.

61. Wang GC et al. Combined intestinal and biliary stenting in gastric outlet and biliary obstruction. Gastroenterol Res. 2009;2(1):29-34.

62. Altonbary AY, Bahgat MH. Endoscopic retrograde cholangiopancreatography in periampullary diverticulum: the challenge of cannulation. World J Gastrointest Endosc. 2016;8(6):282-7.

63. Lobo DN et al. Periampullary diverticula: consequences of failed ERCP. Ann R Coll Surg Engl. 1998;80(5):326-31.

64. Hochberger J et al, "Difficult cannulation and sphincterotomy," Chandrasekhara V et al (eds.), Clinical Gastrointestinal Endoscopy (2019) 3rd edition, Elsevier, pp.563-570.e2.

65. Zimmer V. Gastrointestinal: no touch-guidewire cannulation in periampullary diverticulum. J Gastroenterol Hepatol. 2020;35(8):1261.

66. Hallerbäck B, Enochsson L. A prospective nationwide study on the impact of the level of sedation on cannulation success and complications of endoscopic retrograde cholangiopancreatography. Ann Gastroenterol. 2020;33(3):299-304.

67. Krutsri C et al. Current status of endoscopic retrograde cholangiopancreatography in patients with surgically altered anatomy. World J Gastroenterol. 2019;25(26):3313.

68. Enestvedt BK et al. Devices and techniques for ERCP in the surgically altered GI tract. Gastrointest Endosc. 2016;83(6):1061-75.

69. Moreels TG. Endoscopic retrograde cholangiopancreatography in patients with altered anatomy: How to deal with the challenges? World J Gastrointest Endosc. 2014;6(8):345-51.

70. Kochar B et al. Incidence, severity, and mortality of post-ERCP pancreatitis: a systematic review by using randomized, controlled trials. Gastrointest Endosc. 2015;81(1):143-9.e9.

71. Chandrasekhara V et al. Adverse events associated with ERCP. Gastrointest Endosc. 2017;85(1):32-47.

72. Weiser R et al. Management of endoscopic retrograde cholangiopancreatography–related perforations: experience of a tertiary center. Surgery. 2017;161(4):920-9.

73. Stapfer M et al. Management of duodenal perforation after endoscopic retrograde cholangiopancreatography and sphincterotomy. Ann Surg. 2000;232(2):191-8.

Difficult Biliary Cannulation in Endoscopic Retrograde Cholangiopancreatography: An Overview of Advanced Techniques

Authors: Brian M. Fung,[1,2] Teodor C. Pitea,[2] *James H. Tabibian[3,4]

1. Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Arizona College of Medicine – Phoenix, Arizona, USA
2. Banner – University Medical Center Phoenix, Arizona, USA
3. Division of Gastroenterology, Department of Medicine, Olive View-UCLA Medical Center, Sylmar, California, USA
4. David Geffen School of Medicine at UCLA, Los Angeles, California, USA

*Correspondence to jtabibian@dhs.lacounty.gov

Disclosure: The authors have declared no conflicts of interest.

Author contributions: Fung reviewed the literature for relevant original studies and other content, designed the figures, and drafted the manuscript. Pitea and Tabibian critically reviewed the manuscript. All authors provided critical input and approved the final version of the manuscript.

Acknowledgements: This work was supported in part through the United States National Institutes of Health (NIH) grant UL1 TR000135.

Received: 09.01.21

Accepted: 26.03.21

Keywords: Endoscopic retrograde cholangiopancreatography (ERCP), fistulotomy, papillotomy, post-ERCP pancreatitis (PEP), precut, selective biliary cannulation, sphincterotomy.

Citation: EMJ Hepatol. 2021;9[1]:73-82.

Abstract

Endoscopic retrograde cholangiopancreatography (ERCP) plays a significant role in the treatment of a vast array of pancreatobiliary diseases. However, despite significant progress in the optimisation of ERCP methods and accessories, the technical and clinical success of ERCP can vary significantly due to a variety of patient and operator factors. Over the past several decades, a number of advanced techniques have been developed to improve cannulation success rates, including the use of double-guidewire, pancreatic duct accessory-assisted, precut, and rendezvous techniques. Here, the authors provide an update and overview of the existing advanced techniques used in cases of difficult biliary cannulation, as well as the approach to their selection.

INTRODUCTION

Over the years, endoscopic retrograde cholangiopancreatography (ERCP) has been transformed from primarily a diagnostic modality into a largely therapeutic technique that is now the preferred minimally invasive treatment for many pancreaticobiliary diseases. However, despite significant progress in the optimisation of ERCP methods and accessories, selective biliary cannulation can be one of the most challenging and rate-limiting aspects of ERCP. Even in the hands of experienced endoscopists, failure of biliary cannulation can occur in 5–15% of

cases.[1] Furthermore, there is a non-insignificant risk of post-ERCP pancreatitis (PEP) and other adverse events.[1-3] When confronted with a case of difficult biliary cannulation, an endoscopist must decide whether to continue with standard cannulation techniques, switch to more advanced techniques, consult a more senior colleague, or abort the procedure and consider re-attempting at a later time.

This review provides an overview of the most common advanced techniques currently used when standard selective biliary cannulation techniques are unsuccessful. In particular, the authors discuss the double-guidewire cannulation technique, pancreatic stent-assisted cannulation technique, precut papillotomy, precut fistulotomy, transpancreatic septotomy, rendezvous, and other advanced techniques, as well as the approach to their selection.

PANCREATIC DUCT WIRE- AND STENT-ASSISTED TECHNIQUES

Pancreatic Guidewire and Double-Guidewire Technique

Cannulation of the pancreatic duct is typically easier than cannulation of the bile duct given the angle at which the pancreatic duct inserts into the hepatopancreatic ampulla. Thus, if a guidewire is inadvertently placed in the pancreatic duct, one strategy is to keep the guidewire in the main pancreatic duct and subsequently attempt to cannulate the bile duct using a cannula/sphincterotome and contrast (i.e., single-guidewire technique), while another is to use a second guidewire (double-guidewire technique [DGT]) (Figure 1).[4] Either way, the guidewire in the pancreatic duct can help to straighten the hepatopancreatic ampulla, separate the biliary and pancreatic orifices, and help to identify the respective biliary and pancreatic axes.[5] The guidewire also partially occludes the pancreatic orifice, thus helping to deflect the sphincterotome (or second guidewire) away from the pancreatic duct and towards the common bile duct (CBD), facilitating selective biliary cannulation.[6]

Early studies suggested that DGT could improve biliary cannulation rates.[7] However, subsequent studies have found DGT to be no better than standard biliary cannulation, and the technique even appears to increase the risk of PEP. A recent meta-analysis of seven randomised controlled trials found that DGT significantly increased the risk of PEP compared to other endoscopic techniques (risk ratio [RR]: 1.98; 95% confidence interval [CI]: 1.14–3.42).[8] If employed, the authors recommend placing a temporary plastic pancreatic duct stent to help mitigate this risk, as discussed below.

Pancreatic Stent-Assisted Technique

A variation on DGT is the use of a pancreatic stent.[9] In this technique, a short (3–5 cm long), small-caliber (3–5 Fr) pancreatic stent is immediately placed after cannulating the pancreatic duct. This temporary stent helps to identify the pancreatic axis and occludes the pancreatic duct, thus deflecting the guidewire into the CBD. The stent typically falls out by itself within a week or two after the procedure (depending on the size and type of stent and the depth of insertion); however, a stent removal procedure may be needed if the stent does not pass on its own.[10]

This technique has been demonstrated to have a high rate of successful biliary cannulation, and the placement of a prophylactic pancreatic stent has been shown to lower the rate of PEP.[11] In a study of 70 patients who underwent pancreatic guidewire-assisted biliary cannulation, patients who underwent stent placement had a significantly lower frequency of PEP compared to those without stent placement (2.9% versus 23.0%, respectively; p=0.0096).[12] Similarly, a recent multicentre randomised controlled trial found that prophylactic pancreatic stenting after inadvertent cannulation of the pancreatic duct significantly reduced the rate of PEP (odds ratio [OR]: 0.43).[13] Over the past decade, multiple meta-analyses have supported these findings (OR: 0.22–0.39).[14-20] This technique also appears to be cost-effective for high-risk patients, likely due to the lower rates of PEP associated with this technique.[21,22] The European Society of Gastrointestinal Endoscopy (ESGE) currently recommends prophylactic pancreatic stenting in patients with inadvertent guidewire insertion/opacification of the pancreatic duct or after double-guidewire cannulation.[23]

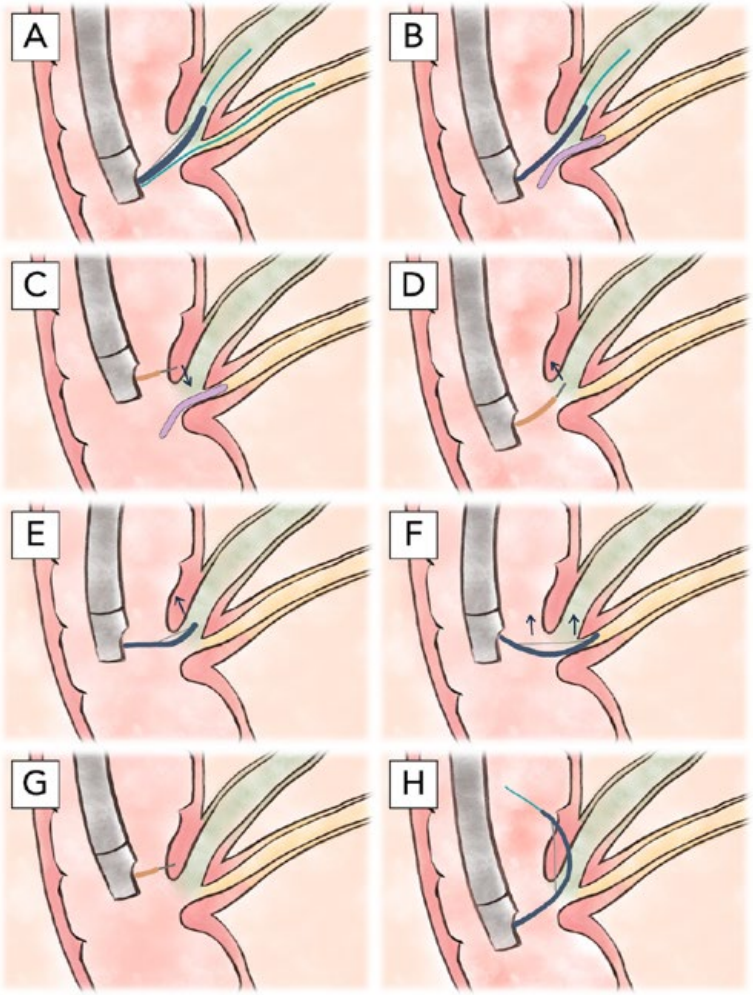


Figure 1: Illustration of various advanced common bile duct cannulation techniques.

A) Double-guidewire technique. A guidewire (light blue) is first placed (often inadvertently) in the main pancreatic duct (yellow). This facilitates adjacent advancement of a second guidewire through a sphincterotome (blue) into the hepatopancreatic ampulla (via either a no-touch or touch technique) and thereafter into the common bile duct (green). **B)** Pancreatic stent-assisted technique. A pancreatic duct stent (purple) is first placed over a guidewire in the main pancreatic duct. Similar to the double-guidewire technique, this technique facilitates adjacent placement of a guidewire through a sphincterotome into the hepatopancreatic ampulla and onward into the common bile duct. **C)** Precut sphincterotomy over a pancreatic duct stent. A needle-knife catheter (orange) is used to cut inferiorly, starting suprapapillary in the (expected) location of the intraduodenal segment of the hepatopancreatic ampulla, towards the pancreatic duct stent. **D)** Precut papillotomy. A needle-knife is used to cut superiorly in a biliary (11–12 o'clock) orientation (i.e., vector), starting from the papillary orifice. **E)** Pull-type precut. A (semi-)seated sphincterotome is used to cut superiorly in a biliary orientation, starting from the papillary orifice. **F)** Transpancreatic precut sphincterotomy. A sphincterotome is inserted into the hepatopancreatic ampulla/distal pancreatic duct and, when repositioning into the distal bile duct is not feasible, used to cut superiorly, cutting through the septum in a biliary orientation. **G)** Precut supra-papillary fistulotomy. A needle-knife is used to incise directly into the intraduodenal segment of the distal bile duct/proximal hepatopancreatic ampulla, superior to the level of the papillary orifice. **H)** Intramural incision. After inadvertent creation of a false tract with a guidewire through the intraduodenal segment of the common bile duct, a sphincterotome is used to unroof the papilla to facilitate direct cannulation. Note: all illustrations show a major papilla with a conventional hepatopancreatic ampulla (i.e., a shared ductal orifice and normal common channel length); however, technique may vary in the case of variant (peri-) ampullary anatomy.

However, of note, placement of a pancreatic stent can compress the biliary orifice and make standard approaches more difficult; thus, the risks and benefits of immediate pancreatic stent placement should be carefully considered.[5]

Precut Sphincterotomy Over Pancreatic Stent

The precut over pancreatic stent (PPS) technique is generally thought of as an extension of the pancreatic stent-assisted technique.[24] As previously mentioned, the placement of a pancreatic stent can often narrow the papillary orifice, thus making cannulation of the bile duct more difficult. To improve access, an incision using a needle-knife can be made, starting from above the papillary orifice and extending downward towards the pancreatic stent. A retrospective study found that PPS was associated with significantly higher biliary cannulation rates (96.9% versus 86.1%; p=0.0189) and fewer adverse events (7.1% versus 33.0%; p<0.001).[25]

PRECUT TECHNIQUES

When biliary cannulation fails with the previously discussed techniques, a variety of precut techniques can be employed to create access to the CBD. These techniques involve the use of a cutting tool, often a needle-knife (a fine, straight, wire-type needle), to make an incision in the ampulla to allow access to the bile duct for successful selective biliary cannulation. Unlike traditional biliary sphincterotomy that enlarges the biliary opening for therapeutic interventions (e.g., extraction of stones or placement of stents), the precut is used solely for biliary access.

Precut Papillotomy

A common precut technique is precut papillotomy.[26] In this technique, a needle-knife is used to carefully dissect the major duodenal papilla to directly visualise and cannulate the CBD. The papillotomy is first initiated by placing the needle-knife at the top of the papillary orifice, near the 12 o'clock position, in the presumed axis of the bile duct. An incision is then made in the cephalad direction by extending the needle-knife upward towards the roof of the papilla, with the electrical current on. While the length and depth of the incision depends on the size and characteristics of the papilla, incisions should generally be made in short increments to avoid cutting too deep and causing bleeding, perforation, or acute pancreatitis.[27] Once the muscle layer of the biliary sphincter muscle is visualised (which can often be recognised by its whitish, concentric circular appearance), the papilla can be cannulated, or an additional cut can be made to transect the biliary sphincter, allowing direct cannulation of the CBD.

Pull-Type Precut Papillotomy

Pull- or traction-type precut papillotomy is a variation of the precut papillotomy technique, originally used after successful cannulation of the bile duct.[28] However, the same device is now used as a method of obtaining access to the bile duct. In this technique, a short-nosed sphincterotome is used instead of a needle-knife. Shallow cannulation of the common channel is first performed using the tip of the sphincterotome. A small 1–2 mm cut is then made, changing the shape of the biliary orifice from a circle to a teardrop and improving access to the CBD. There are four major advantages of this sphincterotome: a lower risk of injury to the pancreatic duct due to protection by the insulated convex catheter tip; a more controlled incision with the tip of the instrument providing stability; the ability to control the direction of the incision by orientation of the cutting wire; and the ability to perform sphincterotomy without another device.[29] This technique has been demonstrated to have a high rate of biliary cannulation, with no increased rate of complications when compared to standard cannulation techniques.[29-31]

Transpancreatic Precut Sphincterotomy

In patients with small or difficult to locate papilla, precut papillotomy or precut fistulotomy using a needle-knife may be difficult.[32] In these cases, the transpancreatic precut sphincterotomy (TPS) technique may be used to achieve biliary access. In this technique, a standard sphincterotome is superficially inserted into the ampulla or main pancreatic duct oriented towards the 11 o'clock position. An incision is then made by pushing the sphincterotome

upward towards the CBD. Both the septum and ampullary sphincter are cut. This technique is thought to allow better control of the depth of incision compared to a standard needle-knife and eliminates the need to exchange the sphincterotome for a needle-knife.[33] The use of a guidewire to assist cannulation of the pancreatic duct prior to TPS has also been described.[34,35] Whether a guidewire is used or not, the TPS technique appears to have a higher rate of biliary cannulation than needle-knife precut papillotomy.[35,36] The associated risk of PEP can also be lowered when a pancreatic stent is placed.[37]

Precut Fistulotomy

Unlike in precut papillotomy where an incision is made extending from the papillary orifice in an upward direction, precut fistulotomy involves the making of an incision using a needle-knife in an area of the papilla above the papillary orifice so that a fistula is made between the duodenal lumen and the CBD lumen.[38-40] This incision can be extended superiorly or inferiorly toward the papillary orifice using the needle-knife, but does not reach the papillary orifice, allowing the sphincter and papillary orifice to remain intact. This method has been reported to have a high cannulation rate and a lower risk of PEP than precut papillotomy, which may be attributed to low likelihood of thermal injury to the papillary orifice and pancreatic duct.[41]

Intramural Incision (for Unroofing False Tracts)

The intramural incision technique (also known as Burdick's technique) can be used when a guidewire inadvertently creates a false tract in the 10–11 o'clock direction through the intraduodenal segment of the bile duct during attempted biliary cannulation.[42] In this scenario, an intramural incision can be made using a sphincterotome or needle-knife, thus unroofing the papilla and exposing the CBD for cannulation. Several small studies have suggested a high rate of success and minimal complications with this technique.[43,44] The intramural incision technique illustrates how an undesired event can be used to the endoscopist's advantage.

COMPARISON AND TIMING OF PRECUT TECHNIQUES

Since the advent of endoscopic biliary sphincterotomy, numerous studies have evaluated the effectiveness and risks of precut techniques. While there is still no consensus on which technique is best, one recent systematic review and meta-analysis of 14 studies found that TPS was associated with a higher success rate than DGW (OR: 2.72; 95% CI: 1.30–5.69) and precut papillotomy (OR: 2.32; 95% CI: 1.37–3.93), but not different from that of precut fistulotomy (OR: 1.38; 95% CI: 0.32–5.96).[45] In the same meta-analysis, the rate of PEP was not significantly different between TPS and DGW (OR: 0.72; 95% CI: 0.24–2.10) and between TPS and precut papillotomy (OR 1.63; 95% CI 0.48–5.47). However, the rate of PEP was higher in TPS compared to precut fistulotomy (OR: 4.62; 95% CI: 1.36–15.72). The rate of bleeding and perforation did not differ between the four advanced techniques. From this study, precut fistulotomy appears to have better outcomes among these four most commonly used advanced techniques; however, it is important to keep in mind that the choice of which technique to pursue can vary depending on a multitude of factors, including experience of the endoscopist, equipment availability, and variations in patient anatomy.

While many studies initially suggested that performing a precut may increase the risk of PEP, this increased risk has not been seen when the precut is done earlier in the procedure (also known as early precut). In many cases, and depending on the clinical scenario, the risk of PEP after early precut may be even lower than when no precut is used.[1,46-49] A recent meta-analysis of six randomised controlled trials, which included 898 patients, found that early precut not only increased the rate of biliary cannulation (RR: 1.87; 95% CI: 1.15–3.04), but also significantly reduced the risk of PEP compared to standard cannulation (RR: 0.49; 95% CI: 0.30–0.80).[46] Thus, it is now thought that a higher risk of PEP after precut is only the consequence of increased papillary manipulation in cases of difficult biliary cannulation, and not the precut itself.[50]

RENDEZVOUS TECHNIQUES

In rare cases where a precut technique fails or is not possible due to anatomical variations of the papilla, a rendezvous technique can be performed. These methods involve the anterograde passage of a wire through the papilla and into the duodenum, with subsequent selective biliary cannulation over the wire or in parallel to the wire. Due to the complex nature of these procedures, they should only be performed by experienced endoscopists and are generally only performed at specialised endoscopy centres. In the following section, the authors provide a brief overview of two rendezvous techniques.

Percutaneous Transhepatic-Endoscopic Rendezvous

The percutaneous-transhepatic-endoscopic rendezvous (PTE-RV) technique is a rendezvous technique that can be used in cases where initial biliary cannulation has failed, particularly in the setting of altered surgical anatomy, failure of selective insertion into the right intrahepatic duct, or a tight hilar biliary stricture where only a guidewire can be passed.[51] In this technique, percutaneous access to the bile duct is first achieved by an interventional radiologist. A guidewire is then threaded anterograde through the needle into the bile duct and advanced through the papilla, where the wire can then be used to facilitate conventional biliary cannulation. A retrospective study found PTE-RV to have a success rate of 92.9%; however, the need for percutaneous access is a significant drawback to this technique.[51]

Endoscopic Ultrasound-Guided Rendezvous

In the endoscopic ultrasound-guided rendezvous (EUS-RV) technique, the bile duct (or pancreatic duct) is punctured via a transgastric or transduodenal approach, and a wire is subsequently passed anterograde through the papilla.[52] A sphincterotome can then be directed over the guidewire or in parallel to the wire for biliary cannulation. Through this technique, a failed standard cannulation attempt can be salvaged during the same session without the need for a precut sphincterotomy.[53] Furthermore, if biliary cannulation is still not possible via EUS-RV, another recently described salvage technique, EUS-hybrid rendezvous technique, can be attempted. In this instance, a dilator is advanced into the biliary system for better guidewire manipulation.[54] In a retrospective study comparing EUS-RV and precut papillotomy techniques, EUS-RV was associated with a higher rate of technical success (98.3% versus 90.3%, respectively; p=0.03), with no difference in complication rate. However, it remains unclear whether this procedure is cost-effective and at what point technical competence is reached.[55]

MANAGEMENT OF PERIDIVERTICULAR OR INTRADIVERTICULAR PAPILLAE

Papillae located near or within a diverticulum are often difficult to visualise and access.[56] Over the years, various techniques for the management of peridiverticular or intradiverticular papillae have been described. The use of an endoscopic clip or forceps to retract an overlying diverticular rim has been reported to be a helpful technique to expose and properly orient the ampulla.[57-61] One study found the attachment of a clear cap to the end of a forward-viewing endoscope helpful in revealing papillae that were difficult to find.[62] Submucosal saline injection has also been described as a method of everting the papillary opening to assist cannulation of the papillary orifice.[63] At times, advancement of the duodenoscope head into the diverticula is needed for cannulation of intradiverticular papillae; however, blind probing of the diverticulum is dangerous and should generally be avoided. Some cases require the use of rendezvous procedures, as described above.[64,65]

SYNOPSIS OF BEST PRACTICES APPROACH

While cannulation techniques vary based on variables such as region of the world, institution, individual physician preference, and papilla- and other patient-specific factors, the following is a recommended approach to selective biliary cannulation based on the authors' experience and the available published data (Figure 2).

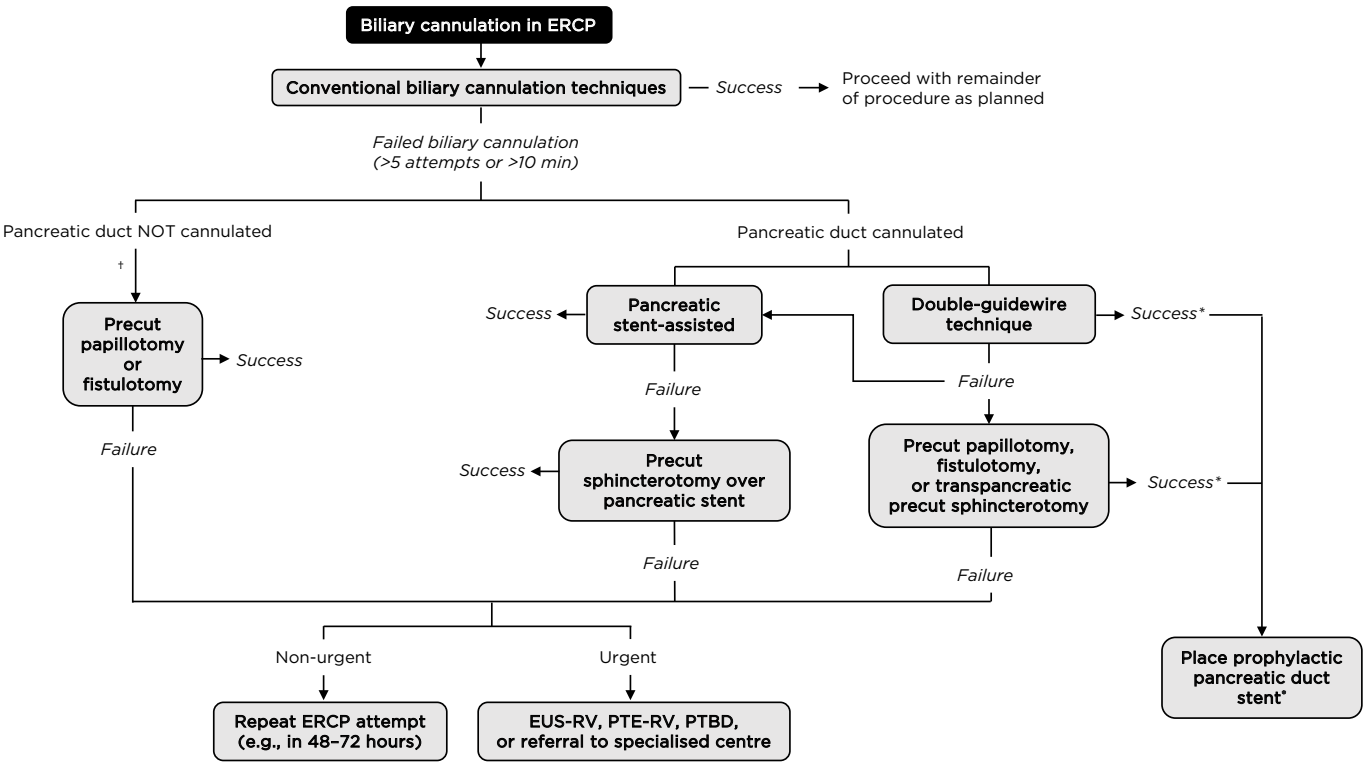


Figure 2: A proposed management algorithm for difficult biliary cannulation.

*Patients who have the pancreatic duct cannulated (intentional or inadvertent) should generally have a pancreatic duct stent placed to reduce the risk of post-ERCP pancreatitis, unless an alternative prophylactic measure is deemed to be equally or more clinically appropriate.

†If false tract created, intramural incision can be attempted.

ERCP: endoscopic retrograde cholangiopancreatography; EUS-RV: endoscopic ultrasound-guided rendezvous; PTE-RV: percutaneous transhepatic-endoscopic rendezvous; PTBD: percutaneous transhepatic biliary drainage.

The authors first visualise the major papilla and assess its size, associated features, and peripapillary anatomy. While the authors generally attempt conventional biliary cannulation techniques in the majority of cases with non-surgically altered anatomy, cases with particularly challenging anatomy may benefit from early adoption of advanced cannulation techniques.

As shown in Figure 2, when starting with conventional cannulation techniques and encountering difficulty with selective biliary cannulation, the authors typically select an advanced technique based on whether or not the pancreatic duct was cannulated. Regardless of the technique chosen, if the pancreatic duct is cannulated, the authors generally recommend the placement of a pancreatic duct stent or an alternative prophylactic measure prior to the end of the procedure to reduce the risk of PEP. Rendezvous techniques are only attempted if other more straightforward techniques fail.

AREAS OF NEED AND FUTURE DIRECTIONS

Over the past several decades, novel techniques and tools have been developed to address difficult biliary cannulation. In addition to continued refinements in these techniques, the authors see several areas that could benefit from further study. Firstly, although risk factors for difficult biliary cannulation have been identified, there are currently no well-established risk assessment tools that can predict, *a priori,* difficult biliary cannulation. The ability to predict which patients

will need advanced techniques could be useful, as this could impact management plans, including the consent process (patients with a higher score may be at higher risk of adverse events and could be counselled or consented accordingly) and scheduling of procedures (patients that potentially require advanced techniques may need to be scheduled for a longer time in the endoscopy suite). An ideal tool would be based on readily available variables, easy to calculate, provide a risk estimate that is easy to understand (e.g., percentage or percentage range), and be accurate. Secondly, it would be useful to have a scoring system, ostensibly based on machine learning models, that can reliably predict the likelihood of adverse events related to difficulty biliary cannulation (e.g., PEP, perforation, or bleeding). Thirdly, patients with surgically-altered anatomy also remain a challenge, and it is unclear to date what the best management approach for biliary cannulation may be (of the various options that currently exist, including the relatively new addition of EUS-directed transgastric ERCP). Lastly, more data are needed to determine which patients (and providers) are best suited for early (or even first-line) implementation of precut sphincterotomy or suprapapillary fistulotomy.

CONCLUSION

ERCP has become an indispensable tool in the diagnosis and management of many pancreaticobiliary diseases. However, it remains a challenging procedure despite technological advances. When selective biliary cannulation is unsuccessful with standard techniques, a variety of advanced cannulation techniques can be attempted, including the use of pancreatic duct-assisted (e.g., double-guidewire and pancreatic stent), precut, and rendezvous techniques. One may also consider calling for back-up (if a more experienced endoscopist is nearby) or stopping the procedure, especially if there appears to be a complication or if the patient is not tolerating the procedure well. Unsuccessful cases can be re-attempted at a later time or referred to a more specialised centre. There are currently many advanced techniques for biliary cannulation; the decision of which technique to pursue should ultimately take into consideration the endoscopist's experience, available equipment and staff, the disease being treated, and anatomical as well as other patient-level considerations.

References

1. Cennamo V et al. Can early precut implementation reduce endoscopic retrograde cholangiopancreatography-related complication risk? Meta-analysis of randomized controlled trials. Endoscopy. 2010;42(5):381-8.
2. Andriulli A et al. Incidence rates of post-ERCP complications: a systematic survey of prospective studies. Am J Gastroenterol. 2007;102(8):1781-8.
3. Harewood GC. An assessment of the learning curve for precut biliary sphincterotomy. Am J Gastroenterol. 2002;97(7):1708-12.
4. Dumonceau JM et al. A new method of achieving deep cannulation of the common bile duct during endoscopic retrograde cholangiopancreatography. Endoscopy. 1998;30(7):S80.
5. Mammen A, Haber G. Difficult biliary access. Gastrointest Endosc Clin N Am. 2015;25(4):619-30.
6. Grönroos JM et al. Double-guidewire-assisted biliary cannulation: experiences from a single tertiary referral center. Surg Endosc. 2011;25(5):1599-602.
7. Maeda S et al. Prospective randomized pilot trial of selective biliary cannulation using pancreatic guide-wire placement. Endoscopy. 2003;35(9):721-4.
8. Tse F et al. Double-guidewire technique in difficult biliary cannulation for the prevention of post-ERCP pancreatitis: a systematic review and meta-analysis. Endoscopy. 2017;49(1):15-26.
9. Goldberg E et al. Pancreatic-duct stent placement facilitates difficult common bile duct cannulation. Gastrointest Endosc. 2005;62(4):592-6.
10. Sofuni A et al. Prophylaxis of post–endoscopic retrograde cholangiopancreatography pancreatitis by an endoscopic pancreatic spontaneous dislodgement stent. Clin Gastroenterol Hepatol. 2007;5(11):1339-46.
11. Singh P et al. Does prophylactic pancreatic stent placement reduce the risk of post-ERCP acute pancreatitis? A meta-analysis of controlled trials. Gastrointest Endosc. 2004;60(4):544-50.
12. Ito K et al. Can pancreatic duct stenting prevent post-ERCP pancreatitis in patients who undergo pancreatic duct guidewire placement for achieving selective biliary cannulation? A prospective randomized controlled trial. J Gastroenterol. 2010;45(11):1183-91.
13. Phillip V et al. Pancreatic stenting to prevent post-ERCP pancreatitis: a randomized multicenter trial. Endosc Int Open. 2019;7(7):E860-8.
14. Choudhary A et al. Pancreatic stents for prophylaxis against post-ERCP pancreatitis: a meta-analysis and systematic review. Gastrointest Endosc. 2011;73(2):275-82.
15. Akbar A et al. Rectal nonsteroidal anti-inflammatory drugs are superior to pancreatic duct stents in preventing pancreatitis

after endoscopic retrograde cholangiopancreatography: a network meta-analysis. Clin Gastroenterol Hepatol. 2013;11(7):778-83.

16. Mazaki T et al. Prophylactic pancreatic stent placement and post-ERCP pancreatitis: an updated meta-analysis. J Gastroenterol. 2014;49(2):343-55.
17. Shi QQ et al. Placement of prophylactic pancreatic stents to prevent post-endoscopic retrograde cholangiopancreatography pancreatitis in high-risk patients: a meta-analysis. World J Gastroenterol. 2014;20(22):7040-8.
18. Fan JH et al. Updated meta-analysis of pancreatic stent placement in preventing post-endoscopic retrograde cholangiopancreatography pancreatitis. World J Gastroenterol. 2015;21(24):7577-83.
19. Vadalà di Prampero SF et al. Endoscopic and pharmacological treatment for prophylaxis against postendoscopic retrograde cholangiopancreatography pancreatitis: a meta-analysis and systematic review. Eur J Gastroenterol Hepatol. 2016;28(12):1415-24.
20. Sugimoto M et al. Pancreatic stents to prevent post-endoscopic retrograde cholangiopancreatography pancreatitis: a meta-analysis. World J Metaanal. 2019;7(5):249-58.
21. Dumonceau JM et al. Prophylaxis of post-ERCP pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Guideline – updated June 2014. Endoscopy. 2014;46(09):799-815.
22. Das A et al. Pancreatic-stent placement for prevention of post-ERCP pancreatitis: a cost-effectiveness analysis. Gastrointest Endosc. 2007;65(7):960-8.
23. Dumonceau J-M et al. ERCP-related adverse events: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2020;52(2):127-49.
24. Fogel EL et al. Increased selective biliary cannulation rates in the setting of periampullary diverticula: main pancreatic duct stent placement followed by pre-cut biliary sphincterotomy. Gastrointest Endosc. 1998;47(5):396-400.
25. Kubota K et al. Needle-knife precut papillotomy with a small incision over a pancreatic stent improves the success rate and reduces the complication rate in difficult biliary cannulations. J Hepatobiliary Pancreat Sci. 2013;20(3):382-8.
26. Katsinelos P et al. Comparison of three types of precut technique to achieve common bile duct cannulation: a retrospective analysis of 274 cases. Dig Dis Sci. 2012;57(12):3286-92.
27. Freeman ML et al. Complications of endoscopic biliary sphincterotomy. N Engl J Med. 1996;335(13):909-18.
28. Classen M, Demling L. [Endoscopic sphincterotomy of the papilla of vater and extraction of stones from the choledochal duct (author's transl)]. 1974;99(11):496-7. (In German)
29. Binmoeller KF et al. Papillary roof incision using the Erlangen-type pre-cut papillotome to achieve selective bile duct cannulation. Gastrointest Endosc. 1996;44(6):689-95.
30. Palm J et al. Safety of Erlangen precut papillotomy: an analysis of 1044 consecutive ERCP examinations in a single institution. J Clin Gastroenterol. 2007;41(5):528-33.
31. de Weerth A et al. Primary precutting versus conventional over-the-wire sphincterotomy for bile duct access: a prospective randomized study. Endoscopy. 2006;38(12):1235-40.
32. Kawakami H et al. Transpapillary selective bile duct cannulation technique: review of Japanese randomized controlled trials since 2010 and an overview of clinical results in precut sphincterotomy since 2004. Dig Endosc. 2016;28(Suppl 1):77-95.
33. Kapetanos D et al. Case series of transpancreatic septotomy as precutting technique for difficult bile duct cannulation. Endoscopy. 2007;39(9):802-6.
34. Chan CH et al. Wire assisted transpancreatic septotomy, needle knife precut or both for difficult biliary access. J Gastroenterol Hepatol. 2012;27(8):1293-7.
35. Zang J et al. Guidewire-assisted transpancreatic sphincterotomy for difficult biliary cannulation: a prospective randomized controlled trial. Surg Laparosc Endosc Percutan Tech. 2014;24(5):429-33.
36. Pécsi D et al. Transpancreatic sphincterotomy has a higher cannulation success rate than needle-knife precut papillotomy – a meta-analysis. Endoscopy. 2017;49(9):874-87.
37. Sakai Y et al. Transpancreatic precut papillotomy in patients with difficulty in selective biliary cannulation. Hepatogastroenterology. 2011;58(112):1853-8.
38. Artifon ELA et al. A new approach to the bile duct via needle puncture of the papillary roof. Endoscopy. 2005;37(11):1158.
39. Artifon ELA et al. Suprapapillary needle puncture for common bile duct access: laboratory profile. Arq Gastroenterol. 2006;43(4):299-304.
40. Artifon ELA et al. Management of common bile duct stones in cirrhotic patients with coagulopathy: a comparison of supra-papillary puncture and standard cannulation technique. Dig Dis Sci. 2011;56(6):1904-11.
41. Mavrogiannis C et al. Needle-knife fistulotomy versus needle-knife precut papillotomy for the treatment of common bile duct stones. Gastrointest Endosc. 1999;50(3):334-9.
42. Burdick JS et al. Intramural incision technique. Gastrointest Endosc. 2002;55(3):425-7.
43. Goenka MK, Rai VK. Burdick's technique for biliary access revisited. Clin Endosc. 2015;48(1):20-3.
44. Misra SP, Dwivedi M. Intramural incision technique: a useful and safe procedure for obtaining ductal access during ERCP. Gastrointest Endosc. 2008;67(4):629-33.
45. Pécsi D et al. Transpancreatic sphincterotomy is effective and safe in expert hands on the short term. Dig Dis Sci. 2019;64(9):2429-44.
46. Chen J et al. Assessing quality of precut sphincterotomy in patients with difficult biliary access: an updated meta-analysis of randomized controlled trials. J Clin Gastroenterol. 2018;52(7):573-8.
47. Sundaralingam P et al. Early precut sphincterotomy does not increase risk during endoscopic retrograde cholangiopancreatography in patients with difficult biliary access: a meta-analysis of randomized controlled trials. Clin Gastroenterol Hepatol. 2015;13(10):1722-9.e2.
48. Choudhary A et al. Effect of precut sphincterotomy on post-endoscopic retrograde cholangiopancreatography pancreatitis: a systematic review and meta-analysis. World J Gastroenterol. 2014;20(14):4093-101.
49. Gong B et al. Does precut technique improve selective bile duct cannulation or increase post-ERCP pancreatitis rate? A meta-analysis of randomized controlled trials. Surg Endosc. 2010;24(11):2670-2680.
50. Manes G et al. An analysis of the factors associated with the development of complications in patients undergoing precut sphincterotomy: a prospective, controlled, randomized, multicenter study. Am J Gastroenterol. 2009;104(10):2412-2417.
51. Yang MJ et al. Usefulness of combined percutaneous-endoscopic rendezvous techniques after failed therapeutic endoscopic retrograde cholangiography in the era of endoscopic ultrasound guided rendezvous. Medicine (Baltimore). 2017;96(48):e8991.
52. Shiomi H et al. Endoscopic ultrasound-guided rendezvous technique for failed biliary cannulation in benign and resectable malignant biliary disorders. Dig Dis Sci. 2018;63(3):787-96.
53. Iwashita T et al. Endoscopic

ultrasound-guided rendezvous for biliary access after failed cannulation. Endoscopy. 2012;44(1):60-5.

54. Iwashita T et al. EUS-guided hybrid rendezvous technique as salvage for standard rendezvous with intra-hepatic bile duct approach. PloS One. 2018;13(8):e0202445.
55. Yoon WJ, Brugge WR. EUS-guided biliary rendezvous: EUS to the rescue. Gastrointest Endosc. 2012;75(2):360-1.
56. Lobo DN et al. Periampullary diverticula: consequences of failed ERCP. Ann R Coll Surg Engl. 1998;80(5):326-31.
57. Cappell MS et al. Endoclips to facilitate cannulation and sphincterotomy during ERCP in a patient with an ampulla within a large duodenal diverticulum: case report and literature review. Dig Dis Sci. 2015;60(1):168-73.
58. Huang CH et al. Endoscopic retrograde cholangiopancreatography (ERCP) for intradiverticular papilla: endoclip-assisted biliary cannulation. Endoscopy. 2010;42(S 2):E223-4.
59. Balkrishanan M et al. Cannulation in patients with large periampullary diverticulum using SpyBite miniforceps. Clin Exp Hepatol. 2018;4(1):41-2.
60. Inamdar S et al. Difficult biliary cannulation achieved in the setting of periampullary diverticulum with the simultaneous use of biopsy forceps and wire-guided cannulation. VideoGIE. 2017;2(2):25-6.
61. Kim KH, Kim TN. A new technique for difficult biliary cannulation using endobiliary forceps in a patient with a periampullary diverticulum. Endoscopy. 2017;49(8):824-6.
62. Myung DS et al. Cap-assisted ERCP in patients with difficult cannulation due to periampullary diverticulum. Endoscopy. 2014;46(4):352-5.
63. Lee K et al. Papillary cannulation facilitated by submucosal saline injection into an intradiverticular papilla. Clin Endosc. 2019;52(1):83-6.
64. Calvo MM et al. The rendezvous technique for the treatment of choledocholithiasis. Gastrointest Endosc. 2001;54(4):511-3.
65. Dhir V et al. EUS-guided biliary rendezvous using a short hydrophilic guidewire. J Interv Gastroenterol. 2011;1(4):153-9.

Frequency Distribution of Hepatitis C Virus Genotypes with Reference to Age and Sex in Various Districts of Khyber Pakhtunkhwa, Pakistan

Authors: Bakht Biland,[1] Mohsina Haq,[1] Sardar Muhammad,[1] Mohsan Subhani,[2] Syed Gardezi,[3] Najibul Haq,[1] *Nadeem Tehami[4]

1. Peshawar Medical College, Pakistan
2. Nottingham Digestive Disease Biomedical Research Centre, University of Nottingham, UK
3. The Grange University Hospital, Aneurin Bevan University, Cwmbran, UK
4. University Hospital Southampton NHS Foundation Trust, UK

*Correspondence to nadeem.tehami@nhs.net

Disclosure: The authors have declared no conflicts of interest.

Received: 26.12.20

Accepted: 07.06.21

Keywords: Cross-sectional study, distribution, epidemiology, genotype, hepatitis C virus (HCV), population study.

Citation: EMJ Hepatol. 2021;9[1]:83-88.

Abstract

Background: Pakistan has the second highest prevalence of hepatitis C in the world after Egypt. Viral hepatitis is a leading cause of morbidity and mortality in Pakistan and, worryingly, reinfection rates are also on the rise. This cross-sectional study was aimed at finding the most common genotypes of hepatitis C in terms of age and sex in a Pakistani cohort.

Materials and methods: The authors collected blood samples from 1,260 patients with diagnosed hepatitis C visiting a primary teaching hospital affiliated with Peshawar Medical College, Pakistan, from different districts of Khyber Pakhtunkhwa, Pakistan, between January 2017 and April 2019. Hepatitis C virus RNA was quantified by real-time polymerase chain reaction and genotyping was then performed.

Results: The authors found that genotype 3a was the most prevalent type followed by 1a, mixed, and 3b, respectively. Genotypes 2a and 1b were the least prevalent in Khyber Pakhtunkhwa. The most common genotype was 3a, observed in 75.87% of cases. The most common mixed genotype was 3a+1a, observed in 39 cases (3.10%); it had a prevalence of 3.49% in females compared with 2.70% in males. Overall, the most common age group affected by hepatitis C virus was 41–50 years (31.35%), followed by the 51–60 years group (24.45%). Infection rate was comparatively low in other age groups. A significant difference was observed in the prevalence of genotype 3a and 2a among different districts.

Conclusion: The authors concluded that genotype 3a was the most prevalent genotype and it was observed more frequently in the female population, with a median age of 45 years.

INTRODUCTION

The goal set by the World Health Organization (WHO) to eradicate hepatitis C by 2030 is a challenge for Pakistan, given that the prevalence of the virus is near that of an epidemic; a meta-analysis published in 2018 estimated a prevalence of 6.2%, which is the second highest worldwide after Egypt.[1] Hepatitis C virus (HCV) is a single-stranded, enveloped RNA virus from the *Flaviviridae* family, genus *Hepacivirus*. The genome of HCV encodes a single polyprotein, which is processed co-translationally. The single polyprotein divides into three structural and seven non-structural polypeptides.[2,3] These enclosed glycoproteins are responsible for the initial stages of infection in a host cell.[4]

At present, HCV has seven recognised genotypes and 67 subtypes. The genotypes are characterised by a discrete geographical dispersion and clinical expression; for example, genotype 1b is predominant in Japan, while in the USA and Europe genotype 1a is more common.[5] The genotype is also correlated to age and transmission route; for example, individuals who use intravenous drugs are mostly reported to have HCV genotypes 3a and 1a, while genotype 1b is common in those who acquired the virus via blood transfusion.[6] It was observed that the older population in the USA mostly acquired HCV iatrogenically, thereby showing a correlation with age. The number of individuals worldwide infected with HCV is 120–130 million, which is approximately 3% of the world population. According to the WHO, 3–4 million individuals in Pakistan are infected with hepatitis C each year, which contributes about 11 million HCV cases to the global disease burden.[7,8]

HCV infection is on a constant rise in Pakistan because of a lack of awareness, poor sterilisation techniques, reuse of contaminated and disposable syringes, and unscreened blood transfusions.[9] Due to non-specific symptoms, it is difficult to pick early HCV infection, even in individuals with high viraemia. The positive cases identified in clinical surveys are usually in individuals with chronic hepatitis C.[10] Acute HCV infection may lead to chronicity because of delay or irregularity in treatment. The majority of chronic cases of hepatitis C lead to cirrhosis and hepatocellular carcinoma.[11] Due to poor socioeconomic circumstances, at present there is no health insurance system in Pakistan. The health department also receives a very small budget from the government and hence, the majority of patients still rely on cheap and easily available treatments, such as interferon and peginterferon.[12] Until recently, direct-acting antiviral agents were extremely expensive; however, due to recent cost regulations, these therapies have become more widely available.[13] Nevertheless, financial constraints still make it difficult for all of the 11 million individuals infected with HCV to be treated with direct-acting antiviral agents. Fortunately, the most prevalent genotype, 3a, responds effectively to the more conveniently available interferon therapy. Proper screening followed by genotyping is imperative before starting antiviral treatment as the efficacy, duration, and outcome of treatment will be dependent upon those tests.[14,15]

MATERIALS AND METHODS

Sample Collection

The authors conducted a cross-sectional study at Prime Teaching Hospital, Peshawar, Pakistan, affiliated with Peshawar Medical College, where patients from almost all districts of Khyber Pakhtunkhwa attend. A total of 1,260 HCV-positive cases, 630 males and 630 females, were included in the study between January 2017 and April 2019.

Virologic Examination

A total of 1,260 HCV-positive serum samples from an equal number of males and females (630 each) were selected for viral RNA extraction. INNO-LiPA (line probe assay) HCV kit (Innogenetics N.V. [now Fujirebio Europe N.V.], Ghent, Belgium) was used for the genotyping of HCV. Two sets of HCV universal biotinylated primers were selected for the amplification of the 5' non-coding region of the HCV genome because of highly conserved base pairs. The HCV RNA-amplified products were hybridised to immobilise oligonucleotides specific for the genotypes of HCV. The HCV RNA quantification method described by Sarrazin et al.[16] was used for CAP/CTM test (Roche Molecular Systems, Inc., Branchburg, New Jersey, USA). The extraction of RNA was performed by automated

COBAS® AmpliPrep using 1 mL of plasma, and the extracted RNA was amplified by automated real-time polymerase chain reaction. The COBAS® TaqMan® 48 Analyzer was used for the detection of amplified RNA. Data analysis was performed using the AMPLILINK software, which presented HCV load as Log10 international units/mL. The real-time-PCR-positive samples were further subjected to genotyping assays.

Statistical Analysis

This study did not test any specific hypothesis. The continuous data were presented in percentages and descriptive statistics were outlined. The independent samples t-test and binomial test were used. A p value of <0.05 was considered significant.

RESULTS

Hepatitis C Virus Genotype Distributions in Different Age Groups

A total of 1,260 patients were divided into seven age groups. Starting from 10 years of age, each group had a difference of 10 years and HCV genotype prevalence was recorded and analysed for each group (Figure 1). The highest prevalence of HCV was observed in the 41–50 years age group with 395 cases (31.35%), followed by the 51–60 years age group with 308 cases (24.45%). The lowest HCV prevalence was noted in the patients >70 years of age (1.43%) and those <20 years of age (3.65%). There was no significant relationship found between age and genotype (p=0.028) when controlled for sex.

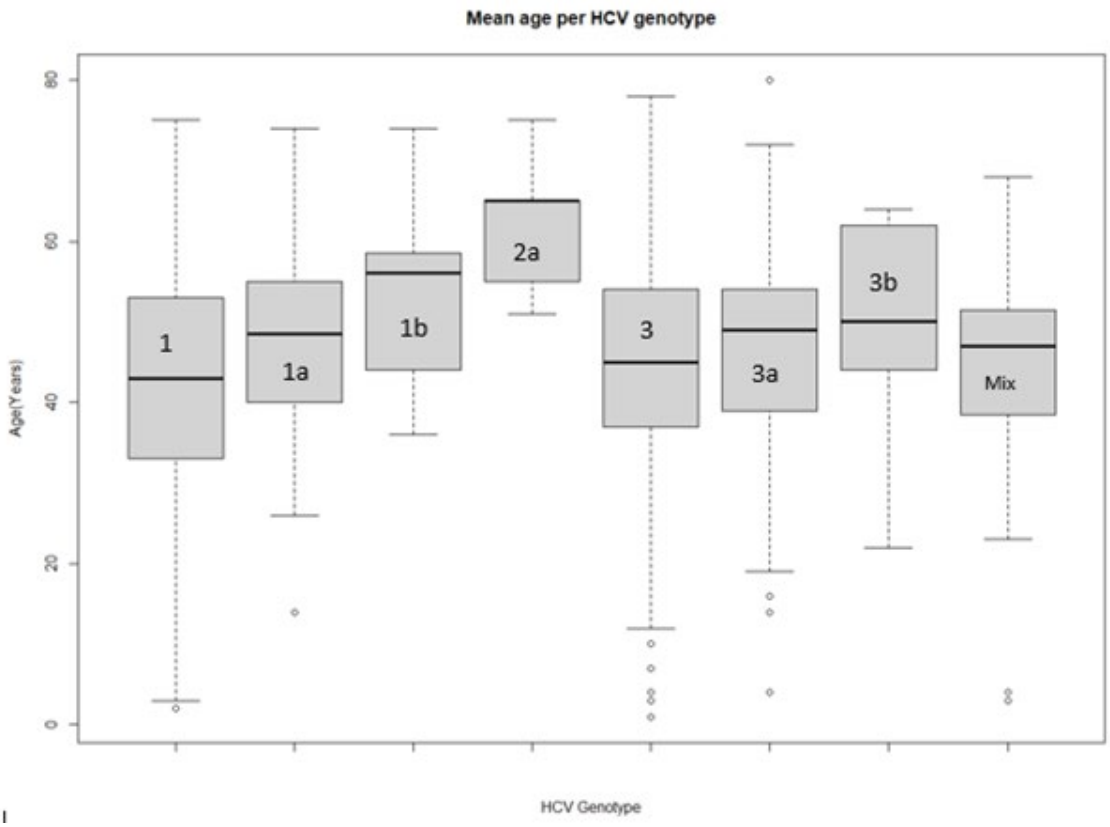


Figure 1: Box plot of distribution of mean age across hepatitis C virus genotypes.
HCV: hepatitis C virus.

Sex-Based Hepatitis C Virus Genotype Distributions

It was observed that the most prevalent HCV genotype in both sexes was 3a, with a total of 956 cases (75.87%); however, the prevalence was slightly higher in females with 487 cases (77.30%) compared to 469 cases (74.44%) in the male population. The second most prevalent HCV genotype observed was 1a with 250

isolates (19.84%). Genotype 1a was, however, slightly more prevalent in males, with 135 isolates (21.43%) as compared to 115 isolates in females (18.25%). A fairly significant number (39; 3.10%) of isolates had mixed genotypes, with a slightly higher prevalence in females: 22 cases (3.49%) compared to 17 cases (2.70%) in the male population. Genotypes 1b, 2a, and 3b were more common in males.

The highest number of patients from a single district of Khyber Pakhtunkhwa was 736 (58.41%) from Peshawar, followed by Mardan with 78 cases (6.19%), Lower Dir with 63 cases (5.00%), and Dera Ismail Khan with 57 cases (4.52%). The most prevalent genotype in all districts was 3a, with slightly differing rates of prevalence. For example, district Buner had 94.12% 3a isolates, which was the highest prevalence of genotype 3a, followed by Nowshera with 91.12%, and Banu with 89.47%; Swabi had comparatively low prevalence of 3a with 57.89%. The second most common genotype, 1a, had the highest prevalence in Khyber Agency with 33.34% isolates, followed by Swabi with 31.58% and Mohmand Agency with 26.09% isolates. There were significant variations in the prevalence of HCV genotypes in the different districts of Khyber Pakhtunkhwa (Figure 2).

Age- and Sex-Based Distribution of Mixed Genotypes

A total of 39 (3.10%) patients with mixed genotypes were observed. Genotypes 3a+1a were found in 32 patients (82.06%), whereas the 3a+2a genotype was observed in three patients (7.69%), 4a+3a in two patients (5.13%), 3a+1b in one patient (2.56%), and 2a-1a in one patient (2.56%). For the mixed genotypes, 22 (56.41%) were female and 17 (43.59%) were male (Table 1).

DISCUSSION

It is now well established that the severity of HCV infection, progression, and patient response to therapy vary according to HCV genotype and subtypes. Worldwide, several regional differences appear to exist in the distribution of HCV subtypes and major genotypes.

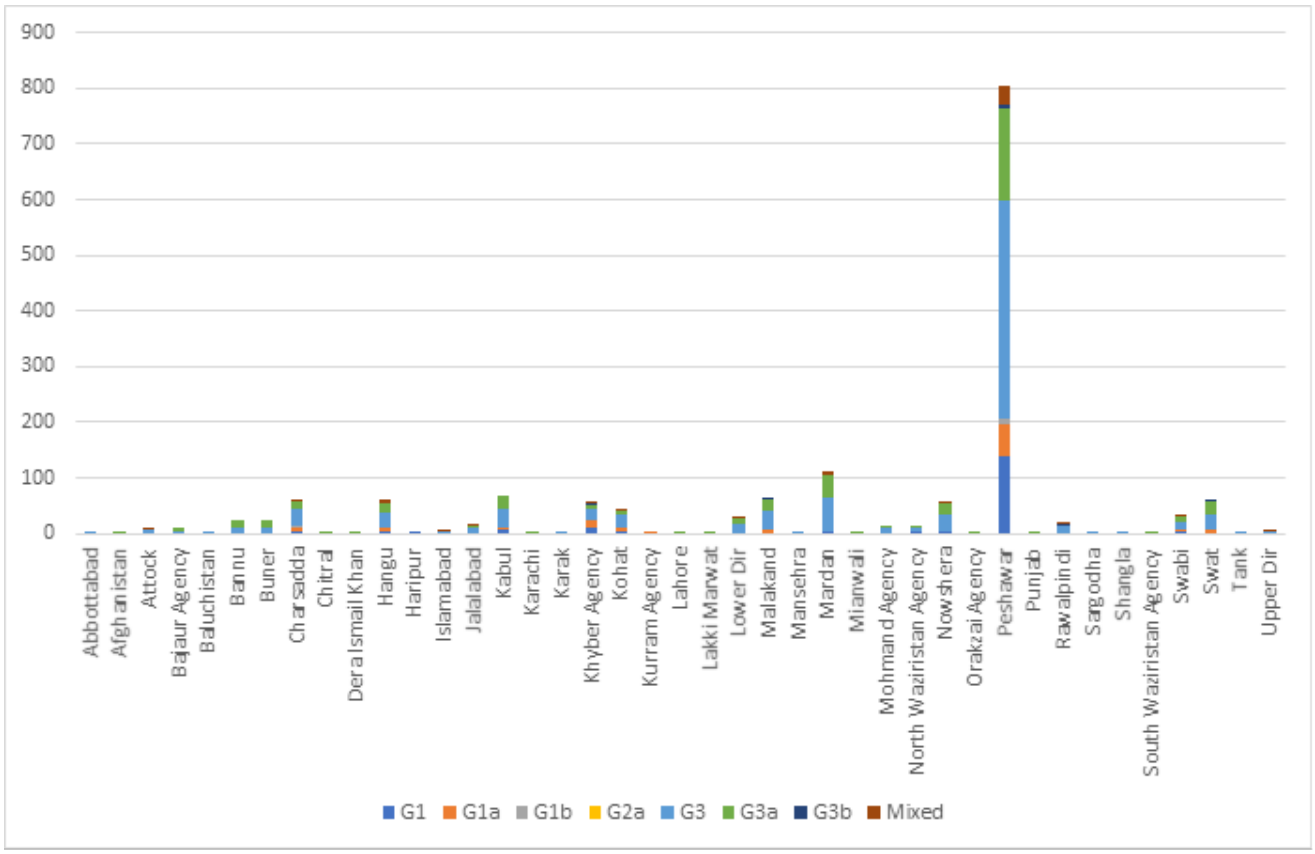


Figure 2: Balance plot of geographic distributions of hepatitis C virus by genotype.

Table 1: Mixed genotype associations with sex and age group in Khyber Pakhtunkhwa, Pakistan.

Mixed genotype	Age group (years)	Male n (%)	Female n (%)	Total n (%)
2a-1a	41–50	0 (0.00)	1 (4.55)	1 (2.56)
3a-1a	29–64	12 (70.59)	20 (90.90)	32 (82.06)
3a-1b	60–64	1 (5.88)	0 (0.00)	1 (2.56)
3a-2a	41–64	3 (17.65)	0 (0.00)	3 (7.69)
4a-3a	34–51	1 (5.88)	1 (4.55)	2 (5.13)
Total	n/a	17 (100.00)	22 (100.00)	39 (100.00)

n/a: not applicable.

Hence, knowledge of the distribution of the various genotypes in Pakistan is essential for the prognostic implications of chronic hepatitis C infection. In this study, the authors reported genotype-based distribution of hepatitis C in relation to age, sex, and geographic locality.

Although several studies have been performed on hepatitis and its genotypes in other parts of the country, a population-based data set is still lacking. It is important to note that Pakistan has the second highest prevalence of hepatitis C in the world next to Egypt; in the Punjab province alone, 7% of individuals are infected with HCV and the rate of infection is still rising.[17-19]

The prevalence of the various genotypes observed in the study is very similar to those reported in previous studies. A key observation in this study was the finding of the most prevalent genotype, 3a, followed by mixed genotypes, which was also reported by Gul et al.[27] However, as reported previously by Idrees et al.[26] in a study based in Khyber Pakhtunkhwa, no cases of genotype 5 or 6 were observed in this study.[20-25] The most prevalent genotypes in the population of Khyber Pakhtunkhwa was 3a and mixed types, which was also reported by Gul et al.[27] The 41–50 years age group was the most infected group, with a slight predominance in female individuals, which has also been previously reported.[28-30]

The frequency distribution of the HCV genotype observed in this Pakistan-based study appears to be similar to the reported genotypes from other South Asian countries such as India[31] and Nepal,[32] with 3a as the predominant genotype; however, this pattern differs from the USA, Western Europe,[33] Thailand,[34] and Japan[35] where the reported predominant HCV genotype is 1 (1a and 1b).

CONCLUSION

This study found that the most prevalent HCV genotype in Khyber Pakhtunkhwa, Pakistan, was type 3a followed by 1a, mixed, 1b, 2a, and 3b. This epidemiological pattern of genotype distribution is seen across South East Asia; however, there is no agreed explanation for this observed pattern.[20] It was also observed in this study that HCV genotype 3a and mixed genotypes infection is slightly more common in females; on the other hand, males showed slightly higher prevalence of genotypes 1a, 1b, 2a, and 3b. The age group most affected was the 41–50 years age group, followed by the 51–60 years age group. Low prevalence was observed in individuals >70 years of age and <20 years of age. To conclude, all possible modes of HCV transmission should be communicated to the masses through awareness campaigns to control the spread of the virus.

References

1. Al Kanaani Z et al. The epidemiology of hepatitis C virus in Pakistan: systematic review and meta-analyses. R Soc Open Sci. 2018;5(4):180257.
2. Grakoui A et al. Expression and identification of hepatitis C virus polyprotein cleavage products. J Virol. 1993;67(3):1385-95.
3. Ali N et al. Hepatitis C virus-induced cancer stem cell-like signatures in cell culture and murine tumor xenografts. J Virol. 2011;85(23):12292-303.
4. Morozov VA, Lagaye S. Hepatitis C virus: morphogenesis, infection and therapy. World J Hepatol. 2018;10(2):186-212.
5. Smith DB et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318-27.
6. Pawlotsky JM et al. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. J Infect Dis. 1995;171(6):1607-10.
7. Hanafiah KM et al. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42.
8. World Health Organization (WHO). Monitoring and evaluation for viral hepatitis B and C: recommended indicators and framework. 2016. Available at: https://www.who.int/publications/i/item/monitoring-and-evaluation-for-viral-hepatitis-b-and-c-recommended-indicators-and-framework. Last accessed: 9 June 2021.
9. Afzal MS, Iqbal MA. Hepatitis C virus in Pakistan: community education is an effective weapon against the killer. Viral Immunol. 2017;30(8):548-51.
10. Afzal MS et al. Hepatitis C virus infection in Iran; viral spread routes in general population and safety measures. Hepat Mon. 2015;15(10):e17343.
11. Afzal MS. Hepatitis C virus and interferon-free antiviral therapeutics revolution: implications for Pakistan. Viral Immunol. 2017;30(4):252-7.
12. Raza H et al. HCV, interferon therapy response, direct acting antiviral therapy revolution and Pakistan: future perspectives. Asian Pac J Cancer Prev. 2015;16(13):5583-4.
13. Ahmad B et al. Conventional interferon therapy response among chronic HCV patients in Khyber Pakhtunkhwa. J Infect Dis Ther. 2013;1:104.
14. Afzal MS, Anjum S. Changing of HCV clade pattern in Iran; the possible means for something good. Hepat Mon. 2014;14(1):e11879.
15. Afzal MS et al. Hepatitis C virus capsid protein and intracellular lipids interplay and its association with hepatic steatosis. Hepat Mon. 2014;14(8):e17812.
16. Sarrazin C et al. Comparison of conventional PCR with real-time PCR and branched DNA-based assays for hepatitis C virus RNA quantification and clinical significance for genotypes 1 to 5. J Clin Microbiol. 2006;44(3):729-37.
17. Janjua N, Nizamy M. Knowledge and practices of barbers about hepatitis B and C transmission in Rawalpindi and Islamabad. J Pak Med Assoc. 2004;54(3):116-9.
18. Jiwani N, Gul RB. A silent storm: hepatitis C in Pakistan. J Pioneer Med Sci. 2011;1(3):89-91.
19. Arshad A, Ashfaq UA. Epidemiology of hepatitis C infection in Pakistan: current estimate and major risk factors. Crit Rev Eukaryot Gene Expr. 2017;27(1):63-77.
20. Attaullah S et al. Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J. 2011;8:433.
21. Ali S et al. Frequency distribution of HCV genotypes among chronic hepatitis C patients of Khyber Pakhtunkhwa. Virol J. 2011;8:193.
22. Afridi SQ et al. Prevalence of HCV genotypes in district Mardan. Virol J. 2013;10:90.
23. Waqas M et al. Distribution pattern of various genotypes of HCV circulating in district Mardan, Khyber Pakhtunkhwa, Pakistan. Advan Biol Res. 2015;9(2):69-74.
24. Tahir M et al. Detection and genotyping of HCV in patients of Sheikh Zayed Hospital, Lahore, Pakistan. JUMDC. 2016;7(1):25-30.
25. Ilyas M et al. Epidemiological study of HCV genotypes circulated in different regions of Pakistan: a lab based study. Int J Biosci. 2014;4(1):477-83.
26. Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC infect Dis. 2008;8:69.
27. Gul A et al. New patterns of HCV subtypes distribution in the Khyber Pakhtunkhwa province of Pakistan. Braz J Infect Dis. 2016;20(1):107-8.
28. Khan N et al. Geographic distribution of hepatitis C virus genotypes in Pakistan. Hepat Mon. 2014;14(10):e20299.
29. Waqar M et al. Determination of hepatitis C virus genotypes circulating in different districts of Punjab (Pakistan). Eur J Gastroenterol Hepatol. 2014;26(1):59-64.
30. Ali A et al. Determination of HCV genotypes and viral loads in chronic HCV infected patients of Hazara Pakistan. Virol J. 2011;8:466.
31. Chowdhury A et al. Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. Hepatology. 2003;37(4):802-9.
32. Tokita H et al. Hepatitis C virus variants from Nepal with novel genotypes and their classification into the third major group. J Gen Virol. 1994;75(Pt 4):931-6.
33. Pujol FH, Loureiro CL. Replacement of hepatitis C virus genotype 1b by genotype 2 over a 10-year period in Venezuela. J Clin Gastroenterol. 2007;41(5):518-20.
34. Tokita H et al. Hepatitis C virus variants from Thailand classifiable into five novel genotypes in the sixth (6b), seventh (7c, 7d) and ninth (9b, 9c) major genetic groups. J Gen Virol. 1995;76(Pt 9):2329-35.
35. Shinji T et al. Analysis of HCV genotypes from blood donors shows three new HCV type 6 subgroups exist in Myanmar. Acta Med Okayama. 2004;58(3):135-42.

Hepatic Encephalopathy: A Review

Authors: Savan Kabaria,[1] Ishita Dalal,[2] Kapil Gupta,[2,3] Abhishek Bhurwal,[2] Carlos D. Minacapelli,[2,3] Carolyn Catalano,[2,3] *Vinod Rustgi[2,3]

1. Rutgers Robert Wood Johnson Medical School, Department of Internal Medicine, New Brunswick, New Jersey, USA
2. Rutgers Robert Wood Johnson Medical School, Department of Medicine, Division of Gastroenterology and Hepatology, New Brunswick, New Jersey, USA
3. Center for Liver Diseases and Masses, Rutgers Robert Wood Johnson School of Medicine, New Brunswick, New Jersey, USA

*Correspondence to vinod.rustgi@rutgers.edu

Disclosure: The authors have declared no conflicts of interest.

Received: 03.02.21

Accepted: 26.03.21

Keywords: Cirrhosis, hepatic encephalopathy, lactulose, liver disease, rifaximin.

Citation: EMJ Hepatol. 2021;9[1]:89-97.

Abstract

Hepatic encephalopathy (HE) is a reversible syndrome observed in patients with liver disease. The syndrome is characterised by a spectrum of neuropsychiatric abnormalities resulting from the accumulation of neurotoxic substances in the bloodstream and ultimately in the brain. HE is a huge burden to patients, caregivers, and the healthcare system. Common treatments for HE, including rifaximin and lactulose, have been shown to reduce the risk of recurrence, frequency of hospitalisations, hospital costs, and mortality. New research and therapeutics exist, including faecal transplants and small-molecule therapies such as branched-chain amino acids. This review article provides a general overview of the current understanding of HE.

INTRODUCTION

Hepatic encephalopathy (HE) is a complex neurological process that is seen in advanced liver disease. HE causes significant morbidity and mortality and is responsible for considerable burden on patients and their caregivers. HE is treated clinically and can be reversible, especially in the situation of an acute underlying process. This review article will briefly review HE in its entirety: its epidemiology, manifestations, diagnosis, pathophysiology, and treatment as well as future research.

The incidence and prevalence of HE can be difficult to assess because of underlying cause, variable severity of the disease manifestations, and the definition of HE (minimal versus overt).[1] Consequently, HE has been reported to occur in a wide range (20–80%) of patients with cirrhosis. The prevalence of overt HE at the time of cirrhosis diagnosis is approximately 10–14%. This rises to 16–21% in patients with decompensated cirrhosis and approximately 10–50% in patients who have undergone transjugular intrahepatic portosystemic shunt (TIPS). Overall, an estimated 30% to 40% of patients with cirrhosis will experience overt HE during their clinical course.[1]

CLINICAL MANIFESTATIONS

HE can present as a diverse spectrum of neurologic, psychiatric, and musculoskeletal

symptoms. In many cases, patients are unaware of the symptoms, but family members or close friends may notice changes and voice their concerns.[2] In the early stages, patients may only report subtle symptoms, such as disturbances in their sleep–wake cycles.[3] As symptoms progress, patients may develop personality changes such as apathy, irritability, and disinhibition. If left unrecognised, symptoms worsen and patients can present with cognitive impairments such as disorientation, memory impairment, slurred speech, confusion, and eventually coma.[4,5] Along with neurologic and psychiatric symptoms, HE can also affect the musculoskeletal system. Patients with minimal HE may show minor issues with coordination, such as changes in their handwriting.[6]

The most widely recognised symptom of HE is asterixis. However, asterixis is not isolated to HE. It can also be seen in other causes of encephalopathy, such as uraemia.[7] Asterixis presents as a flapping tremor that occurs because of negative myoclonus resulting in loss of postural tone.[7] It is elicited when patients hyperextend their wrists but can also be observed in the patient's feet, legs, arms, tongue, and eyelids.[6] If it continues to progress, patients can develop hyperreflexia, clonus, and rigidity.[6] They can also present with cirrhosis-related Parkinsonism symptoms, with extrapyramidal symptoms including masked facies, rigidity, bradykinesia, slowed speech, and tremors.[7,8] These symptoms are seen in approximately 4% of patients, suggesting they are more prevalent than previously thought.[8] In patients with TIPS, HE usually becomes clinically apparent 2–3 weeks after TIPS insertion.[9]

CLASSIFICATION

According to national guidelines, HE is classified using four main axes: the underlying cause, the severity of the disease manifestation, the time course of the disease, and the existence of precipitating factors.[6]

The first criterion is based on the underlying cause of HE. Type A is HE seen in acute liver failure, Type B in the portal-systemic bypass setting with no intrinsic hepatocellular disease, and Type C is in the setting of cirrhosis with portal hypertension or systemic shunting. The most commonly occurring type is Type C in which patients frequently show signs of liver failure, such as jaundice, ascites, spider telangiectasias, and palmar erythema, among other manifestations.[10]

The severity of HE is generally classified using the West Haven Criteria (WHC) and the International Society for Hepatic Encephalopathy (ISHEN) criteria (Table 1).[6] Alternate grading systems, such as HE Scoring Algorithm (HESA), were developed to provide more reliable grading of HE severity by combining both subjective and objective data.[11] Clinical HE Staging Scale (CHESS) is another scoring system that consists of nine clinical items and linearly determines severity using simple terms and questions.[12] Various criteria to define the severity of HE are depicted in Table 1.

The third and fourth axes allow patients to be further classified according to the time course and precipitating factors.[6] Timing is further sub-classified into episodic, recurrent, or persistent. To classify as recurrent, episodes of HE occur within intervals of less than 6 months, whereas persistent HE involves altered behaviour that is always present in the setting of recurrent HE. Examples of precipitating factors include infections, gastrointestinal bleeding, over-diuresis, electrolyte abnormalities, and constipation. Treating precipitating factors can resolve HE in 90% of all patients.[6]

DIAGNOSIS

There is no standardised serologic testing or imaging modality to accurately diagnose HE and assess its severity. Ammonia is classically associated with HE; however, increased blood ammonia level alone does not help with the diagnosis or prognosis of HE. Elevation of ammonia in combination with the clinical picture of HE can be more useful, as isolated increased ammonia level can be seen in other medical conditions.[13]

Table 1: Various criteria to define severity of hepatic encephalopathy.

WHC Grade	ISHEN	HESA	CHESS	Clinical Features
Unimpaired				No encephalopathy at all, no history of HE
Minimal	Covert	Grade 0	0–1	Alterations in psychomotor speed/ executive functions. No evidence of clinical mental status change.
Grade I		Grade I	0–3	Anxiety, attention span deficit, impaired simple mathematic skills, altered sleep pattern, unaware of deficits
Grade II	Overt	Grade II	1–6	Lethargy, not oriented to time, personality changes, bizarre behaviour, dyspraxia, asterixis
Grade III		Grade III	3–6	Somnolence, semi-stupor, responsive to stimulus, increasing confusion and disorientation
Grade IV		Grade IV	9	Coma

The data are reported as mean±SD or median (and interquartile range) as appropriate.
HRS: hepatorenal syndrome; MAP: mean arterial pressure: NA, not available.

Current national guidelines suggest the use of an electroencephalogram and complementary neurophysiological tests to diagnose HE in the absence of other neurological process.[6] Sensitivity and specificity of an electroencephalogram depend on both modality of data analysis and severity of HE and vary from 57–100% and 41–88%, respectively.[14] Neurophysiological tests include psychometric testing of attention as well as working memory and psychomotor speed. Visuospatial ability may also be necessary to identify subtle mental status changes.[15,16] Specific examples of these tests and time required to perform them are depicted in Table 2.[14,17] While these tests can be beneficial, they are limited because they do not account for results secondary to the patient's age or baseline education status. They are also limited by time and lack of adequately trained professionals.[17]

Imaging modalities such as CT and MRI scans of the brain are generally non-specific for HE; however, sequential scans may be a useful marker for acute brain volume change.[14] PET is not widely used because of cost and limitations of availability; however, PET does provide valuable insight into the pathogenesis of HE in regard to calculating blood flow, glucose metabolism, and ammonia metabolism, which may have prognostic value.[14]

PATHOPHYSIOLOGY

The pathophysiology of HE is vital in understanding its management. The most well-understood pathophysiological mechanism and correlate of HE is the neurotoxicity of ammonia in the brain, either due to increased production or impaired excretion.[18]

Table 2: Diagnostic and screening methods of hepatic encephalopathy.

Tests	Equipment required	Tested domains	Time required (min)
PHES	Paper and pencil test	Psychomotor speed, visual perception, visuospatial orientation, visuomotor ability, and attention	20
RBANS	Paper and pencil test	Psychomotor speed, anterograde memory, and working memory	30
Computer-aided test Scan test	Computer test	Working memory, vigilance, and attention	15
CDR assessment battery	Computer test	Reaction time, memory, and recognition	15
ICT	Computer test	Response inhibition, working memory, vigilance, and attention	15
EncephalApp Stroop App	Requires smartphone with application (EncephalApp Stroop Application)	Psychomotor speed and cognitive alertness	10
CFF	Light pulse with portable machine (e.g., Hepatonorm Analyzer*)	Visual discrimination and general arousal. CFF is meant to detect neuropsychological abnormalities that can range from visual signal processing to cognitive functions	10

* Accelab GmbH, Kusterdingen, Germany.

CDR: Cognitive Drug Research; CFF: Critical Flicker Frequency; ICT: Inhibitory Control Test; PHES: Psychometric Hepatic Encephalopathy Score; RBANS: Repeatable Battery for the Assessment of Neuropsychological Status.

The two main sites of ammonia production are the small/large intestine (50%) and the kidneys (40%). In the gastrointestinal system, ammonia is produced by degradation of dietary protein to ammonia from urease-producing bacteria as well as breakdown of glutamine by enterocyte glutaminase. Within the kidneys, the proximal tubular cells generate ammonia from glutamine and create bicarbonate as a by-product. The production of ammonia at these sites can be altered through various mechanisms including gastrointestinal bleeding, hypovolaemic states, over-diuresis, hypokalaemia, acidosis, and excessive protein intake.[18]

The liver is the major site of ammonia catabolism using the urea cycle (Krebs–Henseleit cycle), which converts ammonia into water-soluble urea. The generated urea is subsequently excreted via the intestine and the urine. Due to the reduced capability of the liver to detoxify ammonia, secondary to hepatocellular damage or shunting, ammonia levels increase within the systemic circulation. The kidneys also contribute to decreased ammonia excretion due to acid–base and potassium imbalance, increased protein intake, and dysregulation of glucocorticoid hormones.[19] Skeletal muscle also plays a role in ammonia detoxification through glutamine synthase, which converts ammonia to glutamine. Consequently, sarcopenia, a common complication of cirrhosis, can be an adverse factor in HE.[20]

Dysfunction of the neurons can result from elevations in ammonia in the systemic circulation. Astrocytes, via astrocytic glutamine synthetase, convert ammonia and glutamate into glutamine, which causes increased cerebral volume by osmosis. Consequently, a rapid rise in ammonia

can place the patient at risk of brain oedema, as often evidenced on MRI.[21] Additionally, glutamine is converted to ammonia in the mitochondria of the astrocytes, which can also lead to direct oxidative damage.[22]

Another well-described pathophysiological mechanism is alterations in the gut flora that play a vital role in HE. It is theorised that decreased bile acid production allows urease-producing bacteria to proliferate and overtake protective bacteria (i.e., *Lachnospiraceae*).[18,23] Multiple studies have stressed the importance of the microbiota in the development of HE.[18,24,25] Several molecules other than ammonia, particularly altered neurotransmitters, may also participate in the development of HE. These can include glutamine, histamine, serotonin, γ-aminobutyric acid, and manganese.[18] Further investigation is required to identify additional targets of HE pathophysiology for prompt diagnosis and treatment.

Manifestation of HE is often exacerbated in patients with prior TIPS because of creation of a shunt, allowing portal blood flow to bypass the liver parenchyma. Consequently, ammonia is not broken down or excreted and ultimately crosses the blood–brain barrier, with varying effect on cognitive function.[26]

TREATMENT

The first-line pharmacologic therapy for HE is laxative. The initial goal of therapy is to prevent ammonia absorption through non-absorbable disaccharides (i.e., lactulose). They are potent laxatives and additionally help alter the intestinal microbiome to non-urease-producing bacteria that reduce ammonia production.[27,28] Lactulose also lowers the pH of the colon, causing conversion of ammonia to the ammonium ion, which is not readily absorbable in the colon.[29,30] Disaccharide enemas and polyethylene glycol have also been proven to be useful for removing excess ammonia from the colon.[29,31] Use of prophylactic therapy to prevent post-TIPS HE was not shown to be beneficial.[6,32]

Antimicrobials are used to treat HE by targeting culprit gut flora and thereby leading to decreased ammonia production. The most commonly used antimicrobial is rifaximin, a synthetic antibiotic that targets aerobic and anaerobic Gram-negative and Gram-positive bacteria.[29,33] Rifaximin has been found to reduce hospital admissions and frequency of recurrent episodes.[27,34] In the past, neomycin, vancomycin, and metronidazole have also been used to treat HE but have fallen out of favour.[29,35,36]

Faecal microbiota transplant (FMT) is a promising treatment for refractory overt HE. Enteric bacteria play an important role in the pathophysiology of HE as described previously. The goal of FMT is to manipulate the gut microbiome in an attempt to reverse intestinal dysbiosis. Two trials exploring the use of FMT in HE found improvement after pre-treatment with antibiotics.[29,37,38]

Protein restriction is not recommended as normal protein intake levels do not exacerbate or cause HE. Protein restriction may actually be deleterious in patients who are already sarcopenic.[27] Other pharmacologic treatments that have efficacy in treating HE are L-ornithine L-aspartate and branched-chain amino acids. L-ornithine L-aspartate works to improve HE by upregulation of two enzymes, carbamoyl phosphate synthetase and glutamine synthetase, which are enzymes for urea and glutamine synthesis; these are impaired in cirrhosis.[39] Branched-chain amino acids are theorised to assist with HE through increased detoxification of ammonia in the skeletal muscle. Common treatments for HE are summarised in Table 3.

PROGNOSIS

The development of subclinical or overt HE in a patient with liver pathology has been considered one of the adverse markers of decompensation and carries significant morbidity and more. HE was incorporated into early prognostic tools, including the Child–Turcotte–Pugh score, based on clinical experience at the time. The prognosis related to HE varies significantly depending on the decompensation status of the underlying liver cirrhosis.[6,40]

Subclinical HE has been shown to be present in as many as 80% of patients with cirrhosis in the USA. It may adversely impact activities of daily living, such as driving, which can further affect patients' socioeconomic status.[41]

Table 3: Common agents used in the treatment of hepatic encephalopathy.

Therapy	Mechanism	Benefits	Comments
Lactulose	Osmotic laxative Acidification of the colon ↓ urease-producing bacteria ↓ ammonia production/ absorption	Improved cognitive function Most extensively studied ↓ progression to overt HE	Cost effective Mainstay of HE treatment Result in dehydration if severe diarrhoea
Rifaximin	↓ urease-producing bacteria ↓ ammonia production	Improved cognitive function ↓ HE-related hospitalisations	Modulates gut flora Expensive
BCAA	Promotes synthesis of glutamine from ammonia in the skeletal muscle	Improves recurrent HE	No effect on overall mortality
LOLA	Ammonia scavenging ↑ production of urea in hepatocytes, activating glutamine synthase in hepatocytes and skeletal muscle	↓ progression to overt HE	Further studies required to prove efficacy
Glycerol phenylbutyrate	↑ excretion of glutamine	↑ time to HE recurrence	No benefit for patients on rifaximin
Zinc	If deficient, reduced urea cycle utilisation of ammonia	Improvement in cognitive tests	No evidence on other outcomes
FMT	Manipulate gut microbiome	Improvement in HE recurrence	Currently under investigation Used for recurrent HE

BCAA: branched-chain amino acid; FMT: faecal microbiota transplant; HE: hepatic encephalopathy; LOLA: L-ornithine L-aspartate.

It may adversely impact activities of daily living such as driving; this can further affect patients' socioeconomic status.[42,43] Patients with subclinical HE also have a higher likelihood of developing overt HE within a period of 2 weeks to 2 years after the initial diagnosis.[44,45] Further, subclinical HE has been independently associated with increased mortality and the need for liver transplantation, irrespective of the Model for End-Stage Liver Disease (MELD) score.[46] Therefore, the diagnosis of subclinical HE is important in prognosticating the development of overt HE as well as overall prognosis.

The development of overt HE is one of the events that defines a decompensated phase of cirrhosis. The development of the first episode of overt HE is independently associated with shorter life expectancies. The cumulative survival of patients who developed overt HE has been found to be <50% in 1 year and <25% at 3 years.[47,48] Development of HE has been shown to have a worse 1-year mortality outcome than either variceal bleeding or ascites, which suggests that HE may potentially hold more prognostic value than other events of decompensation.[48]

The duration of HE, with episodes lasting longer than 48 hours, is important and has been correlated with lower survival rates.[49] Higher mortality has also been shown in patients hospitalised with HE and follow-up periods including 28, 90, and 365 days after hospitalisation.[50,51] The presence of overt HE is also of prognostic importance in patients with cirrhosis after placement of TIPS. Compared to a single episode of HE, early recurrent overt HE has been found to have three times the increased mortality after adjusting for the MELD score, ascites, albumin, indication for TIPS, and age.[52]

FUTURE RESEARCH

There are still controversies related to the classification of HE and the role of ammonia measurement in the management of patients

with HE.[53] Multiple tools are in development to accurately diagnose HE that are not yet validated. The challenge is to keep the clinical diagnostic test simple for the patients while providing high accuracy in diagnosing HE. For example, naming the maximum number of animals displayed in 1 minute has been shown to directly correlate with the grade of encephalopathy.[54] A pilot study utilising biomolecular testing has demonstrated the potential use of exhaled volatile organic compounds to detect HE.[55] With further improvements in MR and PET technology, research may help personalise prophylactic and therapeutic approaches for patients with HE.[53] The classification of HE in the setting of acute-on-chronic liver failure (ACLF) also remains undefined. However, HE in patients with ACLF has distinct clinical features, prognosis, and pathophysiology compared to the conventional forms represented in Types A, B, and C. Further investigations into the classification of HE in patients with ACLF and the role of modulating ammonia in this patient population need to be conducted.[50,56]

Ultimately, liver transplantation remains the most effective treatment available for cirrhosis and HE; however, it is not always an available option. Unfortunately, permanent cognitive impairment from pre-transplant HE may persist even after this step.

There are a few promising treatments for HE that are still experimental. Probiotics are being evaluated for efficacy in treatment of HE, given the role of microbiota; however, the data that suggests any benefit have been of low quality with no evidence of mortality improvement.[29] Minocycline, which reduces microglial cell activation, has been shown to potentially reduce brain oedema and plasma and cerebrospinal fluid ammonia levels.[57] Alternative potential treatments include indomethacin, ibuprofen, and phosphodiesterase inhibitors, which restore the function of the glutamate–nitric oxide–cyclic guanine monophosphate pathway in the cerebral cortex in experimental models.[29] Benzodiazepine inverse agonists, such as Ro15-4513, have also been shown to be effective in treating HE in animal models of liver failure.[58] Flumazenil improved clinical symptoms of HE, albeit temporarily, in a double-blind crossover clinical trial, possibly by decreasing γ-aminobutyric acidergic tone.[59] Acetyl-L-carnitine has shown improvement in symptoms of HE as assessed by neuropsychological testing and cognitive abilities, with parallel reductions in serum and brain ammonia levels.[60]

The use of liver support systems, such as the Molecular Adsorbent Recirculating System (MARS®; Gambro Lundia AB, Lund, Sweden) and Prometheus® device (Fresenius Medical Care, Bad Homburg, Germany), have shown some promise in the acute setting. They function by removing circulating toxins that accumulate in the blood due to liver dysfunction. Both devices have been well tolerated by patients with liver failure and pilot studies suggest improvement in survival rates in some groups of patients with liver failure.[61] These devices might have particular utility as bridges to transplantation; however, they might not be appropriate in all patients given the requirement for central venous access and the non-uniform treatment protocols.[62]

Despite several promising avenues of treatment of HE, studies remain in the early phase and await proof. Future research should understand the impact of comorbidities on outcomes, biomolecular diagnostic strategies, targeting systematic, and neuroinflammation and provide clear endpoints for clinical trials.

CONCLUSION

The clinical presentation of HE may be highly variable and may range from defects in cognition, personality, and intellect, to an altered state of consciousness and impaired neuromuscular function with asterixis and hyperreflexia.

The heterogeneous manifestations of HE vary not only between patients but also longitudinally for an individual patient. By categorising HE's severity accurately and efficiently, valuable prognostic information and current clinical status can be ascertained.

In conclusion, HE remains one of the most critical prognosticators of worsening cirrhosis, contributing to and correlating with the mortality and morbidity of patients with cirrhosis. Advances in therapeutics are underway to improve clinical outcomes in patients with HE.

References

1. Elsaid MI, Rustgi VK. Epidemiology of hepatic encephalopathy. Clin Liver Dis. 2020;24(2):157-74.
2. Dellatore P et al. Clinical manifestations of hepatic encephalopathy. Clin Liver Dis. 2020;24(2):189-96.
3. Córdoba J et al. High prevalence of sleep disturbance in cirrhosis. Hepatology. 1998;27(2):339-45.
4. Weissenborn K. Diagnosis of encephalopathy. Digestion. 1998;59(Suppl 2):22-4.
5. Wiltfang J et al. Psychiatric aspects of portal-systemic encephalopathy. Metab Brain Dis. 1998;13(4):379-89.
6. Vilstrup H et al. Hepatic encephalopathy in chronic liver disease: 2014 practice guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. Hepatology. 2014;60(2):715-35.
7. Weissenborn K et al. Neurological and neuropsychiatric syndromes associated with liver disease. Aids. 2005;19(Suppl 3):S93-8.
8. Tryc AB et al. Cirrhosis-related Parkinsonism: prevalence, mechanisms and response to treatments. J Hepatol. 2013;58(4):698-705.
9. Sanyal AJ et al. Portosystemic encephalopathy after transjugular intrahepatic portosystemic shunt: results of a prospective controlled study. Hepatology. 1994;20(1 Pt 1):46-55.
10. Mumtaz K et al. Precipitating factors and the outcome of hepatic encephalopathy in liver cirrhosis. J Coll Physicians Surg Pak. 2010;20(8):514-8.
11. Hassanein TI et al. Introduction to the hepatic encephalopathy scoring algorithm (HESA). Dig Dis Sci. 2008;53(2):529-38.
12. Ortiz M et al. Development of a clinical hepatic encephalopathy staging scale. Aliment Pharmacol Ther. 2007;26(6):859-67.
13. Ge PS, Runyon BA. Serum ammonia level for the evaluation of hepatic encephalopathy. JAMA. 2014;312(6):643-4.
14. Karanfilian BV et al. Laboratory abnormalities of hepatic encephalopathy. Clin Liver Dis. 2020;24(2):197-208.
15. Amodio P et al. Characteristics of minimal hepatic encephalopathy. Metab Brain Dis. 2004;19(3-4):253-67.
16. McCrea M et al. Neuropsychological characterization and detection of subclinical hepatic encephalopathy. Arch Neurol. 1996;53(8):758-63.
17. Nabi E, Bajaj JS. Useful tests for hepatic encephalopathy in clinical practice. Curr Gastroenterol Rep. 2014;16(1):362.
18. Jaffe A et al. Pathophysiology of hepatic encephalopathy. Clin Liver Dis. 2020;24(2):175-88.
19. Weiner ID et al. Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephrol. 2015;10(8):1444-58.
20. Montano-Loza AJ et al. Muscle wasting is associated with mortality in patients with cirrhosis. Clin Gastroenterol Hepatol. 2012;10(2):166-73.
21. Poveda MJ et al. Brain edema dynamics in patients with overt hepatic encephalopathy: a magnetic resonance imaging study. Neuroimage. 2010;52(2):481-7.
22. Dasarathy S et al. Ammonia toxicity: from head to toe? Metab Brain Dis. 2017;32(2):529-38.
23. Liu Q et al. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology. 2004;39(5):1441-9.
24. Ahluwalia V et al. Impaired gut-liver-brain axis in patients with cirrhosis. Sci Rep. 2016;6:26800.
25. Bajaj JS et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. Am J Physiol Gastrointest Liver Physiol. 2012;302(1):G168-75.
26. Riggio O et al. Hepatic encephalopathy after transjugular intrahepatic portosystemic shunt. Incidence and risk factors. Dig Dis Sci. 1996;41(3):578-84.
27. Wijdicks EF. Hepatic encephalopathy. N Engl J Med. 2016;375(17):1660-70.
28. Nielsen K et al. Liver collagen in cirrhosis correlates with portal hypertension and liver dysfunction. Apmis. 2014;122(12):1213-22.
29. Mahpour NY et al. Pharmacologic management of hepatic encephalopathy. Clin Liver Dis. 2020;24(2):231-42.
30. Riggio O et al. Effect of lactitol and lactulose administration on the fecal flora in cirrhotic patients. J Clin Gastroenterol. 1990;12(4):433-6.
31. Rahimi RS et al. Lactulose vs polyethylene glycol 3350--electrolyte solution for treatment of overt hepatic encephalopathy: the HELP randomized clinical trial. JAMA Intern Med. 2014;174(11):1727-33.
32. Riggio O et al. Pharmacological prophylaxis of hepatic encephalopathy after transjugular intrahepatic portosystemic shunt: a randomized controlled study. J Hepatol. 2005;42(5):674-9.
33. Suraweera D et al. Evaluation and management of hepatic encephalopathy: current status and future directions. Gut Liver. 2016;10(4):509-19.
34. Bass NM et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med. 2010;362(12):1071-81.
35. Leise MD et al. Management of hepatic encephalopathy in the hospital. Mayo Clin Proc. 2014;89(2):241-53.
36. Morgan MH et al. Treatment of hepatic encephalopathy with metronidazole. Gut. 1982;23(1):1-7.
37. Bajaj JS. The role of microbiota in hepatic encephalopathy. Gut Microbes. 2014;5(3):397-403.
38. Kao D et al. Fecal microbiota transplantation in the management of hepatic encephalopathy. Hepatology. 2016;63(1):339-40.
39. Jover-Cobos M et al. Ornithine phenylacetate targets alterations in the expression and activity of glutamine synthase and glutaminase to reduce ammonia levels in bile duct ligated rats. J Hepatol. 2014;60(3):545-53.
40. Zipprich A et al. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. Liver Int. 2012;32(9):1407-14.
41. Román E et al. Minimal hepatic encephalopathy is associated with falls. Am J Gastroenterol. 2011;106(3):476-82.
42. Stewart CA, Smith GE. Minimal hepatic encephalopathy. Nat Clin Pract Gastroenterol Hepatol. 2007;4(12):677-85.
43. Bajaj JS et al. Minimal hepatic encephalopathy is associated with motor vehicle crashes: the reality beyond the driving test. Hepatology. 2009;50(4):1175-83.
44. Romero-Gómez M et al. Subclinical hepatic encephalopathy predicts the development of overt hepatic encephalopathy. Am J Gastroenterol. 2001;96(9):2718-23.
45. Yen CL, Liaw YF. Somatosensory evoked potentials and number connection test in the detection of subclinical hepatic encephalopathy. Hepatogastroenterology. 1990;37(3):332-4.
46. Patidar KR et al. Covert hepatic encephalopathy is independently associated with poor survival and increased risk of hospitalization. Am J Gastroenterol. 2014;109(11):1757-63.
47. Bustamante J et al. Prognostic significance of hepatic encephalopathy in patients with cirrhosis. J Hepatol. 1999;30(5):890-5.
48. Jepsen P et al. Clinical course of alcoholic liver cirrhosis: a Danish

population-based cohort study. Hepatology. 2010;51(5):1675-82.

49. Ventura-Cots M et al. Duration of the acute hepatic encephalopathy episode determines survival in cirrhotic patients. Therap Adv Gastroenterol. 2018;11:1756283x17743419.

50. Romero-Gómez M et al. Hepatic encephalopathy in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. J Hepatol. 2015;62(2):437-47.

51. Cai JJ et al. Characteristics, risk factors, and adverse outcomes of hyperkalemia in acute-on-chronic liver failure patients. Biomed Res Int. 2019;2019:6025726.

52. Zuo L et al. Early-recurrent overt hepatic encephalopathy is associated with reduced survival in cirrhotic patients after transjugular intrahepatic portosystemic shunt creation. J Vasc Interv Radiol. 2019;30(2):148-53.e2.

53. Rose CF et al. Hepatic encephalopathy: novel insights into classification, pathophysiology and therapy. J Hepatol. 2020;73(6):1526-47.

54. Campagna F et al. The animal naming test: an easy tool for the assessment of hepatic encephalopathy. Hepatology. 2017;66(1):198-208.

55. Arasaradnam RP et al. Breathomics--exhaled volatile organic compound analysis to detect hepatic encephalopathy: a pilot study. J Breath Res. 2016;10(1):016012.

56. Lee GH. Hepatic encephalopathy in acute-on-chronic liver failure. Hepatol Int. 2015;9(4):520-6.

57. Jiang W et al. Minocycline attenuates oxidative/nitrosative stress and cerebral complications of acute liver failure in rats. Neurochem Int. 2009;55(7):601-5.

58. Bosman DK et al. The effects of benzodiazepine-receptor antagonists and partial inverse agonists on acute hepatic encephalopathy in the rat. Gastroenterology. 1991;101(3):772-81.

59. Barbaro G et al. Flumazenil for hepatic encephalopathy grade III and IVa in patients with cirrhosis: an Italian multicenter double-blind, placebo-controlled, cross-over study. Hepatology. 1998;28(2):374-8.

60. Malaguarnera M et al. Acetyl-L-carnitine treatment in minimal hepatic encephalopathy. Dig Dis Sci. 2008;53(11):3018-25.

61. Hassanein TI et al. Acute-on-chronic liver failure: extracorporeal liver assist devices. Curr Opin Crit Care. 2011;17(2):195-203.

62. Krampitz HE. [Parasitemia and organic involvement in experimental infections of various xerothermophilic wild rodents with Leishmania donovani (Sudan strain)]. Z Tropenmed Parasitol. 1965;16(4):350-64. (In German).