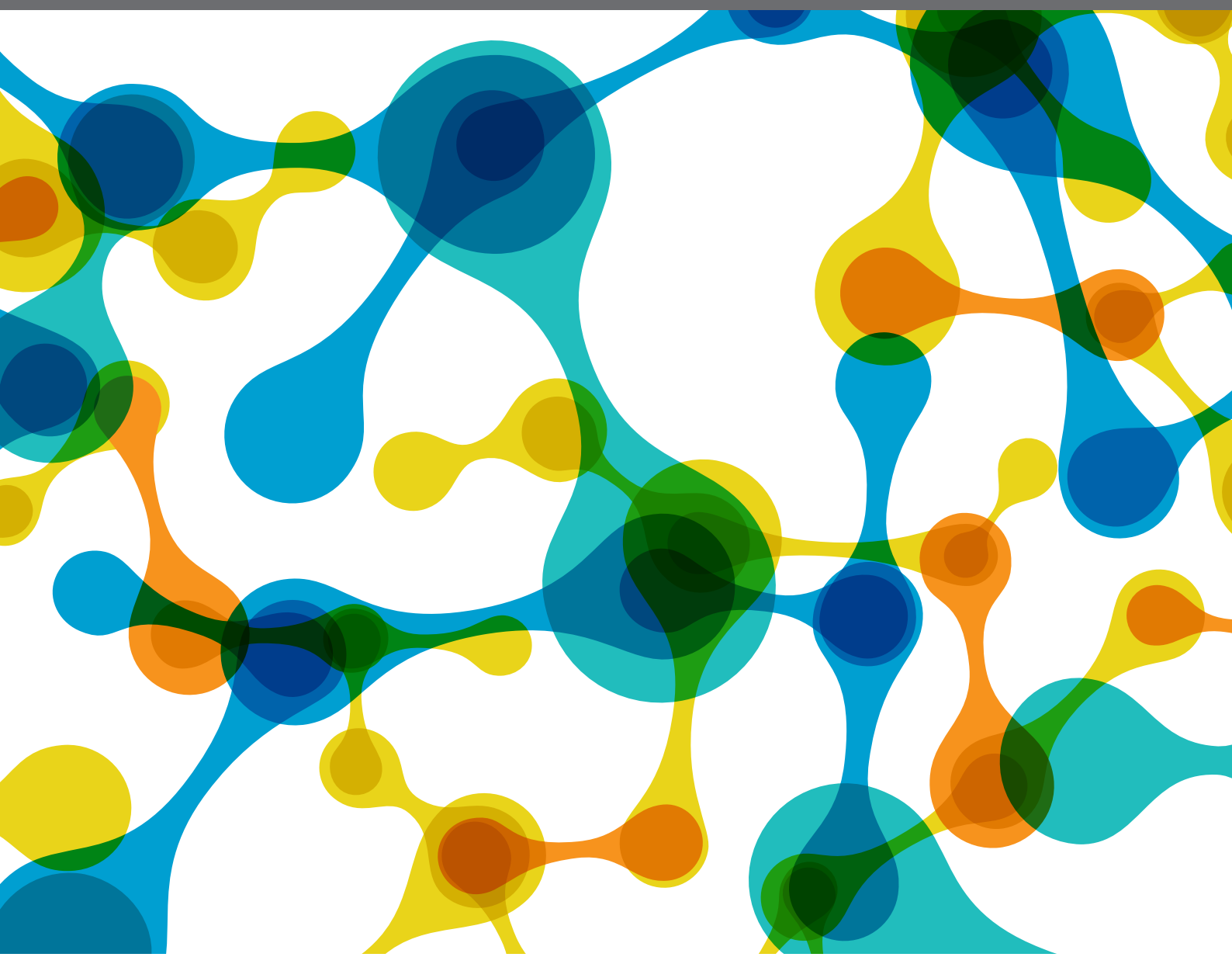
PORTAL HYPERTENSION IN CIRRHOSIS: FROM PATHOGENESIS TO NOVEL TREATMENTS

EDITED BY: Chandana B. Herath, Peter Angus and Jonel Trebicka
PUBLISHED IN: Frontiers in Physiology and Frontiers in Medicine



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88974-898-3
DOI 10.3389/978-2-88974-898-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

PORTAL HYPERTENSION IN CIRRHOSIS: FROM PATHOGENESIS TO NOVEL TREATMENTS

Topic Editors:
Chandana B. Herath, University of New South Wales, Australia
Peter Angus, University of Melbourne, Australia
Jonel Trebicka, Goethe University Frankfurt, Germany

Citation: Herath, C. B., Angus, P., Trebicka, J., eds. (2022). Portal Hypertension in Cirrhosis: From Pathogenesis to Novel Treatments. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-898-3

Table of Contents

frontiers in Physiology

EDITORIAL
published: 21 March 2022
doi: 10.3389/fphys.2022.864083

Editorial: Portal Hypertension in Cirrhosis: From Pathogenesis to Novel Treatments

Chandana B. Herath[1]*, *Peter W. Angus*[2] *and Jonel Trebicka*[3]

[1] Ingham Institute for Applied Medical Research, South Western Sydney Clinical School, University of New South Wales, Liverpool, NSW, Australia, [2] Department of Gastroenterology, Austin Health, University of Melbourne, Melbourne, VIC, Australia, [3] Translational Hepatology, Medical Department, University of Frankfurt, Frankfurt, Germany

Keywords: cirrhosis, portal hypertension, pathophysiology of cirrhosis, novel treatment, portal vein thrombosis (PVT), transjugular intrahepatic portosystemic shunts, macrophage activation markers, liver stiffness measurement (LSM)

Editorial on the Research Topic

Portal Hypertension in Cirrhosis: From Pathogenesis to Novel Treatments

This Editorial summarizes the contributions to the Frontiers Research Topic "*Portal Hypertension in Cirrhosis: From Pathogenesis to Novel Treatments*" with peer-reviewed articles published in Frontiers in Physiology (Gastrointestinal Sciences) and Frontiers in Medicine (Gastroenterology).

Cirrhosis and its complications are responsible for a large number of deaths worldwide annually. Almost 90% of patients with cirrhosis eventually develop portal hypertension (PHT) and this condition is a prequel to the majority of deaths in these patients. Cirrhotic PHT results from increased intrahepatic vascular resistance combined with extrahepatic hyperdynamic circulatory state characterized by a high cardiac output and splanchnic vasodilatation. There have been very few new therapies introduced for the long-term management of PHT over the last 30 years. Thus, the goal of this Research Topic was to highlight recent advances in PHT research and in particular, novel concepts of pathophysiological pathways (e.g., transcription regulation, systemic inflammation), but also to remind the field of already known and possibly forgotten mechanisms (e.g., impact of abdominal surgery, role of bile acids) in the development and modulation of PHT. In addition, this Research Topic elaborates on new and established diagnostics and therapies in PHT.

Portal vein thrombosis (PVT) is an important complication of cirrhosis that aggravates PHT and variceal bleeding. In general, transjugular intrahepatic portosystemic shunts (TIPS) are used as a therapeutic option to establish a shunt between the hepatic vein and the portal vein to reduce portal pressure. Wang et al. conducted a retrospective analysis to study and compare the effectiveness of TIPS in cirrhotic patients with and without PVT and found that cirrhotic patients with PVT were equally able to tolerate TIPS. This suggests that this treatment may be used in the management of cirrhotic PHT with PVT. The Research Topic also included a paper by Chen et al. who conducted a retrospective study to investigate the impact of PVT on cirrhosis decompensation and survival of cirrhotic patients and found that PVT has no significant impact on the progression of cirrhosis.

Developing a novel drug to improve cirrhotic PHT by reducing hepatic vascular resistance to incoming portal blood flow whilst increasing splanchnic vascular resistance to lower mesenteric blood flow is a difficult task because vasoactive drugs can either vasodilate or vasoconstrict the respective vasculature. In this Research Topic, Zhao et al. comprehensively demonstrated that PTUPB, a dual cyclooxygenation-2 (COX-2) and soluble epoxy hydrolase (sEH) inhibitor, given orally to carbon tetrachloride-induced cirrhotic PHT rats markedly improved PHT by reducing

OPEN ACCESS

Edited by:
Stephen J. Pandol,
Cedars Sinai Medical Center,
United States

Reviewed by:
Philipp Schwabl,
Medical University of Vienna, Austria

***Correspondence:**
Chandana B. Herath
c.herath@unsw.edu.au

Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: *28 January 2022*
Accepted: *25 February 2022*
Published: *21 March 2022*

Citation:
Herath CB, Angus PW and Trebicka J (2022) Editorial: Portal Hypertension in Cirrhosis: From Pathogenesis to Novel Treatments. Front. Physiol. 13:864083. doi: 10.3389/fphys.2022.864083

hepatic extracellular matrix deposition and hepatic vascular remodeling while also increasing the wall thickness of the superior mesenteric artery which helped improve splanchnic vascular resistance. It was notable that PTUPB treatment caused a massive ∼70% reduction in portal pressure from its baseline levels. The findings thus suggest that PTUPB which acts on both hepatic and splanchnic vasculatures is a potential drug candidate to treat cirrhotic PHT.

Macrophage activation plays a vital role in the pathogenesis of chronic liver disease and has also been linked to cirrhotic PHT, although a role in non-cirrhotic PHT has not been established. Ørntoft et al. evaluated the role of macrophage activation by measuring well-characterized markers of macrophage activation, soluble CD163 (sCD163) and soluble mannose receptor (sMR), in cohorts of patients with idiopathic PHT, non-cirrhotic patients with PVT and compared with those of cirrhotic patients with and without PVT and healthy subjects. The findings that elevated levels of sCD163 and sMR in cirrhotic patients with or without PVT compared to low levels of the markers in patients with idiopathic PHT and in non-cirrhotic PVT patients suggested that hepatic macrophage activation with elevated sCD163 and sMR levels is closely linked to the underlying liver disease with cirrhosis rather than PHT.

Hepatic surgery is generally contraindicated in patients with advanced liver disease since it increases the chances of acute decompensation of cirrhosis and multiorgan failure, resulting in high mortality. Similarly, extrahepatic surgical procedures have also been recognized as a main cause of mortality in cirrhotic patients with PHT. To further understand this, Chang et al. investigated the pathophysiology of post-operative decompensation of cirrhosis in cirrhotic animals undergoing extrahepatic intestinal manipulation (IM). The authors reported that IM significantly elevated portal pressure, induced systemic inflammation, and accelerated progression of liver fibrosis in the presence of liver injury. The findings thus suggest that these models may be useful to investigate pathophysiology of post-operative decompensation of cirrhosis which may be prevented by controlling portal pressure peri-operatively.

The formation of esophageal varices and variceal bleeding constitutes a major clinical manifestation of PHT in decompensated cirrhosis and has high associated morbidity and mortality. Although liver stiffness measurement (LSM) is an accurate widely used non-invasive tool for the diagnosis of liver fibrosis, its use to predict the occurrence of complications of liver cirrhosis such as esophageal variceal rebleeding in cirrhotic PHT patients has not been reported. In this Research Topic, Liu et al. conducted a prospective study to examine the effectiveness of LSM in predicting rebleeding compared with other non-invasive methods in hepatitis B cirrhotic patients. The authors reported that in comparison with other non-invasive methods including AST to Platelet Ratio Index, Fibrosis-4 score, King's College Criteria, Goteborg University Cirrhosis Index, Fibroindex, Fornsindex and transient elastography, LSM showed a highly reliable prediction performance of variceal rebleeding. The findings thus suggest that LSM can simply and accurately predict variceal rebleeding events in hepatitis B cirrhotic patients with PHT.

Balvey and Fernandez provided a comprehensive review of the literature on "translational control of liver disease," highlighting the abnormalities in the regulation of translation of key mRNA transcripts by RNA-binding proteins during chronic liver disease and their pathological impact on PHT, fibrosis, steatosis, neovascularization, and cancer development. In a second review, Sauerbruch et al. analyzed the current literature to evaluate a possible vasoactive role of bile acids in the cirrhotic hyperdynamic circulatory state, based primarily on *in-vitro* studies, and suggested that long-term RCTs with hemodynamic endpoints are needed in patients with early-stage cirrhosis.

Finally, a case-report of subtotal splenectomy during auxiliary partial orthotopic liver transplantation has been published by Zhou et al. which demonstrated that this is a viable procedure for modulating portal inflow and correcting severe hypersplenism in patients with end-stage liver cirrhosis.

Collectively, whilst the original work published in this Frontiers Research Topic highlighted novel aspects of pathophysiology, diagnosis and treatment of cirrhotic PHT, potential limitations include the lack of mechanistic aspects of the work, for example, the mechanism(s) by which PTUPB improves PHT and investigation of regional blood flows using approaches such as fluorescent-labeled colored microsphere beads in animal models with cirrhotic PHT. Work on these areas potentially leads to future Research Topics.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This study was supported by grant funding to CH and PA from the National Health and Medical Research Council (NHMRC) of Australia (APP1062372 and APP1124125), the German Research Foundation (DFG) project ID 403224013 - SFB 1382 (A09), the German Federal Ministry of Education and Research (BMBF) for the DEEP-HCC project and by the Hessian Ministry of Higher Education, Research and the Arts (HMWK) for the ENABLE and ACLF-I cluster projects. The MICROB-PREDICT (project ID 825694), DECISION (project ID 847949), GALAXY (project ID 668031), LIVERHOPE (project ID 731875), and IHMCSA (project ID 964590) projects have received funding from the European Union's Horizon 2020 research and innovation program. The manuscript reflects only the authors' views, and the European Commission is not responsible for any use that may be made of the information it contains. The funders had no influence on study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

We would like to thank the authors of the articles published in this Research Topic for their valuable contributions to the field and all reviewers for accepting and reviewing the

manuscripts in a rigorous manner. We would also thank the Frontiers specialists, especially Liam Hobbins, for their initial and ongoing support.

Conflict of Interest: JT has received speaking and/or consulting fees from Versantis, Gore, Bayer, Alexion, Norgine, Grifols, and CSL Behring.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Physiology

ORIGINAL RESEARCH
published: 11 June 2021
doi: 10.3389/fphys.2021.649668

Divergences in Macrophage Activation Markers Soluble CD163 and Mannose Receptor in Patients With Non-cirrhotic and Cirrhotic Portal Hypertension

Nikolaj Worm Ørntoft[1], Michel Blé[2], Anna Baiges[2], Jose Ferrusquia[2], Virginia Hernández-Gea[2], Fanny Turon[2], Marta Magaz[2], Søren Møller[3], Holger Jon Møller[4], Juan Carlos Garcia-Pagan[2]† and Henning Gronbaek[1]*†*

[1] Department of Hepatology and Gastroenterology, European Reference Network on Hepatological Diseases (ERN RARE-LIVER), Aarhus University Hospital, Aarhus, Denmark, [2] Barcelona Hepatic Hemodynamic Laboratory, Liver Unit, Hospital Clínic, Centro de Investigación Biomédica en Red Enfermedades Hepáticas y Digestivas (CIBEREHD), European Reference Network on Hepatological Diseases (ERN RARE-LIVER), Institut de Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain, [3] Center of Functional and Diagnostic Imaging and Research, Department of Clinical Physiology and Nuclear Medicine 260, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark, [4] Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark

Introduction: Macrophages are involved in development and progression of chronic liver disease and portal hypertension. The macrophage activation markers soluble (s)CD163 and soluble mannose receptor (sMR), are associated with portal hypertension in patient with liver cirrhosis but never investigated in patients with non-cirrhotic portal hypertension. We hypothesized higher levels in cirrhotic patients with portal hypertension than patients with non-cirrhotic portal hypertension. We investigated sCD163 and sMR levels in patients with portal hypertension due to idiopathic portal hypertension (IPH) and portal vein thrombosis (PVT) in patients *with* and *without* cirrhosis.

Methods: We studied plasma sCD163 and sMR levels in patients with IPH (n = 26), non-cirrhotic PVT (n = 20), patients with cirrhosis *without* PVT (n = 31) and *with* PVT (n = 17), and healthy controls (n = 15).

Results: Median sCD163 concentration was 1.51 (95% CI: 1.24–1.83) mg/L in healthy controls, 1.96 (95% CI: 1.49–2.56) in patients with non-cirrhotic PVT and 2.16 (95% CI: 1.75–2.66) in patients with IPH. There was no difference between non-cirrhotic PVT patients and healthy controls, whereas IPH patients had significantly higher levels than controls ($P < 0.05$). The median sCD163 was significantly higher in the cirrhotic groups compared to the other groups, with a median sCD163 of 6.31 (95% CI: 5.16–7.73) in cirrhotics *without* PVT and 5.19 (95% CI: 4.18–6.46) *with* PVT ($P < 0.01$, all). Similar differences were observed for sMR.

OPEN ACCESS

Edited by:
Chandana Herath,
University of New South Wales,
Australia

Reviewed by:
William Sievert,
Monash Institute of Medical Research, Australia
Christopher Leung,
University of Melbourne, Australia

***Correspondence:**
Henning Gronbaek
henngroe@rm.dk
Juan Carlos Garcia-Pagan
jcgarcia@clinic.cat

† These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: 05 January 2021
Accepted: 06 May 2021
Published: 11 June 2021

Citation:
Ørntoft NW, Blé M, Baiges A, Ferrusquia J, Hernández-Gea V, Turon F, Magaz M, Møller S, Møller HJ, Garcia-Pagan JC and Gronbaek H (2021) Divergences in Macrophage Activation Markers Soluble CD163 and Mannose Receptor in Patients With Non-cirrhotic and Cirrhotic Portal Hypertension. Front. Physiol. 12:649668. doi: 10.3389/fphys.2021.649668

Conclusion: Soluble CD163 and sMR levels are elevated in patients with IPH and patients with cirrhosis, but normal in patients with non-cirrhotic PVT. This suggests that hepatic macrophage activation is more driven by the underlying liver disease with cirrhosis than portal hypertension.

Keywords: portal hypertension, cirrhosis, macrophages, non-cirrhotic portal hypertension, biomarker

INTRODUCTION

Liver macrophages play a significant role in chronic liver disease development and progression, and are also suggested to play a role in portal hypertension (Steib, 2011). The macrophages may be activated by the specific liver disease (e.g., virus, alcohol, steatosis, and drugs) where damage associated molecular patterns (DAMPs) activate macrophages accompanied by inflammation, fibrosis, and finally cirrhosis. Further, patients with liver cirrhosis and portal hypertension have intestinal edema and a leaky gut wall resulting in translocation of endotoxins and gut bacteria, e.g., pathogen associated molecular patterns (PAMPs), stimulating gastrointestinal and liver macrophages (Wiest and Garcia-Tsao, 2005; Wiest et al., 2014; Seitz et al., 2018) with secretion of inflammatory and vasoactive cytokines (Steib et al., 2007, 2010a,b).

Similar mechanisms of macrophage activation especially PAMPs may be involved in patients with non-cirrhotic portal hypertension; however, most often, without underlying liver disease. Non-cirrhotic portal hypertension is mainly caused by vascular disorders, especially portal vein thrombosis (PVT). However, a number of other conditions are associated with non-cirrhotic portal hypertension (Wanless, 1990; Strauss and Valla, 2014; Hernandez-Gea et al., 2018), and yet in some patients, a specific cause for the portal hypertension cannot be identified and these patients are classified as having idiopathic portal hypertension (IPH) currently also known as porto sinusoidal vascular liver disease (Schouten et al., 2012b; Hernandez-Gea et al., 2018). Patients with non-cirrhotic portal hypertension may also display macrophage activation due to portal hypertension and PAMPs; however, it is unknown how macrophage activation differs between patients with non-cirrhotic portal hypertension e.g., PVT and IPH and patients with cirrhosis *with* and *without* PVT. Divergences may partly explain differences in disease severity and prognosis in patients with non-cirrhotic portal hypertension compared to patients with liver cirrhosis, who may develop acute decompensation with risk of progression toward acute-on-chronic liver failure.

As recently reviewed macrophage activation markers soluble (s)CD163 and soluble mannose receptor, sMR, are associated with chronic liver disease severity (Child-Pugh and MELD scores) and portal hypertension (Møller et al., 2016). However, the macrophage activation markers sCD163 and sMR have never been studied in the setting of non-cirrhotic portal hypertension. We therefore aimed to evaluate the role of macrophage activation by sCD163 and sMR in patients with non-cirrhotic portal hypertension (PVT and IPH) and compare this to patients with cirrhotic portal hypertension *with* and *without* PVT and in healthy controls. We hypothesized higher levels in patients with cirrhosis and portal hypertension than patients with non-cirrhotic portal hypertension, which suggest that the underlying liver disease is the main driver of macrophage activation.

Abbreviations: IPH, idiopathic portal hypertension; PVT, portal vein thrombosis; PAMPs, pathogen associated molecular patterns; TLR, toll like receptors; sCD163, soluble CD163; sMR, soluble mannose receptor; HVPG, hepatic venous pressure gradient.

MATERIALS AND METHODS

Patients and Healthy Controls

Ninety-four patients were included in the study from 2003 to 2015 from the outpatient clinic in Barcelona. The patients were divided into four groups according to their underlying disease. Twenty-six patients had IPH, 20 patients had non-cirrhotic PVT, 31 patients had cirrhosis *without* PVT and 17 patients had cirrhosis *with* PVT. All patients with IPH reached this diagnosis after discarding other etiologies for portal hypertension with CT-examination, biochemical screening and liver biopsy. In general, the histological changes were subtle and diverse. The most pronounced histological features present in 48% of the IPH patients were portal tract vascular abnormalities (including vascular multiplication, periportal vascular channels and aberrant vessels). Hepatic sinusoidal dilation, architectural disturbance (irregular distribution of central veins and portal tract) and regenerative nodules was present in 38%, 21%, and 21%, respectively. Two patients with IPH had histological features of obliterative portal venopathy. In 21% of IPH patients there was mild perisinusoidal fibrosis. None of the patients with IPH showed histological features of inflammation in the liver biopsy. Fifteen healthy human subjects were included at Hvidovre Hospital in Denmark. Liver cirrhosis was diagnosed in patients with underlying chronic liver disease (e.g., alcohol, HCV, and HBV) combined with imaging showing nodular surface and collaterals including clinical complications to portal hypertension (e.g., ascites, varices, and hepatic encephalopathy).

All patients and healthy controls had physical examination, measurements of additional biochemical parameters and underwent hemodynamic investigation with liver vein catheterization for measurement of hepatic venous pressure gradient (HVPG), physical examination and measurements of additional biochemical parameters (**Table 1**). HVPG was determined as the difference between the wedged and the free hepatic venous pressure. No patients or healthy subjects had fever or other signs of infections. Blood samples were collected from a peripheral vein for measurements of sCD163 and sMR and

TABLE 1 | Baseline characteristics.

	Controls (*n* = 15)	IPH (*n* = 26)	Non-cirrhotic PVT (*n* = 20)	Cirrhosis with PVT (*n* = 17)	Cirrhosis without PVT (*N* = 31)
Male/female (*n*)	6/9*,#	19/7	15/5	9/8	15/16
Age (years)	53 (51, 65)	43 (31, 56)€,&	53 (38, 62)€	61 (51, 70)	60 (51, 68)
Child-Pugh score	–	5 (5, 6)€,&	5 (5, 6)€,&	7 (7, 9)	7 (5, 8)&
HVPG (mmHg)	3.0 (2.0, 4.0)*,€,&	6.8 (5.5, 11)#,€,&	4.0 (2.75, 4.5)€,&	21.5 (16, 23)	18.0 (14.5, 19.5)&
Varices (% with small and large varices)	0%; 0%*, #,€,&	12%; 77%€, &	25%; 60%€,&	12%; 76%	29%; 52%
Ascites (% with non-tense and tense ascites)	0%, 0%€,&	12%; 0%€,&	15%; 0%€,&	71%; 18%	48%; 3%&
BMI (kg/m^2)	25 (19, 28)	23 (21, 25)€,&	23 (21, 26)€,&	25 (24, 30)	25 (23, 29)
Bilirubin (mg/dL)	0.4 (0.2, 1)€,&	1 (0.6, 1.5)	0.8 (0.6, 1.7)	1.6 (1.4, 3.0)	1.4 (0.8, 2.5)
Alkaline phosphatase (IU/l)	113 (69, 193)	142 (114, 287)	163 (135, 216)	133 (108, 286)	173 (96, 215)
Albumin (g/dl)	4.0 (3.5, 4.2)	4.0 (3.6, 4.2)€,&	4.1 (3.6, 4.4)€,&	3.6 (3.1, 3.8)	3.6 (3.2, 3.9)
INR	1.1 (1.0, 1.2)€,&	1.2 (1.1, 1.4)&	1.2 (1.1, 1.3)&	1.4 (1.3, 1.6)	1.3 (1.2, 1.4)&
AST (IU/l)	–	33 (27, 46)€	30 (25, 45)€	43 (33, 53)	83 (38, 115)&
ALT (IU/l)	28 (23, 35)€	25 (21, 42)€	29 (23, 48)€	26 (21, 38)	58 (24, 101)&
Creatinine (mg/dl)	0.84 (0.74, 1.1)	0.77 (0.61, 0.99)	0.86 (0.78, 0.94)	0.86 (0.72, 1.02)	0.70 (0.59, 0.94)
MELD score	–	9.8 (8.2, 11.7)€,&	9.5 (8.0, 10.6)€,&	13.1 (10.3, 15.2)	12.4 (10.1, 15.8)
Sodium (mmol/l)	143 (140, 145)	140 (139, 143)	140 (137, 141)€	141 (137, 142)	142 (140, 144)
Potassium (mmol/l)	4.3 (3.8, 4.4)	4.1 (3.8, 4.5)	4.4 (4.1, 4.7)	4.2 (4.0, 4.5)	4.3 (4.0, 4.6)
Hemoglobin (g/dl)	8.6 (7.8, 9.0)	12.9 (10.7, 14.5)	12.3 (10.5, 14.7)	11.2 (10.4, 12.5)	12.8 (11.3, 14.2)
Thrombocytes (10^9/l)	372 (250, 447)*,#,€, &	82 (60, 112)#	159 (105, 281)€,&	62 (44, 102)	97 (70, 125)
Leukocytes (10^9/l)	–	4.0 (3.4, 5.0)	5.4 (3.3, 8.4)	3.4 (2.9, 4.3)	4.0 (3.2, 5.7)

Values are reported as median with 25 and 75% interquartile range in brackets unless other is specified.
** Significantly different from patients with IPH ($P < 0.05$).*
 # Significantly different from patients with non-cirrhotic PVT ($P < 0.05$).
€ Significantly different from patients with cirrhosis without PVT ($P < 0.05$).
& Significantly different from patients with cirrhosis with PVT ($P < 0.05$).

frozen at −80°C until analysis. Informed consent was obtained from all participants according to the Helsinki Declaration.

Soluble CD163 and Soluble MR

Levels of sCD163 and sMR in plasma samples were measured by an in-house sandwich enzyme-linked immunosorbent assay as previously described (Moller et al., 2002; Rodgaard-Hansen et al., 2014).

Statistics

Normality was assessed visually by using quantile-quantile-plots and histograms. The values of the biomarkers were not normally distributed. To obtain normal distribution, the data were log-transformed. Accordingly, data are presented as median with a 95% CI of the median. Multiple linear regression was used to test if the values of sCD163 and sMR were different between the groups. Age was used as a control variable in the regression of both sCD163 and sMR, as sCD163 is known to increase with age (Moller, 2012). Model assumptions was checked and fulfilled. For all other parameters the Mann–Whitney *U* test was used to test statistical differences between groups. A p-value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed using STATA software, release 11 (StataCorp, College Station, TX, United States).

RESULTS

Patient Groups

Gender was evenly distributed for the healthy controls and the patients with cirrhosis, but in the groups with IPH and non-cirrhotic PVT there was an overweight of males (75%). Although HVPG was significantly higher in the patients with IPH compared to healthy controls and patients with non-cirrhotic PVT, there was no difference in the clinical signs of portal hypertension, with no significantly difference in the degree of varices or the degree of ascites between the groups ($P = 0.26$ and $P = 0.73$, **Table 1**). HVPG was significantly higher in the patient groups with cirrhosis compared to the non-cirrhotic patients ($P < 0.05$) and they also had a significantly higher degree of ascites ($P < 0.05$ for all), whereas there was no significantly difference in the grade of varices ($P > 0.05$, for all, **Table 1**). Both HVPG and the degree of ascites was significantly higher in the cirrhotic group *with* PVT compared to the cirrhotic group *without* PVT ($P = 0.02$ and $P = 0.006$, **Table 1**).

Soluble CD163

In the healthy controls and in patients with non-cirrhotic PVT, where the liver can be assumed to be normal or near normal, the plasma concentration of sCD163 was low and within the normal

range (0.69–3.86 mg/L). The median plasma concentration was 1.51 mg/L (1.24–1.83) in healthy controls and 1.96 mg/L (1.49–2.56) in patients with non-cirrhotic PVT (**Figure 1A**); and with no significant difference between the two groups ($P = 0.09$).

In the patients with IPH sCD163 was slightly but significantly elevated [2.16 mg/L (1.75–2.66)], when compared to the healthy controls ($P = 0.007$) (**Figure 1A**). The median plasma sCD163 concentration in patients with IPH was slightly but insignificantly higher than patients with non-cirrhotic PVT ($P = 0.35$).

In patients with cirrhosis sCD163 levels were high with a median of 6.31 mg/L (5.16–7.73) in the patients *without* PVT and 5.19 mg/L (4.18–6.46) in the patients *with* PVT (**Figure 1A**). The two patient groups with cirrhosis had significantly elevated sCD163 compared to healthy controls, patients with IPH and patients with non-cirrhotic PVT (all $P < 0.001$). There was no difference between the concentration of sCD163 in the cirrhotic patients *with* or *without* PVT ($P = 0.23$).

Soluble MR

Soluble mannose receptor was not measured in healthy controls, but reference values have been established with a mean of 0.28 mg/L and 95% reference interval of 0.10 mg/L to 0.43 mg/L (Rodgaard-Hansen et al., 2014). The median concentration of sMR was 0.27 mg/L (0.22–0.33) in patients with IPH and 0.24 mg/L (0.19–0.31) in the patients with non-cirrhotic PVT (**Figure 1B**). In both cirrhotic patient groups, median sMR was approximately two times higher than the concentration in the patients without cirrhosis with 0.58 mg/L (0.49–0.68) in cirrhotic patients *without* PVT and 0.51 mg/L (0.39–0.66) in cirrhotic patients *with* PVT (**Figure 1B**).

Similar, to the results of sCD163 the median concentration of sMR was higher but not significantly different in patients with IPH compared to patients with non-cirrhotic PVT ($P = 0.46$) and there was no difference between the cirrhosis groups ($P = 0.41$). The concentration of sMR was significantly higher in both patient groups with cirrhosis compared to the patients with IPH and non-cirrhotic PVT ($P < 0.001$ for all cases), see **Figure 1B**.

Hepatic Venous Pressure Gradient

Median HVPG was 3.0 mmHg in the healthy controls, 6.8 mmHg in patients with IPH, and 4.0 mmHg in patients with non-cirrhotic PVT (**Table 1**). In the patients with cirrhosis, median HVPG was 18.0 mmHg in patients without PVT and 21.5 mmHg in patient with PVT (**Table 1**). There was no correlation between HVPG and sCD163. Soluble MR was only correlated to HVPG in patients with cirrhosis without PVT (Rho = 0.46, $P = 0.01$).

DISCUSSION

This is to our knowledge the first study to investigate macrophages and macrophage activation markers in patients with IPH and non-cirrhotic PVT. The main finding of the present study was the significantly elevated sCD163 levels in patients with underlying liver disease and cirrhosis *with* and *without* PVT, and being lesser in IPH and non-cirrhotic PVT. This may suggest that the primary driver for hepatic macrophage activation and elevated sCD163 and sMR levels is the underlying liver disease with cirrhosis rather than portal hypertension.

A major strength of the present study is the inclusion of well-characterized patients and healthy controls who all had invasive measurements of HVPG. The study limitations include the relatively small number of patients within each category and the cross-sectional design, which does not permit determination of changes in macrophage activation marker levels with prognosis or progression or regression of inflammation and fibrosis. However, this may not affect the rather clear distinction between

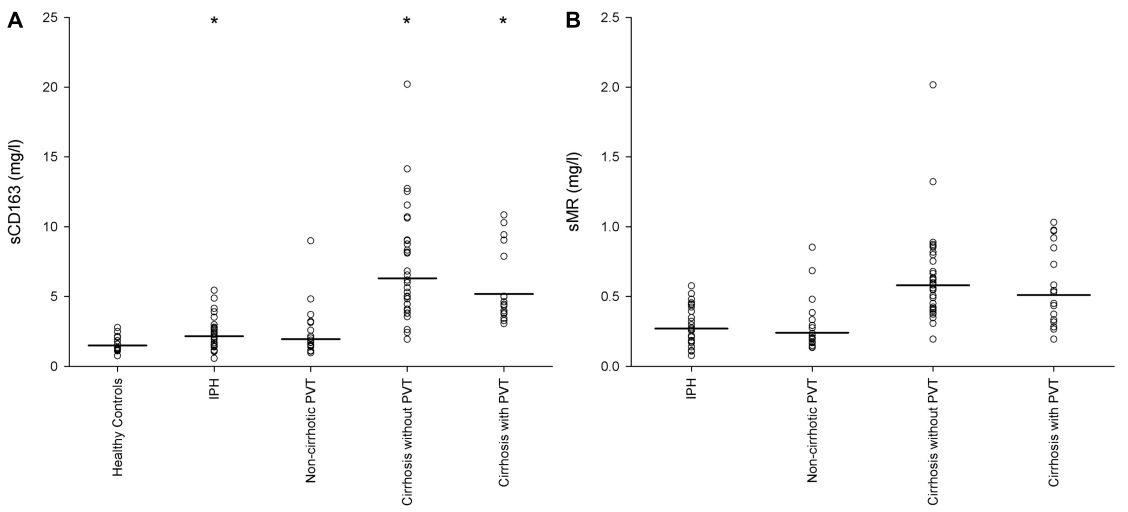


FIGURE 1 | Plasma concentration and median s163 **(A)** and sMR **(B)** levels in patients with idiopathic portal hypertension (IPH), non-cirrhotic portal vein thrombosis, cirrhosis without portal vein thrombosis, cirrhosis with portal vein thrombosis and healthy controls. sCD163 was significantly elevated in IPH and the two cirrhosis groups when compared to healthy controls ($P < 0.05$). Both sCD163 and sMR was significantly higher in the cirrhosis groups compared to the non-cirrhosis groups ($P < 0.01$). There was no difference between the two cirrhosis groups. *$P < 0.05$ compared to healthy controls.

healthy controls and patients with IPH or cirrhosis. Additionally, while included patients had stable disease it is not possible to control for subclinical events, such as minor infections, which could affect macrophage activation; however, none of the patients showed any signs of infections at inclusion or during HVPG measurements.

CD163 is a monocyte/macrophage lineage specific scavenger receptor for the hemoglobin and haptoglobin complex (Kristiansen et al., 2001). The soluble form is present in plasma under normal circumstances but substantially increased during macrophage activation (Moller, 2012). Over the past decade, studies have established macrophage activation as an important factor in liver disease development, progression and prognosis. Macrophage activation, as measured by sCD163, is associated with liver fibrosis and cirrhosis, liver disease severity (Child-Pugh- and MELD scores), and portal hypertension (Holland-Fischer et al., 2011; Gronbaek et al., 2012; Rode et al., 2013; Kazankov et al., 2014, 2015, 2016; Grønbæk et al., 2020). Furthermore, sCD163 levels are associated with prognosis, predict the risk of variceal bleeding (Rode et al., 2013; Waidmann et al., 2013) and correlates to disease severity and treatment response in patients with non-alcoholic fatty liver disease (Kazankov et al., 2015), alcoholic hepatitis (Sandahl et al., 2014), hepatitis B and C virus infection (Dultz et al., 2015; Laursen et al., 2018, 2019) and autoimmune liver diseases (Gronbaek et al., 2016a; Bossen et al., 2020, 2021). The magnitude of macrophage activation and corresponding elevated plasma markers depends on the underlying pathogenesis, being more pronounced in conditions with a high hepatic inflammatory load and fibrosis like acute liver failure and advanced cirrhosis (Hiraoka et al., 2005; Moller et al., 2007; Gronbaek et al., 2012). In acute liver failure, macrophage activation is dynamic and resolves with disease regression in contrast to the stable increase in activation seen in advanced cirrhosis (Hiraoka et al., 2005).

The mannose receptor is able to bind various ligands of microbial and endogenous origin and is involved in antigen presentation and macrophage activation (Martinez-Pomares, 2012). The receptor is expressed on selected inflammatory cells, including subsets of macrophages, dendritic and endothelial cells and shed during inflammation and subsequently measurable as soluble MR (Martinez-Pomares, 2012; Rodgaard-Hansen et al., 2014). Consequently, sMR is not as specific a marker of macrophage activation as sCD163. However, elevated sMR levels have previously been described in patients with liver disease and are shown to be associated to disease severity, portal hypertension and mortality (Gronbaek et al., 2016b; Laursen et al., 2017; Sandahl et al., 2017; Grønbaek et al., 2021).

Hepatic venous pressure gradient measures the post-sinusoidal pressure gradient. Consequently, the patients without cirrhosis who have elevated pre-sinusoidal pressure and a normal pressure gradient across the liver, e.g., patients with non-cirrhotic PVT or IPH, can still suffer from significant splenic and portal hypertension without it being detectable with standard HVPG-measurement (Keiding et al., 2004; Sørensen et al., 2018). The patients with IPH had a significantly higher HVPG compared to healthy controls (**Table 1**), but no association to macrophage activation, as measured by sCD163 and sMR. Same lack of association was seen for cirrhotic patients, except for cirrhotic patients without PVT where HVPG correlated with sMR. This is in opposition to previous findings in patients with liver cirrhosis (Holland-Fischer et al., 2011; Gronbaek et al., 2012) and could pertain to the small sample size.

As previously observed, macrophage activation was substantially higher in patients with underlying chronic liver disease and cirrhosis compared to controls and as a novel finding, this also applied to patients with IPH where the structural changes, however, are less severe. This may suggest that macrophage activation is a pronounced feature of the underlying liver disease *per se* and not related to vascular or hemodynamic changes. In the cirrhotic patients, there was a tendency toward less macrophage activation in the group with PVT, who had a significantly higher HVPG and Child-Pugh score. This likewise supports that vascular changes are less important for macrophage activation. Additionally, findings of comparable patterns for both sCD163 and sMR corroborate the observed associations. Furthermore, we suggest that the central mechanisms behind macrophage activation in cirrhosis and IPH is not only driven by translocation of gut-derived PAMPs but represents a constitutive inflammatory upregulation in the liver disease as also demonstrated in TIPS treated patients (Holland-Fischer et al., 2011). In addition to the constitutive macrophage activation, this may be further enhanced by a general systemic inflammatory state as seen in cirrhosis, leading to immune activation and production of pro-inflammatory cytokines, like tumor necrosis factor and interleukin 8, which are known to be involved in recruitment of inflammatory cells to the liver (Seitz et al., 2018). In the event of acute exacerbation of inflammation or infection as seen in e.g., ACLF we have observed even more pronounced macrophage activation by sCD163 and sMR levels (Gronbaek et al., 2016b).

Patients with IPH in general have a better prognosis than patients with cirrhosis with 10-year transplant free survival of 82% (Siramolpiwat et al., 2014). The current treatment of IPH is restricted to the management of portal hypertension and this does not prevent disease progression (Schouten et al., 2012a; Hernandez-Gea et al., 2018). Our study shows that macrophages to some degree are activated in IPH patients and consequently therapeutic strategies aimed at decreasing macrophage activation might be relevant in the treatment of IPH in the future. However, the association between IPH and macrophage activation needs further investigations.

CONCLUSION

Macrophage activation, as measured by elevated sCD163 and sMR, were only observed in patients with cirrhosis with and without PVT and in IPH patients, and not in patients with non-cirrhotic PVT. This suggest that the main determinant of macrophage activation in chronic inflammatory liver diseases is

associated to the underlying liver disease with cirrhosis and not portal hypertension.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board, Hospital Clinic, Barcelona. The Informed consent was obtained from all participants according to the Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NØ: data analysis and drafting the manuscript. MB, AB, JF, VH-G, FT, MM, SM, and HM: patient collection and data aquisition. JG-P and HG: concept and design, data analysis, and finalising the manuscript. All authors approved the final version of the manuscript.

FUNDING

JG-P received support in part through grants from the Spanish Ministry of Education and Science (SAF-2016-75767-R), Fondo Europeo de Desarrollo Regional (FEDER): (PIE15/00027), "Commissioner for Universities and Research of the Generalitat de Catalunya" (AGAUR SGR 2017), and The Spanish Health Ministry (National Strategic Plan against Hepatitis C). CIBERehd was funded by the Instituto de Salud Carlos III and Secretariat for Universities and Research of the Department of Economy and Knowledge and (SGR17_00517). HG received funding from the NOVO Nordisk Foundation and "Savvaerksejer Jeppe Juhl og hustru Ovita Juhls mindelegat."

ACKNOWLEDGMENTS

We acknowledge the skillful technical assistance by Helle Hauser Ryom and Christina Stande Sønderskov, Department of Clinical Biochemistry, Aarhus University Hospital. We are indebted to the IDIBAPS Biobank, integrated in the Spanish National Biobank Network and to the Xarxa de Bancs de Tumors de Catalunya (XBTC) financed by the Pla Director d'Oncologia de Catalunya, for the sample and data procurement. We also acknowledge administrative support by Oliva Ros Fargas and Nini Johana Bustos, Angels Falgà and M. Carmen Alcantara for nursing support and expert technical hemodynamic assistance.

REFERENCES

Bossen, L., Rebora, P., Bernuzzi, F., Jepsen, P., Gerussi, A., Andreone, P., et al. (2020). Soluble CD163 and mannose receptor as markers of liver disease severity and prognosis in patients with primary biliary cholangitis. *Liver Int.* 40, 1408–1414. doi: 10.1111/liv.14466

Bossen, L., Vesterhus, M., Hov, J. R., Färkkilä, M., Rosenberg, W. M., Møller, H. J., et al. (2021). Circulating Macrophage Activation Markers Predict Transplant-Free Survival in Patients With Primary Sclerosing Cholangitis. *Clin. Transl. Gastroenterol.* 12:e00315. doi: 10.14309/ctg.0000000000000315

Dultz, G., Gerber, L., Farnik, H., Berger, A., Vermehren, J., Pleli, T., et al. (2015). Soluble CD163 is an indicator of liver inflammation and fibrosis in patients chronically infected with the hepatitis B virus. *J Viral. Hepat.* 22, 427–432. doi: 10.1111/jvh.12309

Grønbæk, H., Gantzel, R. H., Laursen, T. L., Kazankov, K., and Møller, H. J. (2020). Macrophage markers and innate immunity in cirrhosis. *J. Hepatol.* 73, 1586–1588. doi: 10.1016/j.jhep.2020.07.033

Gronbaek, H., Kreutzfeldt, M., Kazankov, K., Jessen, N., Sandahl, T., Hamilton-Dutoit, S., et al. (2016a). Single-centre experience of the macrophage activation marker soluble (s)CD163 - associations with disease activity and treatment response in patients with autoimmune hepatitis. *Aliment Pharmacol. Ther.* 44, 1062–1070. doi: 10.1111/apt.13801

Grønbaek, H., Møller, H. J., Saliba, F., Zeuzem, S., Albillos, A., Ariza, X., et al. (2021). Improved prediction of mortality by combinations of inflammatory markers and standard clinical scores in patients with acute-on-chronic liver failure and acute decompensation. *J. Gastroenterol. Hepatol.* 36, 240–248. doi: 10.1111/jgh.15125

Gronbaek, H., Rodgaard-Hansen, S., Aagaard, N. K., Arroyo, V., Moestrup, S. K., Garcia, E., et al. (2016b). Macrophage activation markers predict mortality in patients with liver cirrhosis without or with acute-on-chronic liver failure (ACLF). *J. Hepatol.* 64, 813–822. doi: 10.1016/j.jhep.2015.11.021

Gronbaek, H., Sandahl, T. D., Mortensen, C., Vilstrup, H., Moller, H. J., and Moller, S. (2012). Soluble CD163, a marker of Kupffer cell activation, is related to portal hypertension in patients with liver cirrhosis. *Aliment Pharmacol. Ther.* 36, 173–180. doi: 10.1111/j.1365-2036.2012.05134.x

Hernandez-Gea, V., Baiges, A., Turon, F., and Garcia-Pagan, J. C. (2018). Idiopathic Portal Hypertension. *Hepatology* 68, 2413–2423.

Hiraoka, A., Horiike, N., Akbar, S. M., Michitaka, K., Matsuyama, T., and Onji, M. (2005). Soluble CD163 in patients with liver diseases: very high levels of soluble CD163 in patients with fulminant hepatic failure. *J. Gastroenterol.* 40, 52–56. doi: 10.1007/s00535-004-1493-8

Holland-Fischer, P., Gronbaek, H., Sandahl, T. D., Moestrup, S. K., Riggio, O., Ridola, L., et al. (2011). Kupffer cells are activated in cirrhotic portal hypertension and not normalised by TIPS. *Gut* 60, 1389–1393. doi: 10.1136/gut.2010.234542

Kazankov, K., Barrera, F., Møller, H. J., Bibby, B. M., Vilstrup, H., George, J., et al. (2014). Soluble CD163, a macrophage activation marker, is independently associated with fibrosis in patients with chronic viral hepatitis B and C. *Hepatology* 60, 521–530. doi: 10.1002/hep.27129

Kazankov, K., Barrera, F., Moller, H. J., Rosso, C., Bugianesi, E., David, E., et al. (2016). The macrophage activation marker sCD163 is associated with morphological disease stages in patients with non-alcoholic fatty liver disease. *Liver. Int.* 36, 1549–1557. doi: 10.1111/liv.13150

Kazankov, K., Tordjman, J., Moller, H. J., Vilstrup, H., Poitou, C., Bedossa, P., et al. (2015). Macrophage activation marker soluble CD163 and non-alcoholic fatty liver disease in morbidly obese patients undergoing bariatric surgery. *J. Gastroenterol. Hepatol.* 30, 1293–1300. doi: 10.1111/jgh.12943

Keiding, S., Solvig, J., Gronbaek, H., and Vilstrup, H. (2004). Combined liver vein and spleen pulp pressure measurements in patients with portal or splenic vein thrombosis. *Scand. J. Gastroenterol.* 39, 594–599. doi: 10.1080/00365520410005171

Kristiansen, M., Graversen, J. H., Jacobsen, C., Sonne, O., Hoffman, H. J., Law, S. K., et al. (2001). Identification of the haemoglobin scavenger receptor. *Nature* 409, 198–201. doi: 10.1038/35051594

Laursen, T. L., Rodgaard-Hansen, S., Moller, H. J., Mortensen, C., Karlsen, S., Nielsen, D. T., et al. (2017). The soluble mannose receptor is released from the

liver in cirrhotic patients, but is not associated with bacterial translocation. *Liver Int.* 37, 569–575. doi: 10.1111/liv.13262

Laursen, T. L., Siggaard, C. B., Kazankov, K., Sandahl, T. D., Moller, H. J., Tarp, B., et al. (2019). Time-dependent improvement of liver inflammation, fibrosis and metabolic liver function after successful direct-acting antiviral therapy of chronic hepatitis C. *J. Viral. Hepat.* 27, 28–35. doi: 10.1111/jvh.13204

Laursen, T. L., Wong, G. L., Kazankov, K., Sandahl, T., Moller, H. J., Hamilton-Dutoit, S., et al. (2018). Soluble CD163 and mannose receptor associate with chronic hepatitis B activity and fibrosis and decline with treatment. *J. Gastroenterol. Hepatol.* 33, 484–491. doi: 10.1111/jgh.13849

Martinez-Pomares, L. (2012). The mannose receptor. *J. Leukoc. Biol.* 92, 1177–1186.

Moller, H. J. (2012). Soluble CD163. *Scand. J. Clin. Lab. Invest.* 72, 1–13.

Moller, H. J., Gronbaek, H., Schiodt, F. V., Holland-Fischer, P., Schilsky, M., Munoz, S., et al. (2007). Soluble CD163 from activated macrophages predicts mortality in acute liver failure. *J. Hepatol.* 47, 671–676. doi: 10.1016/j.jhep.2007.05.014

Moller, H. J., Hald, K., and Moestrup, S. K. (2002). Characterization of an enzyme-linked immunosorbent assay for soluble CD163. *Scand. J. Clin. Lab. Invest.* 62, 293–299. doi: 10.1080/003655102760145852

Møller, H. J., Kazankov, K., Rødgaard-Hansen, S., Nielsen, M. C., Sandahl, T., Vilstrup, H., et al. (2016). "Soluble CD163 (sCD163): Biomarker of Kupffer Cell Activation in Liver Disease," in *Biomarkers in liver disease*, ed. V. R. Preedy (Dordrecht: Springer Science+Business Media).

Rode, A., Nicoll, A., Moller, H. J., Lim, L., Angus, P. W., Kronborg, I., et al. (2013). Hepatic macrophage activation predicts clinical decompensation in chronic liver disease. *Gut* 62, 1231–1232. doi: 10.1136/gutjnl-2012-304135

Rodgaard-Hansen, S., Rafique, A., Christensen, P. A., Maniecki, M. B., Sandahl, T. D., Nexo, E., et al. (2014). soluble form of the macrophage-related mannose receptor (MR/CD206) is present in human serum and elevated in critical illness. *Clin. Chem. Lab. Med.* 52, 453–461.

Sandahl, T. D., Gronbaek, H., Moller, H. J., Stoy, S., Thomsen, K. L., Dige, A. K., et al. (2014). Hepatic macrophage activation and the LPS pathway in patients with alcoholic hepatitis: a prospective cohort study. *Am. J. Gastroenterol.* 109, 1749–1756. doi: 10.1038/ajg.2014.262

Sandahl, T. D., Stoy, S. H., Laursen, T. L., Rodgaard-Hansen, S., Moller, H. J., Moller, S., et al. (2017). The soluble mannose receptor (sMR) is elevated in alcoholic liver disease and associated with disease severity, portal hypertension, and mortality in cirrhosis patients. *PLoS One* 12:e0189345. doi: 10.1371/journal.pone.0189345

Schouten, J. N., Nevens, F., Hansen, B., Laleman, W., van den Born, M., Komuta, M., et al. (2012a). Idiopathic noncirrhotic portal hypertension is associated with poor survival: results of a long-term cohort study. *Aliment Pharmacol. Ther.* 35, 1424–1433. doi: 10.1111/j.1365-2036.2012.05112.x

Schouten, J. N., Van der Ende, M. E., Koeter, T., Rossing, H. H., Komuta, M., Verheij, J., et al. (2012b). Risk factors and outcome of HIV-associated idiopathic noncirrhotic portal hypertension. *Aliment Pharmacol. Ther.* 36, 875–885. doi: 10.1111/apt.12049

Seitz, H. K., Bataller, R., Cortez-Pinto, H., Gao, B., Gual, A., Lackner, C., et al. (2018). Alcoholic liver disease. *Nat. Rev. Dis. Primers* 4:16.

Siramolpiwat, S., Seijo, S., Miquel, R., Berzigotti, A., Garcia-Criado, A., Darnell, A., et al. (2014). Idiopathic portal hypertension: natural history and long-term outcome. *Hepatology* 59, 2276–2285. doi: 10.1002/hep.26904

Sørensen, M., Larsen, L. P., Villadsen, G. E., Aagaard, N. K., Grønbæk, H., Keiding, S., et al. (2018). β-Blockers Improve Presinusoidal Portal Hypertension. *Dig. Dis. Sci.* 63, 3153–3157. doi: 10.1007/s10620-018-5186-1

Steib, C. J. (2011). Kupffer cell activation and portal hypertension. *Gut* 60, 1307–1308. doi: 10.1136/gut.2011.242560

Steib, C. J., Bilzer, M., Hartl, J. M., Beitinger, F., Gulberg, V., Goke, B., et al. (2010a). Kupffer cell activation by hydrogen peroxide: a new mechanism of portal pressure increase. *Shock* 33, 412–418. doi: 10.1097/shk.0b013e3181b85934

Steib, C. J., Gerbes, A. L., Bystron, M., Op den Winkel, M., Hartl, J., Roggel, F., et al. (2007). Kupffer cell activation in normal and fibrotic livers increases portal pressure via thromboxane A(2). *J. Hepatol.* 47, 228–238. doi: 10.1016/j.jhep.2007.03.019

Steib, C. J., Hartmann, A. C., Hesler, C., Benesic, A., Hennenberg, M., Bilzer, M., (2010b). Gerbes AL. Intraperitoneal LPS amplifies portal hypertension in rat liver fibrosis. *Lab. Invest.* 90, 1024–1032. doi: 10.1038/labinvest.2010.60

Strauss, E., and Valla, D. (2014). Non-cirrhotic portal hypertension–concept, diagnosis and clinical management. *Clin. Res. Hepatol. Gastroenterol.* 38, 564–569. doi: 10.1016/j.clinre.2013.12.012

Waidmann, O., Brunner, F., Herrmann, E., Zeuzem, S., Piiper, A., and Kronenberger, B. (2013). Macrophage activation is a prognostic parameter for variceal bleeding and overall survival in patients with liver cirrhosis. *J. Hepatol.* 58, 956–961. doi: 10.1016/j.jhep.2013.01.005

Wanless, I. R. (1990). Micronodular transformation (nodular regenerative hyperplasia) of the liver: a report of 64 cases among 2,500 autopsies and a new classification of benign hepatocellular nodules. *Hepatology* 11, 787–797. doi: 10.1002/hep.1840110512

Wiest, R., and Garcia-Tsao, G. (2005). Bacterial translocation (BT) in cirrhosis. *Hepatology* 41, 422–433. doi: 10.1002/hep.20632

Wiest, R., Lawson, M., and Geuking, M. (2014). Pathological bacterial translocation in liver cirrhosis. *J. Hepatol.* 60, 197–209. doi: 10.1016/j.jhep.2013.07.044

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 28 June 2021
doi: 10.3389/fmed.2021.685944

The Impact of Portal Vein Thrombosis on the Prognosis of Patients With Cirrhosis: A Retrospective Propensity-Score Matched Study

Zhiji Chen, Tao Ran, Haiyan Cao, Feng Xu, Zhi-hang Zhou and Song He**

Department of Gastroenterology, Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

Objectives: To investigate the impact of portal vein thrombosis (PVT) on cirrhosis decompensation and survival of cirrhosis.

Methods: In this retrospective observational study between January 2012 and August 2020, 117 patients with cirrhotic PVT and 125 patients with cirrhosis were included. Propensity score matching (PSM) was applied to reduce the bias. The clinical characteristics of non-tumoral PVT in cirrhosis and its influence on cirrhosis decompensation and survival were analyzed.

Results: The median follow-up for the PVT group was 15 (8.0–23.0) months and for the non-thrombosis group 14 (8.0–23.5) months. The presence of PVT was related with esophageal varices, higher Child-Pugh score and MELD score ($P < 0.05$). Most PVTs were partial (106/117). Non-occlusive PVT disappeared on later examinations in 32/106 patients (30.19%), of which six patients reappeared. All the 11 patients with occlusive PVT remained occlusive, among which five patients (45.45%) developed portal cavernoma. There was no significant correlation between PVT and decompensation or survival before or after PSM. Multivariate analysis identified only Child-Pugh score (HR = 2.210, 95% CI: 1.332–3.667) and serum sodium level (HR = 0.818, 95% CI: 0.717–0.933) as independent factors for death.

Conclusion: Though PVT is associated with greater Child-Pugh score and MELD score, it has no significant impact on the progression of cirrhosis.

Keywords: cirrhosis, survival, decompensation, portal vein thrombosis, propensity score matching

OPEN ACCESS

Edited by:
Chandana Herath, University of New South Wales, Australia

Reviewed by:
Wei Zhao, City University of Hong Kong, Hong Kong, SAR China
Li Yang, Sichuan University, China

****Correspondence:***
Zhi-hang Zhou
zhouzhihang@cqmu.edu.cn
Song He
hedoctor65@cqmu.edu.cn

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *26 March 2021*
Accepted: *31 May 2021*
Published: *28 June 2021*

Citation:
Chen Z, Ran T, Cao H, Xu F, Zhou Z-h and He S (2021) The Impact of Portal Vein Thrombosis on the Prognosis of Patients With Cirrhosis: A Retrospective Propensity-Score Matched Study. Front. Med. 8:685944. doi: 10.3389/fmed.2021.685944

INTRODUCTION

Portal vein thrombosis (PVT) is a common complication of patients with cirrhosis. It is associated with relative venous stasis caused by portal hypertension, endothelial injury, hypercoagulability, splenectomy and other factors. Acute PVT can have severe abdominal pain, while chronic PVT can be asymptomatic. Many asymptomatic PVT have been accidentally discovered by the widespread application of medical imaging technology. It was reported that the 5-year cumulative incidence of PVT was 10.7% after regular follow-up of 1,243 Child A and B cirrhosis (1). Non-tumoral PVT is present at liver transplantation in 5–26% of cirrhotic patients (2). Based on current data, the annual incidence of PVT in patients with advanced cirrhosis may be 10–15% (3). The incidence

of PVT is significantly higher in decompensated cirrhosis (10–25%) than in compensated cirrhosis (1–5%) (4). Although it was estimated from clinical experience that non-tumoral PVT increases the incidence of refractory ascites, worsens liver function, and ultimately reduces the survival rate of patients, the conclusions from clinical studies are controversial, largely due to the bias in baseline features. Propensity score matching (PSM) is a good way to reduce selection bias.

We retrospectively explored the impact of PVT on the hepatic decompensation and survival rate in 117 patients with cirrhotic PVT and 125 patients without PVT. We found that the Child-Pugh score and MELD score of the PVT group were higher than those of the non-thrombosis group ($P < 0.05$). PVT was mostly partial and the most common clinical outcome was unchanged or improvement. However, there was no significant correlation between PVT and decompensation or survival before or after PSM. All together, though PVT is associated with greater Child-Pugh score and MELD score, it has no impact on the progression of cirrhosis.

MATERIALS AND METHODS

Patients and Study Design

This study selected patients who were hospitalized for cirrhosis in the Second Affiliated Hospital of Chongqing Medical University from 2012 to 2020. Inclusion criteria included: Age >18 and <80 years, clinical diagnosis of cirrhosis (presence of irregular margins on ultrasound, portal hypertension with laboratory evidence of chronic liver disease) (5). The exclusion criteria were as follows: patients with malignant diseases (including history of hepatocellular carcinoma); patients who received anticoagulant treatment during follow-up; prior transjugular intrahepatic portosystemic shunt (TIPS) or surgical shunt; (6) patients with history of bleeding or blood products (red blood cells, platelets, plasma) transfusion in the past 2 weeks. This study was approved by the institutional ethics committee of the Second Affiliated Hospital of Chongqing Medical University.

Portal Vein Thrombosis Diagnosis

When abdominal ultrasound found solid endoluminal material in the trunk and branches of the portal vein, it was suspected that there was a portal vein thrombosis. Patients with suspected PVT underwent abdominal computed tomography (CT) or magnetic resonance (MRI) to confirm the diagnosis. Occlusive PVT was defined as no flow visible in portal vein lumen on imaging or Doppler study (7). Otherwise PVT was considered non-occlusive. The natural course of thrombosis was observed in our study, which was classified into three categories based on the changes in the degree or extension seen on the imaging: improved or worsened appearance for 50% change or more and unchanged appearance for less than that (6).

Follow-UP

All patients performed imaging and laboratory tests at least every 6–12 months. Primary endpoint: all-cause death during follow-up; secondary endpoint: decompensation (refractory ascites, hepatic encephalopathy, variceal bleeding, jaundice, or serum bilirubin >45 mol/L) (1). Esophageal varices were graded according to the Paquet's classification (8). The management of complications of cirrhosis was carried out according to current international guidelines (9–12).

Statistical Analysis

Kolmogorov-Smirnov test was used to test the normality of continuous variables. Normally distributed variables were compared with Student's T-tests, expressed as mean ± standard deviation (SD). Non-normally distributed variables were compared with the Mann-Whitney U-test, expressed as the medians with interquartile ranges (IQRs). Categorical variables were compared with the χ^2 or Fisher's exact tests, expressed as counts and percentages. Cox proportional hazards regression model with forward stepwise elimination was used to determine risk factors for decompensations and survival. Ninety-five percent confidence intervals (95% CI) were computed. Multivariate models included variables significantly associated with the outcome in univariate analyses at a level of 0.1.

To reduce the probability of selection bias, propensity score matching (PSM) was performed. Propensity scores were estimated using based on serum albumin level, hemoglobin level, Child-Pugh score, MELD score, the history of splenectomy, varices grade III/IV according to Paquet, portal vein diameter and D-dimer. Patients in the PVT group were matched to those in the non-thrombosis group (1:1), with the nearest neighbor estimated propensity score within a range of 0.02 standard deviation.

Data analysis used IBM SPSS Statistics version 25.0. $P < 0.05$ was considered as statistically significant.

RESULTS

Patient Baseline Characteristics

Initially, 1,187 patients with cirrhosis were evaluated, of which 945 were excluded (221 with malignant diseases, 112 with insufficient laboratory data, 86 with anticoagulant treatment during follow-up, 74 with transjugular intrahepatic portosystemic shunt or surgical shunt, 323 with history of bleeding or blood products transfusion in the past 2 weeks, 89 with inadequate follow-up duration, 40 with patients with hepatic encephalopathy, refractory ascites, and recent variceal bleeding in the baseline). Finally, 117 patients with cirrhotic PVT and 125 patients with cirrhosis who were hospitalized during the same period were enrolled. All patients with PVT did not receive anticoagulation and TIPS treatment before or during the follow-up period. No significant difference was observed between the two group in sex, age, or cirrhosis etiology. The serum albumin and hemoglobin level of the PVT group were significantly lower than those of the non-thrombosis group ($P < 0.05$), and the Child-Pugh score and MELD score were higher than those of the non-thrombosis group ($P < 0.05$). There were also differences in the history of splenectomy, varices grade III/IV according to Paquet, portal vein diameter, and D-dimer between the two groups ($P < 0.05$). Detailed patient characteristics are presented in **Supplementary Table 1**.

TABLE 1 | Characteristics of PVT in patients with cirrhosis.

Patients with PVT (n = 117)	
Site of PVT, *n* (%)	
Only trunk	32 (27.35%)
Only branch	23 (19.66%)
Trunk and branches	62 (52.99%)
Degree of PVT, *n* (%)	
Occlusive	11 (9.40%)
Non-occlusive	106 (90.60%)
Extension of PV system occlusion, *n* (%)	
PV alone	65 (55.56%)
Extension into SV	4 (3.42%)
Extension into MV	36 (30.77%)
Extension into SV and MV	12 (10.25%)

PVT, portal vein thrombosis; PV, portal vein; SV, splenic vein; MV, mesenteric Vein.

Characteristics and Natural Course of PVT

Among the 117 PVT patients, 62 patients (52.99%) had thrombosis involving the trunk and branches of the portal vein, and only 11 patients (9.40%) had occlusive PVT. During the follow up period, 44.44% of PVT extended to the splenic vein or superior mesenteric vein (**Table 1**). Non-occlusive PVT disappeared on later examinations in 32/106 patients (30.19%), of which six patients reappeared. Totally, 11/117 (9.40%) patients with PVT had progression of the thrombosis (**Supplementary Table 2**). All the 11 patients with occlusive PVT remained occlusive, but 5/11 patients with occlusive PVT (45.45%) developed portal cavernoma.

Clinical Outcomes

The median follow-up for the PVT group was 15 (8.0–23.0) months and for the on-thrombosis group 14 (8.0–23.5) months. There was no significant difference in the incidence of refractory ascites, hepatic encephalopathy, variceal bleeding, and decompensation between the two groups ($P > 0.05$) (**Supplementary Table 3**). Occlusive PVT also had no significant effect on decompensation ($\chi^2 = 0.031$, $P = 0.861$). Factors associated with decompensation of cirrhosis by Cox univariate regression analysis are shown in **Supplementary Table 4**. Multivariate COX regression analysis found that the independent influencing factors of decompensation in patients with cirrhosis were esophageal varices (HR = 3.187, 95% CI: 1.601–6.343, $P = 0.001$), endoscopic treatment (HR = 0.834, 95% CI: 0.706–0.984, $P = 0.032$), serum sodium level (HR = 0.903, 95% CI: 0.853–0.955, $P < 0.001$) and spontaneous portosystemic shunts (HR = 2.338, 95% CI: 1.314–4.162, $P = 0.004$) (**Table 2**). No relationship has been observed between PVT and decompensation in different Child-Pugh class and MELD score.

Overall, 10/242 (4.13%) patients died, among which five are associated with multiple organ failure and the other five with gastrointestinal bleeding. Factors associated with death by univariate Cox regression analysis are shown in **Supplementary Table 4**. There was no influence of PVT on survival. Multivariate analysis identified only Child-Pugh score (HR = 2.210, 95% CI: 1.332–3.667, $P = 0.002$) and serum sodium level (HR = 0.818, 95% CI: 0.717–0.933, $P = 0.003$) as independent factors (**Table 2**).

TABLE 2 | Multivariate analysis to determine predictive factors for decompensation and death.

	***P*-values**	**HR**	**95% CI**
Decompensation			
Esophageal varices (Paquet's grade III/IV)	0.001	3.187	1.601–6.343
SPSS	0.004	2.338	1.314–4.162
Endoscopic treatment	0.032	0.834	0.706–0.984
Serum sodium level	<0.001	0.903	0.853–0.955
Death			
Child-Pugh score	0.002	2.210	1.332–3.667
Serum sodium level	0.003	0.818	0.717–0.933

SPSS, Spontaneous portosystemic shunts.

TABLE 3 | Propensity-matched study cohort.

	With PVT (n = 44)	**Without PVT (n = 44)**	***P*-values**
Age (years)	54.0 ± 11.4	54.6 ± 11.7	0.775
Male gender	30 (68.18%)	26 (59.09%)	0.375
Etiology of cirrhosis (HBV/alcohol/other)	29/5/10	28/5/11	0.968
Child-Pugh score	7.0 (6.0–8.0)	7.0 (6.0–8.0)	0.594
MELD score	9.3 (8.5–11.8)	8.8 (8.1–10.7)	0.367
Serum albumin (g/L)	35.5 ± 5.1	35.1 ± 6.3	0.785
Hemoglobin (g/L)	99.4 ± 24.3	99.8 ± 25.5	0.935
Portal vein diameter (mm)	16.0 (15.0–17.2)	16.0 (13.3–18.4)	0.970
D-dimer (μg/L)	0.4 (0.1–0.6)	0.2 (0.1–0.7)	0.468
History of splenectomy	2 (4.55%)	2 (4.55%)	1.000
Diabetes	8 (18.18%)	7 (15.91%)	0.777
Esophageal varices (Paquet's grade III/IV)	32 (72.73%)	33 (75.00%)	0.808
SPSS	8 (18.18%)	9 (20.45%)	0.787

PVT, portal vein thrombosis; HBV, hepatitis B virus; MELD, Model for End-Stage Liver Disease; SPSS, Spontaneous portosystemic shunts.

Propensity-Matched Cohort

After propensity score matching, 44 patients remained in each group (**Table 3**). There was no significant difference in the baseline features between the two groups. We found that PVT had no effect on variceal bleeding (**Figure 1A**) and decompensation of cirrhosis (**Figure 1B**). No significant difference in survival time was found between the two groups (**Figure 1C**). Meanwhile, esophageal varices (HR = 4.428, 95% CI: 1.633–12.008, $P = 0.003$), serum sodium level (HR = 0.921, 95% CI: 0.862–0.984, $P = 0.015$) and spontaneous portosystemic shunts (HR = 3.062, 95% CI: 1.363–6.880, $P = 0.007$) independently predicted cirrhosis decompensations

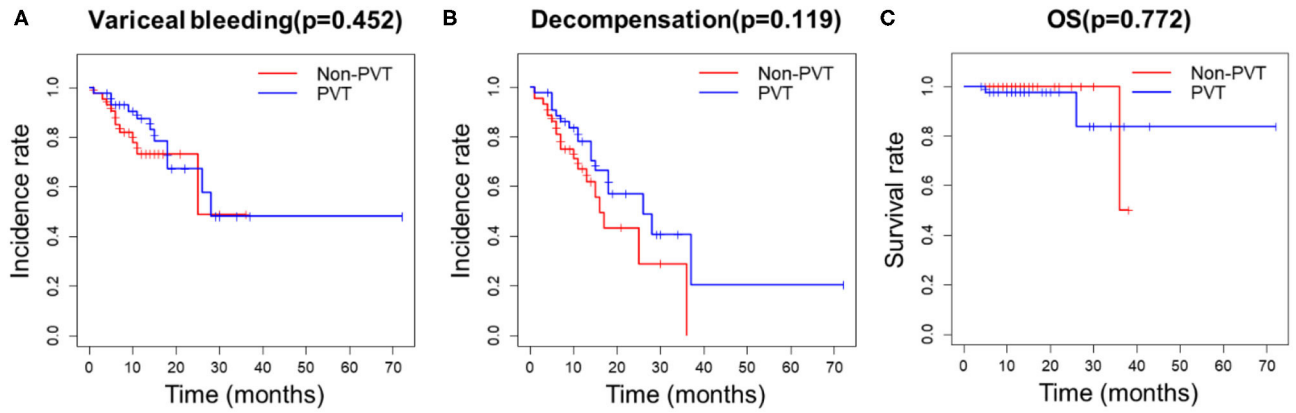


FIGURE 1 | Kaplan-Meier curves for PVT and non-PVT groups. **(A)** PVT had no effect on variceal bleeding, **(B)** decompensation of cirrhosis and **(C)** survival after PSM.

TABLE 4 | Multivariate analysis to determine predictive factors for decompensation and death (propensity-matched study cohort).

	P-values	HR	95% CI
Decompensation			
Esophageal varices (Paquet's grade III/IV)	0.003	4.428	1.633–12.008
SPSS	0.007	3.062	1.363–6.880
Serum sodium level	0.015	0.921	0.862–0.984
Death			
Child-Pugh score	0.085	1.996	0.909–4.380

SPSS, Spontaneous portosystemic shunts.

(**Table 4**). Because of the small number of deaths, we did not find independent risk factors for death after PSM.

DISCUSSION

PVT is a common complication in patients with cirrhosis, and its pathogenesis can be explained by the Virchow's triad (blood flow stasis caused by portal hypertension, vascular endothelial damage and blood hypercoagulability). With the progress of liver disease, the flow of the portal vein decreases and ectopic intestinal bacteria increase, which lead to vascular endothelial damage (13). In addition, elevated endothelial-derived factor VWF and decreased protein C resulted in a relatively hypercoagulable state (14). As a result, the incidence of PVT is higher in patients with more advanced cirrhosis. Nery et al. followed up 1,243 patients with cirrhosis for 47 months and found that the independent risk factors for development of PVT were baseline esophageal varices and prothrombin time, but not with prothrombotic mutations (1). Consistently, our study also found that patients with PVT have worse liver function and a higher proportion of severe esophageal varices.

There are few studies on the natural course of PVT. A retrospective study found that thrombosis was improved in 47.60 %, unchanged in 45.20 %, and worsened in 7.20% (6). Our data showed that 90.59% of PVT were partial thrombosis, 27.35% of PVT disappeared completely during follow-up, and only 9.40% of PVT had progressed significantly. It can be seen that PVT is mostly partial thrombosis and the most common clinical outcome is unchanged or improvement. Qi X et al. proposed risk stratification for PVT: transient PVT was defined if a thrombus within the portal vein spontaneously disappears within 3 months in the absence of antithrombotic treatment (15). But so far, no predictor of transient PVT has been found. All the 11 patients with occlusive PVT remained occlusive, but 5/11 patients with occlusive PVT (45.45%) developed portal cavernoma. Portal cavernoma is considered to be one of the characteristics of chronic PVT and can maintain blood supply to the liver.

The effect of PVT on the decompensation or survival is controversial. Some studies did report that PVT was associated with increased mortality (16–18). But other studies showed that partial thrombosis is common in the clinic and there is no significant association (1, 6, 19, 20). Nery et al. (1) adjusted for baseline liver function and found that the formation of PVT did not increase the risk of decompensation in patients with cirrhosis. The independent risk factors for decompensation were esophageal varices (≥grade2) and prothrombin time. A recent prospective study (19) followed up 241 patients with cirrhosis for 29 months and found that PVT development did not independently predict cirrhosis decompensations or lower OLT-free survival. This can be at least partially caused by the fact that some of these studies did not appropriately adjust for differences in baseline liver function between PVT and non-thrombosis patients. Because PVT is more likely to occur in advanced cirrhosis, differences in liver function have a greater impact on decompensation and mortality (21). We used propensity score matching to adjust for confounding factors and found that the independent risk factors for decompensation were esophageal varices, serum sodium level and spontaneous portosystemic shunts. We consider that PVT has little effect on liver blood flow as most of the PVT is partial and occlusive thrombosis can form collateral vessels, which will reduce the portal vein tension. PVT may be a marker of liver disease decompensation, rather than a direct cause of portal hypertension and liver disease

decompensation. Large prospective studies are needed to reveal the effect of PVT on the outcome of cirrhosis patients.

A systematic review analyzed 25,753 liver transplant patients and found that only patients with occlusive thrombosis had a reduced survival rate after transplantation (22). Qi X et al. called occlusive PVT or PVT with extensive superior mesenteric vein thrombosis as clinically significant PVT (23) and believe that when clinically PVT is present, the prognosis of cirrhosis will be significantly compromised and anticoagulation therapy will benefit in such patients. However, our data showed that the occlusive PVT was 11.1% (5/45) in decompensated liver disease and was 8.3% (6/72) in the compensated group. The difference between two groups is not statistically significant ($P = 0.861$). Due to limited data, whether clinically significant PVT affects the prognosis of cirrhosis needs more data to verify.

Anticoagulation could be considered in selected cases. Acute symptomatic PVT can cause intestinal ischemia, anticoagulation therapy is recommended. The presence of severe PVT increases the complexity of the operation, reduces the blood supply of the transplanted organ, and decrease survival after transplantation (22). Therefore, the current guidelines generally recommend anticoagulation therapy for patients with PVT who are liver transplantation candidates (9, 24). With newly diagnosed PVT, comprehensive consideration should be given to extent of the thrombosis, presence or absence of attributable symptoms and risk of bleeding (24). When PVT progresses significantly and extends to the superior mesenteric vein, anticoagulation therapy should be considered. If no treatment, regular follow-up is required, and a considerable part of non-occlusive PVT will disappear.

Limitations of our study are mainly related to its retrospective nature. Most of the patients we included were Child-Pugh class A and B, the number of deaths during the observation period was small. Therefore, exploring the independent risk factors of death in cirrhosis has limited significance. Despite these limitations, our study has several strengths. This is a large retrospective study to observe the course of PVT under natural conditions. PSM analysis was applied to control selection bias.

In conclusion, the incidence of PVT is higher in patients with more advanced cirrhosis. The development of PVT cannot independently predict clinical outcome.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZC, Z-hZ, and SH: conception and design. ZC, HC, and FX: collection and assembly of data. ZC, TR, and HC: analysis and interpretation of the data. ZC and TR drafting of the manuscript. Z-hZ and SH supervised the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by Kuanren Talents Program of the Second Affiliated Hospital of Chongqing Medical University (13-002-011, 13-004-009), Chongqing Appropriate technology for health (06-123, 06-137), and National Science Foundation of China (81972285).

ACKNOWLEDGMENTS

ZC wants to thank his parents for their nurturing and selfless love.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.685944/full#supplementary-material

REFERENCES

1. Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PE, et al. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology*. (2015) 61:660–7. doi: 10.1002/hep.27546
2. Bhangui P, Lim C, Levesque E, Salloum C, Lahat E, Feray C, et al. Novel classification of non-malignant portal vein thrombosis: a guide to surgical decision-making during liver transplantation. *J Hepatol*. (2019) 71:1038–50. doi: 10.1016/j.jhep.2019.08.012
3. Intagliata NM, Caldwell SH, Tripodi A. Diagnosis, development, and treatment of portal vein thrombosis in patients with and without cirrhosis. *Gastroenterology*. (2019) 156:1582–99.e1. doi: 10.1053/j.gastro.2019.01.265
4. Zanetto A, Rodriguez-Kastro KI, Germani G, Ferrarese A, Cillo U, Burra P, et al. Mortality in liver transplant recipients with portal vein thrombosis - an updated meta-analysis. *Transpl Int*. (2018) 31:1318–29. doi: 10.1111/tri.13353
5. Benvegnù L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. *Gut*. (2004) 53:744–9. doi: 10.1136/gut.2003.020263
6. Maruyama H, Okugawa H, Takahashi M, Yokosuka O. *De novo* portal vein thrombosis in virus-related cirrhosis: predictive factors and long-term outcomes. *Am J Gastroenterol*. (2013) 108:568–74. doi: 10.1038/ajg.2012.452
7. Sarin SK, Philips CA, Kamath PS, Choudhury A, Maruyama H, Nery FG, et al. Toward a comprehensive new classification of portal vein thrombosis in patients with cirrhosis. *Gastroenterology*. (2016) 151:574–7. doi: 10.1053/j.gastro.2016.08.033

8. Paquet KJ. Prophylactic endoscopic sclerosing treatment of the esophageal wall in varices – a prospective controlled randomized trial. *Endoscopy*. (1982) 14:4–5. doi: 10.1055/s-2007-1021560
9. de Franchis R, Baveno VI Faculty. Expanding consensus in portal hypertension: report of the baveno VI consensus workshop: stratifying risk and individualizing care for portal hypertension. *J Hepatol*. (2015) 63:743–52. doi: 10.1016/j.jhep.2015.05.022
10. European Association for the Study of the Liver. EASL clinical practice guidelines for the management of patients with decompensated cirrhosis. *J Hepatol*. (2018) 69:406–60. doi: 10.1016/j.jhep.2018.03.024
11. Ge PS, Runyon BA. Treatment of patients with cirrhosis. *N Engl J Med*. (2016) 375:767–77. doi: 10.1056/NEJMra1504367
12. Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: risk stratification, diagnosis, and management: 2016 practice guidance by the American association for the study of liver diseases. *Hepatology*. (2017) 65:310–35. doi: 10.1002/hep.28906
13. Praktiknjo M, Trebicka J, Carnevale R, Pastori D, Queck A, Ettorre E, et al. Von willebrand and factor VIII portosystemic circulation gradient in cirrhosis: implications for portal vein thrombosis. *Clin Transl Gastroenterol*. (2020) 11:e00123. doi: 10.14309/ctg.0000000000000123
14. Sinegre T, Duron C, Lecompte T, Pereira B, Massoulier S, Lamblin G, et al. Increased factor viii plays a significant role in plasma hypercoagulability phenotype of patients with cirrhosis. *J Thromb Haemost*. (2018) 16:1132–40. doi: 10.1111/jth.14011
15. Qi X, Guo X, Yoshida EM, Méndez-Sánchez N, De Stefano V, Tacke F, et al. Transient portal vein thrombosis in liver cirrhosis. *BMC Med*. (2018) 16:83. doi: 10.1186/s12916-018-1069-8
16. Qi X, Dai J, Yang M, Ren W, Jia J, Guo X. Association between portal vein thrombosis and survival in non-liver-transplant patients with liver cirrhosis: a systematic review of the literature. *Gastroenterol Res Prac*. (2015) 2015:480842. doi: 10.1155/2015/480842
17. Amitrano L, Guardascione MA, Brancaccio V, Margaglione M, Manguso F, Iannaccone L, et al. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. *J Hepatol*. (2004) 40:736–41. doi: 10.1016/j.jhep.2004.01.001
18. Violi F, Corazza GR, Caldwell SH, Talerico G, Romiti GF, Napoleone L, et al. Incidence and recurrence of portal vein thrombosis in cirrhotic patients. *Thromb Haemost*. (2019) 119:496–9. doi: 10.1055/s-0038-1676981
19. Noronha Ferreira C, Marinho RT, Cortez-Pinto H, Ferreira P, Dias MS, Vasconcelos M, et al. Incidence, predictive factors and clinical significance of development of portal vein thrombosis in cirrhosis: a prospective study. *Liver Int*. (2019) 39:1459–67. doi: 10.1111/liv.14121
20. Luca A, Caruso S, Milazzo M, Marrone G, Mamone G, Crinò F, et al. Natural course of extrahepatic nonmalignant partial portal vein thrombosis in patients with cirrhosis. *Radiology*. (2012) 265:124–32. doi: 10.1148/radiol.12112236
21. Berry K, Taylor J, Liou IW, Ioannou GN. Portal vein thrombosis is not associated with increased mortality among patients with cirrhosis. *Clin Gastroenterol Hepatol*. (2015) 13:585–93. doi: 10.1016/j.cgh.2014.10.010
22. Rodríguez-Castro KI, Porte RJ, Nadal E, Germani G, Burra P, Senzolo M. Management of non-neoplastic portal vein thrombosis in the setting of liver transplantation: a systematic review. *Transplantation*. (2012) 94:1145–53. doi: 10.1097/TP.0b013e31826e8e53
23. Qi X, Li H, Liu X, Yao H, Han G, Hu F, et al. Novel insights into the development of portal vein thrombosis in cirrhosis patients. *Expert Rev Gastroenterol Hepatol*. (2015) 9:1421–32. doi: 10.1586/17474124.2015.1083856
24. O'Leary JG, Greenberg CS, Patton HM, Caldwell SH. AGA clinical practice update: coagulation in cirrhosis. *Gastroenterology*. (2019) 157: 34–43.e1. doi: 10.1053/j.gastro.2019.03.070

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 28 July 2021
doi: 10.3389/fmed.2021.690825

Liver Stiffness Is a Predictor of Rebleeding in Patients With Hepatitis B-Related Cirrhosis: A Real-World Cohort Study

*Linxiang Liu†, Yuan Nie†, Yue Zhang, Qi Liu and Xuan Zhu**

Department of Gastroenterology, The First Affiliated Hospital of Nanchang University, Nanchang, China

Background: Esophageal vein rebleeding is a life-threatening complication of liver cirrhosis. However, the role of non-invasive methods that were developed to evaluate the severity of chronic liver disease, especially in rebleeding, remains unclear.

Aims: To evaluate the performance of liver stiffness and non-invasive fibrosis scores in predicting esophageal vein rebleeding in hepatitis B virus (HBV) cirrhotic patients.

Methods: A prospective analysis of 194 HBV patients between 2017 and 2021 was performed. Receiver operating characteristic (ROC) curves and time-dependent ROC curves were used to assess the power for predicting rebleeding with non-invasive fibrosis score and liver stiffness.

Results: During the median follow-up time of 68.28 weeks, 55 patients experienced rebleeding. In the entire cohort, the area under the ROC curve for liver stiffness measurement (LSM) predicting for rebleeding was 0.837, with a cut-off value of 17.79 kPa, and the time-dependent ROC curve also showed stable prediction performance of LSM. The predictive ability of the non-invasive fibrosis score was less than that of LSM, and there were statistical differences. Moreover, patients using non-selective beta-blockers and HBV DNA-negative patients experienced significantly reduced rebleeding.

Conclusions: Compared with non-invasive fibrosis scores, LSM can more simply and accurately predict rebleeding events of hepatitis B liver cirrhosis.

Keywords: cirrhosis complications, hepatitis B virus, liver stiffness, non-invasive fibrosis score, rebleeding

OPEN ACCESS

Edited by:
Jonel Trebicka,
Goethe University Frankfurt, Germany

Reviewed by:
JayaKrishna Chintanaboina,
University of California, San Francisco, United States
Michael Praktiknjo,
University of Bonn, Germany

***Correspondence:**
Xuan Zhu
waiyongtg@163.com

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *04 April 2021*
Accepted: *24 June 2021*
Published: *28 July 2021*

Citation:
Liu L, Nie Y, Zhang Y, Liu Q and Zhu X (2021) Liver Stiffness Is a Predictor of Rebleeding in Patients With Hepatitis B-Related Cirrhosis: A Real-World Cohort Study. Front. Med. 8:690825. doi: 10.3389/fmed.2021.690825

INTRODUCTION

Liver cirrhosis is caused by various injury mechanisms which can induce liver necrosis and fibrosis. It is considered not a single disease entity but a disease that can be subdivided into different clinical prognostic stages (1). Esophageal variceal bleeding (EVB) is a common complication of liver cirrhosis, and it can also be a life-threatening complication due to high morbidity and high mortality (2). Despite improvements in the efficacy of endoscopic, pharmacologic, surgical, and radiologic techniques, the 6-week mortality and total mortality after bleeding are 17.5 and 33.5%, respectively (3). Not only is the mortality rate of the first esophageal venous bleeding high, but the 6-week rebleed rate is up to 60% in patients who have not undergone secondary prevention patients (4). Therefore, the occurrence of rebleeding events has received increasing attention in cirrhosis patients.

Recent studies pooling available evidence have demonstrated that the severity of liver fibrosis, especially the presence of advanced fibrosis defined as stage F3 or F4 fibrosis, is the main driver of prognosis in cirrhosis and the main risk factor for developing not only liver-related events but also extrahepatic complications (5–7). In this context, there is a good correlation between the non-invasive fibrosis score and the degree of liver fibrosis (5, 8–10), and the degree of liver fibrosis was correlated with the degree of portal hypertension. However, the use of the fibrosis score to predict the occurrence of complications of liver cirrhosis, especially the rebleeding of the esophageal vein of liver cirrhosis, is a major unmet need.

Liver stiffness measurement (LSM) is a widely used non-invasive tool for the diagnosis of liver fibrosis and has high accuracy (11), and combined platelets are also used to identify patients at high risk for esophageal varices without the need for endoscopic screening (12). Previous studies have demonstrated that liver stiffness can reflect the prognosis of patients with liver cirrhosis because it can indirectly reflect portal hypertension (11, 13). Liver stiffness measured using transient elastography (TE) has been validated as a prognostic quantitative marker for the occurrence of liver-related complications, survival without liver-related death, and overall survival (6, 14–16). However, LSM has not been well-verified in the esophageal variceal rebleeding, which is a critical event.

Chronic hepatitis B (CHB) is a major health burden, with an estimated 240 million chronic carriers of the hepatitis B virus (HBV) surface antigen (HBsAg) worldwide, resulting in 815,000 people dying annually due to its complications (17, 18). TE and fibrosis scores currently focus on the accuracy of the pathological classification of liver cirrhosis. However, there are few studies predicting the complications of esophageal venous rebleeding in liver cirrhosis, which makes it difficult for clinicians to accurately and rapidly evaluate such patients and increases the burden of public health. To address this limitation, our study aims to evaluate non-invasive serological indices, namely, the AST to Platelet Ratio Index (APRI), Fibrosis-4 score (FIB-4), King's College Criteria (King's Score), Goteborg University Cirrhosis Index (GUCI), FibroIndex, and FornsIndex, and determine their accuracy in predicting bleeding events in hepatitis B liver cirrhosis patients. Simultaneously, we compared the predictive performance of transient elastography.

METHODS

Study Patients

This was a prospective cohort study, and consecutive hospitalized patients with hepatitis B liver cirrhosis were admitted to the Department of Gastroenterology, the First Affiliated Hospital of Nanchang University in China, between February 2017 and January 2021. The patient inclusion criteria were as follows: (1) age $\geq$ 18, (2) diagnosis of hepatitis B cirrhosis (positive hepatitis B surface antigen, and diagnosed with cirrhosis by liver biopsy or imaging examinations together with clinical features such as ascites, thrombocytopenia or gastro-esophageal varices), (3) first bleeding in the past and received secondary prevention of variceal rebleeding (endoscopic variceal ligation (EVL) combined with a non-selective beta-blocker (NSBB) or EVL alone), and (4) had a liver transient elastography measurement before the second episode of variceal bleeding, The exclusion criteria included the following: (1) a diagnosis of HCC at inclusion or during the first 6 months of follow-up, (2) known HIV, (3) the first bleeding is non-esophagogastric vein bleeding under digestive endoscopy, (4) history of liver transplantation, (5) combination with other types of liver disease such as alcoholic cirrhosis or hepatitis C cirrhosis, (6) the patient had a large amount of ascites at the time of admission or a status of Child–Pugh C class, and (7) severe heart and lung disease. The treatment of the included patients was individualized according to Baveno VI standards. The study protocol was approved by the institutional ethics committee of the First Affiliated Hospital of Nanchang University (No. 2015–1206). Informed written consent was obtained from all the study participants.

Clinical Data Collection and Follow-Up

Clinical data such as age, sex, diabetes, hypertension, etiology, white blood cell (WBC), hemoglobin (HB), platelet (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), albumin (ALB), gamma-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), creatinine (Cr), international normalized ratio (INR), prothrombin time (PT), fibrinogen, blood urea nitrogen (BUN), HBV DNA, hepatitis B e antigen (HBeAg), and liver stiffness measurements were collected at the time of the first acute variceal bleeding. The Child–Pugh score and model for end-stage liver disease (MELD) score were also recorded. The data were collected independently by two physicians and checked by a third person. All included patients were followed up for rebleeding and survival. The primary outcome was a rebleeding event due to esophageal varices.

Liver Stiffness Measurements and Calculation of Scores

The liver stiffness measurements were completed within 1 week after the patient underwent ligation for acute bleeding. For patients with ascites at the time of admission, the LSM was measured after the ascites subsided. Transient elastography was performed with FibroScan (Echosens, Paris, France) using the standard-probe, and on a fasting (4 h) patient lying flat on his/her back, with the right arm tucked behind the head to facilitate access to the right upper quadrant. The probe is positioned perpendicular to the skin surface in one of the intercostal spaces adjacent to the right lobe of the liver. LSM was considered reliable only if 10 successful acquisitions were obtained and the ratio of the interquartile range over the median (IQR/LSM) was $\leq$0.3. LSM was expressed in kilopascals. Patients with unreliable LSM results had the examination repeated immediately; the results were not analyzed if they remained unreliable. The operators were blinded to all clinical data and the diagnoses of the patients.

A total of four non-invasive models were performed for all included patients:

a. APRI: AST (U/L)/upper limit of normal/PLT (10^9/L) $\times$ 100

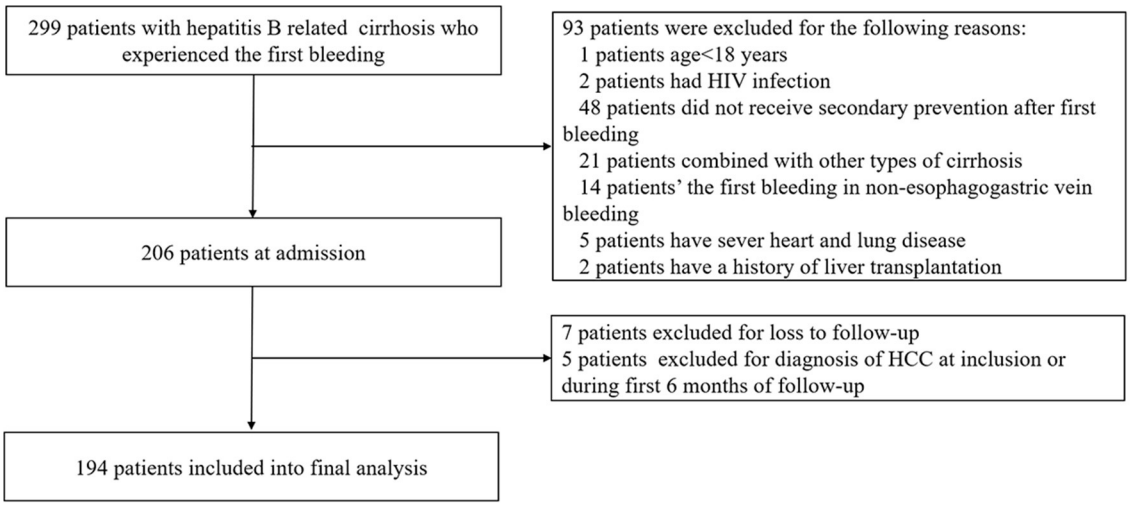


FIGURE 1 | The flowchart of our study.

b. FIB-4: [age (years) × AST (U/L)]/[PLT (10^9/L) × $\sqrt{}$ALT (U/L)]
c. King's score: age (years) × AST (U/L) × INR/PLT (10^9/L)
d. GUCI: [AST (U/L)/upper limit of normal] × [(prothrombin – INR × 100)/PLT (10^9/L)]
e. Fibrosis index: 8.28 – 0.01 × PLT (10^9/L)—serum albumin (g/dl)
f. Forns score: 7.811 – 3.131 × ln [PLT (109/L)] + 0.781 × ln [GGT (U/L)] + 3.467 × ln [age (years)] – 0.014 [cholesterol (mg/dl)]
g. MELD: 3.78 × ln [TBil (μmol/L)] + 11.2 × ln (INR) + 9.57 × ln [creatinine (mg/dl)] + 6.43
h. MELD-Na: MELD + 1.59 × [135-Na^+ (mmol/L)]
i. ALBI: −0.085 × [albumin (g/L)] + 0.66 × lg [TBil (μmol/L)].

NSBB Treatment and EVL Procedure

For patients receiving NSBB treatment, either carvedilol or propranolol was used. Carvedilol was started at an initial dose of 6.25 mg once daily and adjusted gradually to the maximum tolerated dose, keeping the heart rate at >55 beats per minute and systolic blood pressure at >90 mmHg. Propranolol was started at an initial dose of 10 mg three times daily and adjusted gradually to the maximum tolerated dose, keeping the heart rate at >55 beats per minute and the systolic blood pressure at >90 mmHg. EVL was performed using commercial multiband devices under sedation with propofol. The varices were ligated from the cardia to the oral side.

Statistical Analysis

Continuous variables are shown as the mean and standard deviation (SD) or median and interquartile range (IQR), while categorical variables are shown as frequencies (%). The rebleeding rate for the study population was generated using the Kaplan–Meier method, and differences in rebleeding rate were examined using the log-rank test. We tested whether the explanatory variable had an interaction and found no significant interactions within the included variables. Student's *t*-tests or Mann–Whitney *U*-test were performed for group comparisons. The diagnostic accuracy of rebleeding was assessed by receiver operating characteristic (ROC) analysis. Areas under the ROC curves (AUROCs) were compared by the method of DeLong et al. A time-dependent ROC curve was employed to evaluate the time-dependent predictive performance of the model to be tested for rebleeding. All levels of significance were set at a two-sided 5% level. All analyses were performed using SPSS 25.0 IBM (IBM Corp., Armonk, NY, USA) and R 3.5.2 (R Project for Statistical Computing, Vienna, Austria). The R statistical packages "pROC," "survival," "compareGroups," and "survminer" were used to calculate the clinical characteristics table, Kaplan–Meier curves, ROC curve, and time-dependent ROC curves.

RESULT

A total of 299 patients with suspected HBV-related liver cirrhosis underwent series of examinations. Of these, 93 patients were excluded for the exclusion criteria. The remaining 206 patients were followed up. Finally, 194 patients were included in the final analysis. A flow chart for the study enrollment is summarized in **Figure 1**.

Patients' Characteristics

The baseline characteristics of the 194 patients with HBV-related liver cirrhosis are shown in **Table 1**. The average age of the whole population was 52.83 years, and the majority of them were male (81.96%). The baseline median LSM value was 14.57 KPa, and the BMI value was 22.01. The median Child–Pugh, MELD, MELD-Na, and ALBI scores were 6, 9.64, 3.83, and −2.073, respectively. Patients of Child–Pugh class A (86.08%) comprised the majority of this cohort. Various median fibrosis scores were as follows:

TABLE 1 | Baseline characteristics of patients.

Variable	All patients (*N* = 194)
Male	159 (81.96)
Follow-up time (weeks)	63.28 (17.64–112.5)
Age (years)	52.83(11.43)
BMI	22.01(3.16)
ALT (U/L)	25 (17–40)
AST (U/L)	35 (27–50)
Hemoglobin (g/L)	98.23(29.3)
Platelet count (10^9/L)	69.5 (47–114)
White Blood Count (10^9/L)	4.3(2.3)
Albumin (g/L)	34.67 (5.43)
Globulins (g/L)	26.95 (7.89)
Total bilirubin (μmol/L)	20.25 (14–31.9)
INR	1.24 (1.14–1.36)
PT (s)	13.9 (12.7–15)
γ-GT (U/L)	30 (19–59.5)
Cholesterol (mmol/L)	3.18 (2.55–3.86)
Creatinine (μmol/L)	66.4 (57.3–79.1)
Serum sodium (mmol/L)	139.4 (135.8–141.2)
HBV DNA (log10 IU/ml)*	3.121(1.701)
HBeAg (positivity rate, %)	33 (17.01)
LSM (kPa)	14.57 (11.22–18.81)
APRI	1.537 (0.807–2.523)
FIB-4	5.029 (2.887–9.495)
King's score	32.765 (17.829–63.320)
GUCI	1.846 (0.942–3.232)
FibroIndex	2.588 (2.242–2.881)
Forns score	9.254 (7.882–10.609)
MELD	9.64 (8.13–11.59)
MELD-Na	3.83 (−1.41 to 9.40)
ALBI	−2.073 (−2.424 to 1.694)
Child–Pugh score	6 (5–7)
Child A	167 (86.08)
Child B	27 (13.92)
Rebleeding events	55 (28.35)
6-week rebleeding event	13 (6.70)
3-month rebleeding events	26 (13.40)
1-year rebleeding events	47 (24.23)

**For patients with liver cirrhosis who are positive for HBV DNA.*
BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LSM, liver stiffness measurement; PT, prothrombin time; γ-GT, gamma-glutamyl transpeptidase.

APRI, FIB-4, King's score, GUCI, FibroIndex, and Forns score were 1.537, 5.029, 32.765, 1.846, 2.588, and 9.254, respectively.

When they were hospitalized due to bleeding esophageal varices for the first time, eight patients developed hepatic encephalopathy below stage III, five patients had fever with bacteremia or spontaneous peritonitis, and one developed hepatorenal syndrome. All patients who suffered complications during hospitalization had fully recovered from the above complications when they were discharged.

TABLE 2 | Characteristics between the no rebleeding group and rebleeding group.

Variable	No rebleeding (*N* = 139)	Rebleeding (*N* = 55)	*P*-value
Follow-up time (weeks)	85.4 (51.9–168)	14.9 (6.43–35.8)	**<0.001**
Age	52.5 (12.1)	53.7 (9.69)	0.461
BMI	22.1 (3.33)	22.1 (2.73)	0.878
ALT (U/L)	25.0 (17.0–38.0)	27.0 (18.5–41.5)	0.243
AST (U/L)	34.0 (27.0–48.0)	35.0 (26.5–55.0)	0.474
Hemoglobin (g/L)	102 (29.5)	88.6 (26.4)	**0.003**
Platelet count (10^9/L)	82.0 (54.5–134)	54.0 (38.5–77.0)	**<0.001**
White blood count (10^9/L)	4.56 (2.22)	3.64 (2.24)	**0.011**
Albumin (g/L)	35.0 (5.68)	33.9 (4.71)	0.155
Golbulins (g/L)	27.1 (7.93)	26.5 (7.83)	0.602
Total bilirubin (μmol/L)	19.6 (12.6–30.2)	24.6 (18.2–37.0)	**0.007**
INR	1.22 (1.12–1.35)	1.28 (1.20–1.42)	**0.012**
PT (s)	13.7 (12.6–14.9)	14.2 (13.0–15.2)	0.205
γ-GT	32.0 (19.5–67.5)	25.0 (18.0–47.5)	0.086
Cholesterol (mmol/L)	3.19 (2.63–3.91)	3.12 (2.45–3.56)	0.171
Serum sodium (mmol/L)	139 (136–141)	140 (134–141)	0.587
Creatinine (μmol/L)	66.3 (59.5–78.2)	67.8 (56.1–82.8)	0.883
HBV-DNA			**<0.001**
Negative	77 (55.4%)	6 (10.9%)	
Positive	62 (44.6%)	49 (89.1%)	
NSBB drugs			**<0.001**
no used	36 (25.9%)	31 (56.4%)	
Used	103 (74.1%)	24 (43.6%)	
APRI	1.28 (0.70–2.00)	2.32 (1.15–3.27)	**<0.001**
FIB-4	4.13 (2.53–7.87)	6.77 (4.41–11.8)	**<0.001**
King's score	47.0 (79.0)	95.7 (191)	0.072
GUCI	1.64 (0.86–2.59)	3.01 (1.52–4.26)	**<0.001**
FibroIndex	2.53 (2.17–2.80)	2.71 (2.53–2.97)	**0.001**
Forns score	8.78 (7.30–10.4)	10.2 (9.08–11.0)	**<0.001**
Child–Pugh	6.00 (5.00–7.00)	6.00 (6.00–7.00)	0.118
MELD	9.43 (8.13–11.4)	10.1 (8.67–12.2)	**0.039**
MELD-Na	3.38 (−1.59 to 8.26)	4.89 (0.41–11.2)	0.067
ALBI	−2.20 (−2.45 to −1.74)	−1.87 (−2.20 to −1.63)	**0.012**
LSM (kPa)	12.9 (10.5–15.7)	20.0 (17.9–23.0)	**<0.001**

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LSM, liver stiffness measurement; PT, prothrombin time; γ-GT, gamma-glutamyl transpeptidase.
Data are given as mean ± standard deviation, median (interquartile range), or as percentage of cases (%).
The bold values indicates that these variables are significantly different and specific details has been explained in the Methods - Statistical analysis Section.

Patients were followed up until the occurrence of a rebleeding episode. At a median follow-up of 68.28 weeks (range, 1.0–208.5 weeks), rebleeding occurred in 55 out of 194 patients (28.35%). Among them, the rates of rebleeding within 6 weeks, 3 months, and 1 year were 6.7, 13.4, and 24.23%, respectively. At the same time, three people developed hepatocellular carcinoma during the follow-up period and three people died: two of

TABLE 3 | Baseline comparison of patients with and without NSBB drugs.

	No used NSBB drugs			Used NSBB		
	No rebleeding	Rebleeding	*P*-value	No rebleeding	Rebleeding	*P*-value
	N = 36	*N* = 31		*N* = 103	*N* = 24	
Following-up time (weeks)	64.9 (27.0–167)	11.3 (5.43–30.0)	**<0.001**	87.0 (56.4–160)	19.3 (8.43–46.4)	**<0.001**
Age (years)	55.9 (11.3)	54.2 (10.8)	0.539	51.3 (12.2)	53.1 (8.24)	0.392
BMI	21.9 (3.47)	22.2 (3.31)	0.739	22.2 (3.29)	21.9 (1.77)	0.525
ALT (U/L)	31.0 (24.8–47.0)	28.0 (22.0–47.0)	0.910	23.0 (15.5–34.0)	21.5 (16.0–35.0)	0.753
AST (U/L)	37.5 (32.8–56.8)	42.0 (28.5–53.5)	0.651	32.0 (25.0–41.5)	30.5 (24.0–55.5)	0.121
Hemoglobin (g/L)	113 (33.7)	92.7 (27.9)	**0.009**	98.3 (27.1)	83.3 (23.8)	**0.011**
Platelet count (10^9/L)	96.5 (51.0–122)	52.0 (34.5–74.0)	**<0.001**	77.0 (55.5–135)	56.5 (41.5–83.8)	**0.009**
White blood count (10^9/L)	4.85 (1.92)	3.62 (2.57)	**0.034**	4.46 (2.32)	3.65 (1.79)	0.069
Albumin (g/L)	34.3 (6.53)	34.1 (5.29)	0.931	35.2 (5.36)	33.5 (3.93)	0.072
Golbulins (g/L)	28.1 (7.98)	25.6 (8.40)	0.234	26.8 (7.93)	27.6 (7.04)	0.645
Total bilirubin (μmol/L)	25.8 (15.0–39.3)	23.0 (18.4–34.5)	0.602	18.5 (12.3–25.8)	26.4 (17.2–40.1)	**0.015**
INR	1.21 (1.15–1.38)	1.28 (1.23–1.42)	0.178	1.22 (1.12–1.34)	1.28 (1.16–1.39)	0.086
PT (s)	13.8 (12.4–15.4)	14.2 (13.1–14.9)	0.615	13.7 (12.7–14.8)	14.2 (12.9–15.6)	0.294
γ-GT (U/L)	46.0 (24.5–102)	24.0 (16.5–45.5)	**0.012**	30.0 (19.0–56.0)	27.0 (18.0–52.0)	0.714
Cholesterol (mmol/L)	3.37 (2.88–4.11)	3.18 (2.58–3.50)	0.170	3.18 (2.58–3.89)	2.80 (2.35–3.60)	0.253
Serum sodium (mmol/L)	139 (137–141)	140 (134–142)	0.826	139 (136–141)	138 (135–141)	0.407
Creatinine (μmol/L)	64.8 (57.2–78.2)	66.3 (55.1–78.1)	0.651	66.4 (60.5–78.2)	68.7 (60.1–84.2)	0.390
HBV-DNA			**<0.001**			**<0.001**
Negative	20 (55.6%)	3 (9.68%)		57 (55.3%)	3 (12.5%)	
Positive	16 (44.4%)	28 (90.3%)		46 (44.7%)	21 (87.5%)	
APRI	1.59 (0.92–2.24)	2.68 (1.55–3.61)	**0.011**	1.20 (0.65–1.98)	1.88 (1.05–2.56)	**0.037**
FIB-4	4.30 (3.29–8.92)	7.02 (5.42–13.0)	0.061	4.10 (2.44–7.46)	6.25 (3.70–9.34)	0.034
King's score	60.3 (86.9)	88.4 (93.0)	0.208	42.3 (76.0)	105 (272)	0.275
GUCI	2.03 (0.96–3.30)	3.21 (1.90–4.72)	**0.010**	1.52 (0.79–2.42)	2.16 (1.39–3.77)	**0.025**
FibroIndex	2.61 (2.23–2.94)	2.71 (2.50–3.02)	0.200	2.50 (2.15–2.75)	2.72 (2.53–2.92)	**0.004**
Forns score	8.73 (7.88–10.4)	10.2 (9.18–11.6)	**0.012**	8.78 (7.02–10.3)	10.1 (8.44–10.7)	**0.025**
Child–Pugh	6.00 (5.75–7.25)	6.00 (6.00–7.00)	0.698	6.00 (5.00–7.00)	6.00 (6.00–8.00)	0.080
MELD	9.16 (8.13–11.4)	10.1 (8.56–11.8)	0.187	9.45 (8.13–11.4)	10.1 (8.77–13.0)	0.151
MELD-Na	2.94 (−1.84 to 7.56)	4.50 (−0.79 to 11.7)	0.155	3.41 (−1.57 to 8.26)	6.04 (1.44–10.3)	0.158
ALBI	−2.14 (−2.43 to −1.64)	−1.83 (−2.20 to −1.63)	0.386	−2.23 (−2.49 to −1.77)	−1.93 (−2.17 to −1.67)	**0.025**
LSM (kPa)	13.6 (11.0–16.4)	20.1 (17.9–22.4)	**<0.001**	12.5 (10.5–15.3)	19.2 (16.6–23.4)	**<0.001**

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LSM, liver stiffness measurement; PT, prothrombin time; γ-GT, gamma-glutamyl transpeptidase. Data are given as mean ± standard deviation, median (interquartile range), or as percentage of cases (%).
The bold values indicates that these variables are significantly different and specific details has been explained in the Methods - Statistical analysis Section.

them from respiratory failure and one from hypovolemic shock during rebleeding.

Comparison of Baseline Characteristics of Patients With or Without Rebleeding

The comparison of clinical baseline characteristics between the non-rebleeding group and the rebleeding group is shown in **Table 2**. Some variables in the rebleeding group, such as follow-up time, hemoglobin, platelet count, and white blood count, were smaller than those in the no rebleeding group ($p < 0.05$). However, total bilirubin, INR, APRI, FIB-4, GUCI, FibroIndex, and Forns score were higher in the rebleeding group than in the no rebleeding group ($p < 0.05$).

Considering the differences between the NSBB drugs used and HBV-DNA positivity in the two groups of patients, further subgroup analysis was performed. In the two groups of people who used and did not use NSBB, their rebleeding rates were 46.27% (31/67) and 18.89% (24/127), respectively. There was a significant difference in the occurrence of rebleeding events and LSM between the two groups. The remaining clinical features are summarized in **Table 3**. In this context, the same method was used to compare whether HBV-DNA was positive for patients with liver cirrhosis. Among HBV DNA-negative patients, those who experienced rebleeding and those who did not experience rebleeding, only LSM was significantly different, and the complete comparison is summarized in **Table 4**.

TABLE 4 | Baseline comparison of patients with and without HBV-DNA positivity.

	HBV-DNA (–)			HBV-DNA (+)		
	No rebleeding	Rebleeding	*P*-value	No rebleeding	Rebleeding	*P*-value
	N = 77	*N* = 6		*N* = 62	*N* = 49	
Follow-up time (weeks)	76.3 (40.3–145)	40.1 (21.5–68.1)	0.077	88.7 (57.4–172)	12.1 (6.43–30.0)	**<0.001**
Age (years)	53.1 (12.6)	59.0 (11.1)	0.261	51.7 (11.5)	53.1 (9.42)	0.492
BMI	22.0 (3.24)	21.5 (1.47)	0.449	22.3 (3.45)	22.1 (2.85)	0.830
ALT (U/L)	23.0 (15.0–31.0)	25.5 (16.8–32.8)	0.666	27.0 (19.5–47.0)	27.0 (19.0–44.0)	0.891
AST (U/L)	32.0 (24.0–40.0)	28.0 (27.0–35.0)	0.647	36.0 (30.0–53.0)	39.0 (26.0–55.0)	0.677
Hemoglobin (g/L)	99.6 (28.9)	74.0 (27.7)	0.074	105 (30.3)	90.4 (25.9)	**0.007**
Platelet count (10^9/L)	82.0 (52.0–138)	64.5 (36.2–78.5)	0.152	80.5 (55.5–122)	54.0 (39.0–71.0)	**<0.001**
White blood cell count (10^9/L)	4.59 (2.50)	3.92 (2.33)	0.526	4.53 (1.84)	3.60 (2.26)	**0.022**
Albumin (g/L)	35.0 (4.78)	32.1 (5.66)	0.266	34.9 (6.67)	34.1 (4.61)	0.417
Globulins (g/L)	27.2 (7.31)	24.8 (9.84)	0.579	27.0 (8.70)	26.7 (7.65)	0.824
Total bilirubin (μmol/L)	20.3 (13.8–31.0)	18.6 (18.3–26.1)	0.799	18.2 (12.3–28.8)	24.8 (18.2–38.8)	**0.007**
INR	1.20 (1.12–1.33)	1.31 (1.23–1.65)	0.124	1.25 (1.14–1.36)	1.28 (1.19–1.41)	0.248
PT (s)	13.4 (12.6–15.0)	14.8 (13.8–18.3)	0.142	13.9 (12.7–14.8)	14.2 (12.9–14.8)	0.605
γ-GT (U/L)	31.0 (18.0–73.0)	35.0 (19.5–50.5)	0.979	34.0 (22.2–60.5)	25.0 (18.0–46.0)	**0.040**
Cholesterol (mmol/L)	3.18 (2.61–3.91)	2.83 (2.23–3.29)	0.308	3.20 (2.70–3.88)	3.12 (2.47–3.57)	0.362
Serum sodium (mmol/L)	139 (136–141)	138 (134–141)	0.752	140 (136–142)	140 (135–141)	0.318
Creatinine (μmol/L)	66.5 (58.4–78.0)	67.2 (50.7–70.5)	0.712	65.8 (59.8–78.6)	67.8 (56.4–83.0)	0.873
NSBB drugs			0.340			**0.002**
No used NSBB	20 (26.0%)	3 (50.0%)		16 (25.8%)	28 (57.1%)	
Used NSBB	57 (74.0%)	3 (50.0%)		46 (74.2%)	21 (42.9%)	
ALBI	−2.16 (−2.44 to −1.84)	−1.77 (−2.23 to −1.41)	0.225	−2.22 (−2.54 to −1.68)	−1.88 (−2.17 to −1.64)	0.077
APRI	1.19 (0.68–1.84)	1.99 (0.91–3.01)	0.268	1.60 (0.84–2.35)	2.32 (1.16–3.33)	**0.011**
FIB-4	4.10 (2.38–7.60)	7.16 (3.71–13.3)	0.149	4.19 (3.00–8.58)	6.77 (4.51–11.8)	**0.010**
King's score	35.4 (33.7)	57.2 (38.6)	0.231	61.4 (111)	100 (202)	0.228
GUCI	1.40 (0.83–2.26)	3.07 (1.18–4.22)	0.176	1.95 (0.94–3.15)	3.01 (1.64–4.29)	**0.015**
FibroIndex	2.53 (2.15–2.73)	2.63 (2.50–2.89)	0.493	2.53 (2.18–2.87)	2.72 (2.53–2.97)	**0.010**
Forns score	8.68 (7.14–10.5)	10.1 (9.75–11.7)	0.088	8.99 (7.65–10.3)	10.2 (9.01–10.9)	**0.003**
Child–Pugh	6.00 (5.00–7.00)	7.00 (5.50–7.75)	0.287	6.00 (5.00–7.00)	6.00 (6.00–7.00)	0.431
MELD	8.72 (6.96–10.7)	12.5 (8.36–16.6)	0.128	10.4 (8.32–12.4)	10.1 (8.72–12.1)	0.896
MELD-Na	3.41 (−1.48 to 7.28)	8.09 (5.58 to 16.4)	0.073	3.13 (−1.61 to 9.31)	4.50 (0.33 to 10.7)	0.297
LSM (kPa)	12.5 (9.96–15.3)	27.5 (22.9–30.7)	**0.001**	13.6 (10.9–16.1)	19.4 (17.8–21.9)	**<0.001**

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LSM, liver stiffness measurement; PT, prothrombin time; γ-GT, gamma-glutamyl transpeptidase. Data are given as mean ± standard deviation, median (interquartile range), or as percentage of cases (%).
The bold values indicates that these variables are significantly different and specific details has been explained in the Methods - Statistical analysis Section.

According to the two parameters of NSBB drugs and HBV-DNA positivity, two subgroups were divided, and then survival analysis of the two subgroups was performed while drawing Kaplan–Meier curves (**Figure 2**). In the above two subgroups, there was a significant difference in the survival probability of the two groups of patients in each subgroup ($p < 0.0001$).

Comparison of Parameters for Prediction of Rebleeding

As shown in **Table 5**, the area under the ROC curve (AUC) of each parameter at 6 weeks, 3 months, and 1 year was compared. At 6 weeks, 3 months, and 1 year, the maximum and minimum AUC parameters used to predict rebleeding events were LSM (AUC: 0.698) and FibroIndex (AUC: 0.549); LSM (AUC: 0.732) and MELD (AUC: 0.522); and LSM (AUC: 0.735) and MELD-Na (AUC:0.574), respectively. At the above three time points, the AUC of LSM was always the largest ($p < 0.01$). In contrast, the AUC of MELD-Na was <0.6 ($p > 0.05$).

To further illustrate the changes in the AUCs of various parameters over time, we drew a time-dependent ROC curve. This curve was simple and represented a method to visually understand the AUC values corresponding to different time points. As shown in **Figure 3**, various parameters were divided into three categories: the non-invasive fibrosis score-related group (such as APRI, FIB-4, King's Score, GUCI, FibroIndex, FornsIndex), liver function-related score group (such as Child–Pugh, MELD, MELD-Na, ALBI), and LSM. During the follow-up period, it was found that the AUC of LSM remained

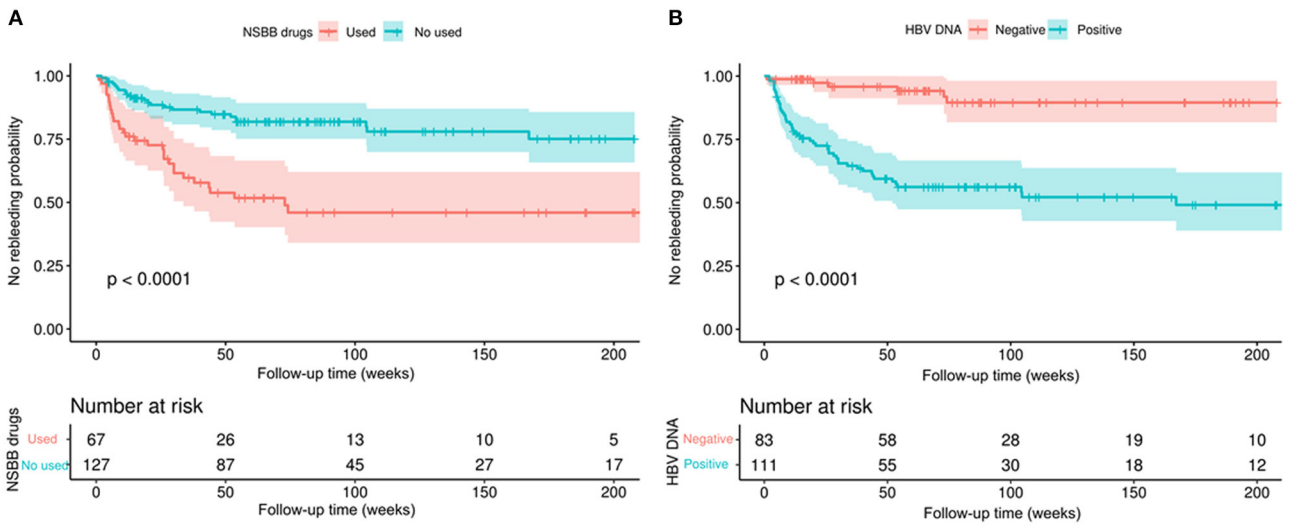


FIGURE 2 | Kaplan–Meier curves demonstrating rebleeding in patients with HBV-related cirrhosis according to different clinical characteristics. **(A)** According to whether NSBB drugs were used to compare the no rebleeding probability between the two groups. **(B)** According to whether HBV-DNA positivity was used to compare the no rebleeding probability between the two groups. *p*-values are from the log-rank test.

relatively stable, and the value was high. At this time, the AUC of LSM was 0.837, and the cut-off value was 17.79 kPa. However, the APRI, King and GUCI time-dependent ROC curves almost fit, which means that they had similar predictive capabilities. ALBI performed best in the liver function-related score group, but over time, the AUC predicting rebleeding gradually decreased.

Prediction of Rebleeding When Combining the Non-invasive Fibrosis Score and LSM

We combined APRI, FIB-4, King's Score, GUCI, FibroIndex, FornsIndex, and LSM and then drew ROC curves separately (**Figure 4**). Compared with the ROC curve of the non-invasive fibrosis score before the combined diagnosis, the ROC curve obtained after the combined diagnosis was statistically significant ($p < 0.05$), but compared with the ROC curve of LSM, it was not statistically significant ($p > 0.05$). In the entire cohort, the AUCs of LSM and the parameters after combined diagnosis were both over 0.8, suggesting that they have excellent performance in predicting rebleeding events.

DISCUSSION

In the present study, we first compared the different approaches that use non-invasive tools to predict esophageal venous rebleeding in HBV-related liver cirrhosis, and we found that baseline LSM accurately predicts rebleeding events in our cohort, while different clinical characteristics of patients, such as the use of NSBB drugs and HBV DNA positivity can affect the prognosis of patients. Furthermore, when predicting the occurrence of rebleeding events in the entire cohort, the AUC of LSM reached 0.837 (0.777–0.886), and the cut-off point was 17.79 kPa. At this time, the sensitivity and specificity were 76.36 and 87.77%, respectively, which means that LSM showed excellent performance compared with the non-invasive fibrosis score.

The formation and appearance of varices are driven by various factors, increased portal pressure, collateral blood flow, and vascular endothelial growth factor, all of which contribute to variceal bleeding (1). Spontaneous portosystemic shunting due to portal hypertension is seen in patients with cirrhosis, and it may predict a poor clinical outcome (19, 20). The hepatic venous pressure gradient (HVPG) directly reflects portal hypertension and is a reliable predictor of bleeding due to esophageal varices (21). However, measuring HVPG is an invasive operation that is expensive and requires highly skilled operators. In this case, transient elastography, a non-invasive tool that can indirectly reflect the pathological staging of liver fibrosis and the degree of HVPG, has been widely verified (22, 23). Throughout our follow-up process, LSM predicted the AUC of total rebleeding events was 0.837, which is an exciting result, indicating that this parameter has excellent predictive performance. Indeed, LSM also demonstrated excellent performance in another study on non-alcoholic fatty liver disease (NAFLD) and primary biliary cirrhosis (PBC) (24–26). However, the clinical outcome of these studies focused on death or liver-related events, rather than a certain type of complication, such as recurrent bleeding from cirrhosis. Our research verifies that LSM can reliably predict rebleeding events in hepatitis B-related cirrhosis and further expands the spectrum of diseases in which LSM can be used to predict rebleeding.

TABLE 5 | The predictive value of various parameters at different time points.

	Variable	AUROC	*P*-value	Cut-off point	Sensitivity (%)	Specificity (%)
6 weeks	Child–Pugh	0.629 (0.557–0.697)	0.0834	6	66.67	67.6
	MELD	0.582 (0.510–0.653)	0.2968	12.91	40	87.15
	MELD-Na	0.569 (0.496–0.640)	0.4259	6.853	53.33	69.83
	ALBI	0.656 (0.585–0.723)	0.0696	−1.889	73.33	64.25
	APRI	0.624 (0.552–0.692)	0.1417	2.295	60	73.18
	FIB-4	0.622 (0.549–0.690)	0.0964	5.623	66.67	55.87
	King's score	0.632 (0.560–0.700)	0.085	51.694	53.33	71.51
	GUCI	0.632 (0.560–0.700)	0.1049	2.915	60	72.07
	FibroIndex	0.549 (0.476–0.621)	0.5362	2.957	33.33	81.01
	Forns score	0.657 (0.586–0.724)	0.0225	8.999	86.67	48.04
	LSM	0.698 (0.628–0.762)	0.0072	17.77	66.67	72.63
3 months	Child–Pugh	0.583 (0.510–0.653)	0.1034	6	50	68.12
	MELD	0.522 (0.449–0.594)	0.6838	8.78	52.94	37.5
	MELD-Na	0.523 (0.451–0.595)	0.6732	5.282	67.65	43.13
	ALBI	0.561 (0.488–0.632)	0.2752	−1.734	41.18	76.25
	APRI	0.652 (0.581–0.719)	0.0024	2	58.82	71.25
	FIB-4	0.628 (0.555–0.696)	0.0085	5.623	64.71	58.13
	King's score	0.630 (0.558–0.698)	0.0071	14.796	100	23.75
	GUCI	0.646 (0.574–0.713)	0.0029	2.915	52.94	74.37
	FibroIndex	0.607 (0.534–0.676)	0.033	2.628	64.71	57.5
	Forns score	0.613 (0.541–0.682)	0.0236	8.515	82.35	40.63
	LSM	0.732 (0.664–0.793)	<0.0001	17.77	67.65	77.50
1 year	Child–Pugh	0.618 (0.546–0.687)	0.0026	6	45.12	72.32
	MELD	0.604 (0.532–0.674)	0.0108	10.36	50	69.64
	MELD-Na	0.574 (0.501–0.644)	0.0822	3.849	58.54	58.04
	ALBI	0.649 (0.578–0.716)	0.0002	−2.045	64.63	64.29
	APRI	0.686 (0.616–0.751)	<0.0001	2	53.66	80.36
	FIB-4	0.666 (0.595–0.732)	<0.0001	5.554	63.41	65.18
	King's score	0.695 (0.625–0.759)	<0.0001	28.432	75.61	55.36
	GUCI	0.693 (0.623–0.757)	<0.0001	2.62	53.66	82.14
	FibroIndex	0.725 (0.656–0.786)	<0.0001	2.62376	70.73	69.64
	Forns score	0.675 (0.604–0.740)	<0.0001	9.133	70.73	59.82
	LSM	0.735 (0.667–0.796)	<0.0001	14.61	73.17	68.75

AUROC, Area under receiver operating characteristic.

Numerous attempts have been made to develop non-invasive fibrosis scores to evaluate the degree of hepatitis B-related liver fibrosis, and these non-invasive fibrosis scores are highly accurate in diagnosing liver fibrosis grading (27), which indirectly reflects the degree of portal hypertension. In our study, six non-invasive fibrosis models were included. Among them, GUCI had the largest AUC (0.686) for predicting rebleed events during the entire follow-up episode, which was similar to the AUC of APRI (0.681). The end point of our study was different, constituting the event of clinical rebleeding, not the pathological stage of liver fibrosis, so the area under the ROC curve we calculated was small. Indeed, a study evaluating the prognostic effect of non-invasive fibrosis scores in patients with non-alcoholic fatty liver disease has pointed out that these scores can help identify patients with NAFLD who are at increased risk for liver-related complications or death (6). The main connection between the above research and this research is to propose that the non-invasive fibrosis score can be related to the prognosis of patients with liver disease rather than just reflecting the degree of liver fibrosis. However, the accuracy of the non-invasive fibrosis score in predicting rebleeding events is not as high as that of LSM. The reason can be found in another article describing LSM as superior to non-invasive fibrosis in the staging of liver fibrosis (28). We tried to combine LSM and non-invasive fibrosis scores to predict rebleeding events, but the combined model did not significantly improve the diagnostic performance. Some studies have confirmed that the abovementioned combined model can improve the accuracy of diagnosing liver fibrosis and avoid liver biopsy (29, 30). In addition to TE, two-dimensional shear wave elastography is a promising marker in predicting esophageal varices and portal hypertension with high accuracy (31–33).

Interestingly, when we drew the time-dependent ROC curve, the scores curves, such as those for the APRI, King's score, and GUCI, almost fit, and their AUCs were also similar. These three scores were included in the two variables of AST and PLT. In our entire cohort, the variation range of AST was not as large as that of PLT, so when calculating the score, the weight of PLT change was high. On the other hand, these three scores showed similar performance in diagnosing liver fibrosis (10, 27), which can also give such results.

In our cohort, we included the scores that reflect the functional status of the liver in the analysis and found that ALBI had the highest accuracy among these scores. Furthermore, our results are consistent with previous studies (34), and ALBI is

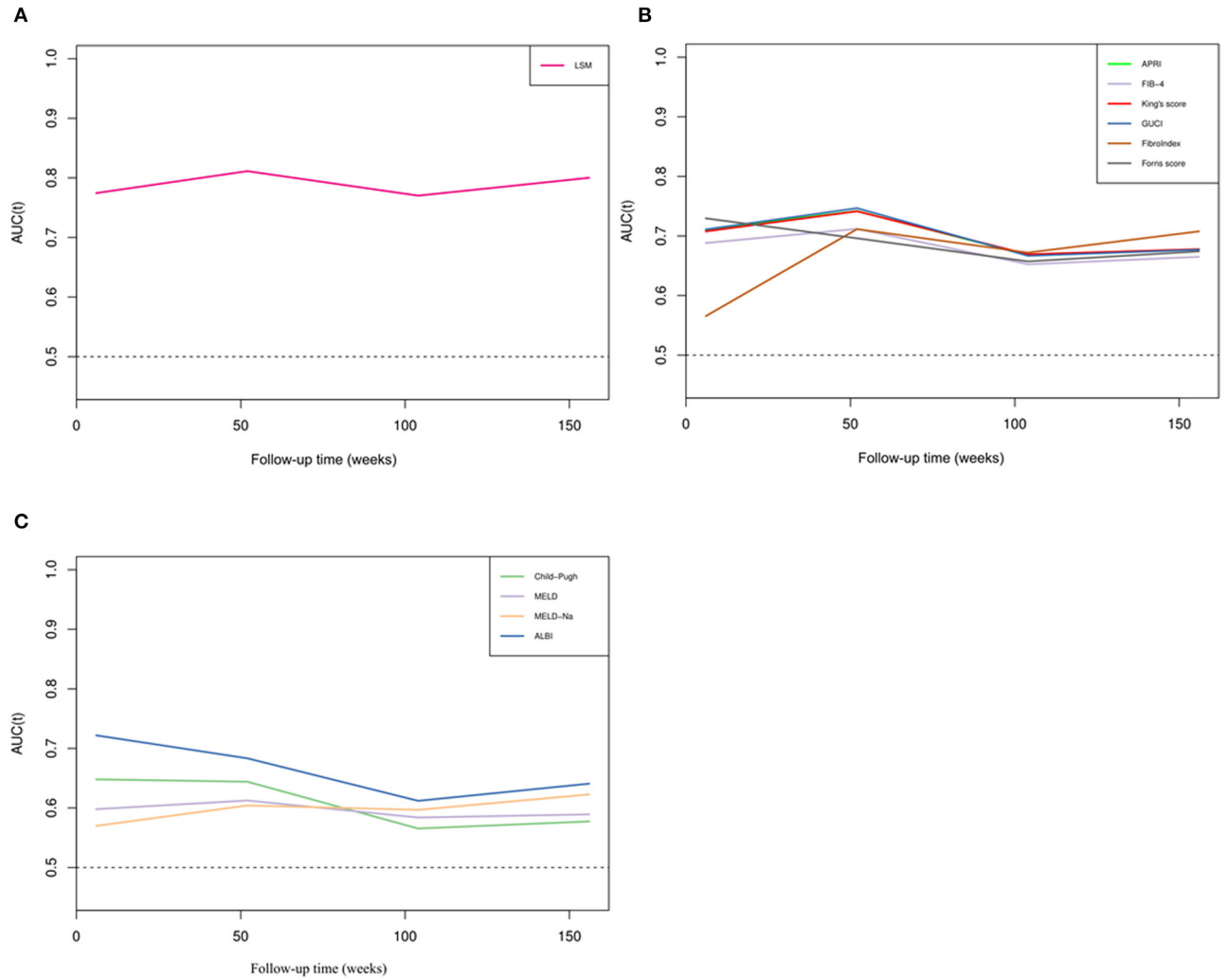


FIGURE 3 | Time-dependent ROC curves of different parameters. **(A)** Time-dependent ROC curve of LSM. **(B)** Time-dependent ROC curves of non-invasive fibrosis scores. **(C)** Time-dependent ROC curves of liver function scores.

not just a prognostic score for primary hepatocellular carcinoma. However, the predictive performance of ALBI was lower than that of LSM in our study, and its performance in predicting rebleeding events was lower than that in predicting survival (35). In fact, a study suggested that the ALBI score can reflect liver fibrosis and portal pressure in cirrhosis (36). Considering that the patients we included were Child grade A or B, the subjectivity and ceiling effect of the Child score were amplified at this time, and the change spectrum of MELD and MELD-Na was not large, making these predictions less accurate than the ALBI score.

Antiviral drugs and the NSBB drugs are the first-line treatments recommended by the guidelines (12). Among the people who meet the criteria for the guidelines, the incidence of rebleeding events is significantly lower than that of those who do not meet the recommended guidelines (37). Treatment with oral antiviral drugs in patients with HBV cirrhosis is effective in improving liver function and survival in decompensated cirrhosis, and several studies have demonstrated that they are able to reverse liver fibrosis (38). We also found that patients who used NSBB had a lower rebleeding rate than those who did not. This effect can be interpreted as reducing portal blood flow and lowering portal pressure. Moreover, NSBB could also reduce overall mortality or rebleeding (39, 40).

The major limitation of this study lies in the potentially limited external validity of the results for different populations and settings. Since our study is based on a single-center cohort in China, the results may need to be verified by international multi-center trials. Another limitation is that our study failed to record the change in LSM during the follow-up process and thus lacked verification of the change in LSM. However, our rigorous study design concluded that the baseline LSM has excellent predictive performance. Finally, most patients were in Child–Pugh class A, suggesting that the number of patients with late

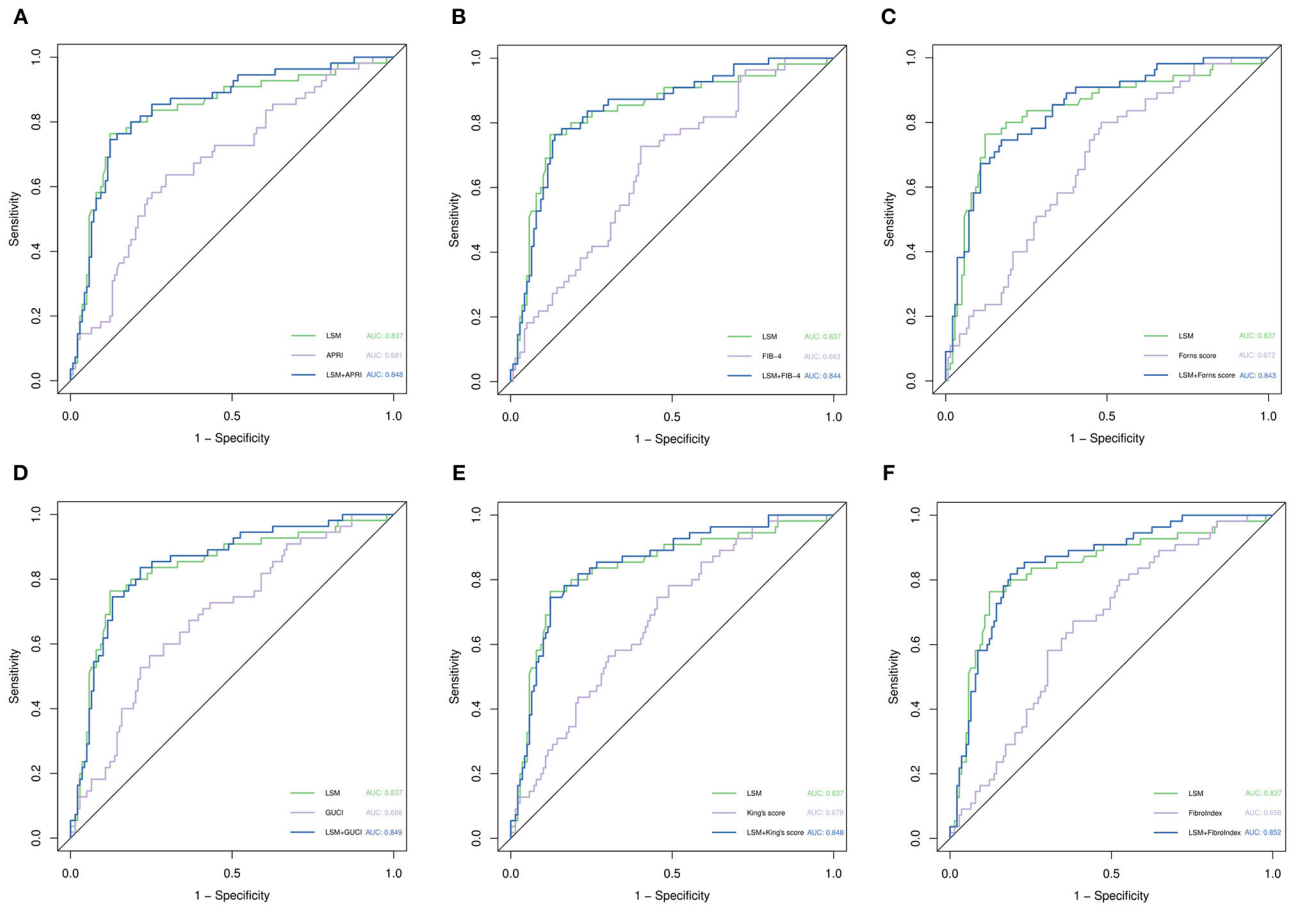


FIGURE 4 | ROC curve analysis for predicting rebleeding by various parameters. **(A)** Comparison of LSM, APRI, and their combined ROC curves. **(B)** Comparison of LSM, FIB-4, and their combined ROC curves. **(C)** Comparison of LSM, Forns score, and their combined ROC curves. **(D)** Comparison of LSM, GUCI, and their combined ROC curves. **(E)** Comparison of LSM, King's score, and their combined ROC curves. **(F)** Comparison of LSM, Fibrosis index, and their combined ROC curves. *p*-values are from the DeLong test (41).

decompensated cirrhosis is relatively low. Thus, our findings may not be readily applicable in a population predominantly with advanced decompensated cirrhosis.

In conclusion, our study demonstrates that LSM seems to be a promising parameter for predicting rebleeding in patients with hepatitis B-related cirrhosis. At the same time, compared with six included non-invasive fibrosis scores and four liver function scores, the prediction performance of LSM was significantly reliable. It is worth emphasizing that different clinical characteristics will also affect the prognosis of patients. However, after the combination of LSM and the non-invasive fibrosis score, the performance of predicting rebleeding cannot be improved further. Hence, we do not recommend such time-consuming work in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of the First Affiliated Hospital of Nanchang University (No. 2015-1206). Written informed consent to participate in this study was provided by the participants.

AUTHOR CONTRIBUTIONS

LL and YN contributed equally to this study. LL designed and wrote the original draft. YN analyzed the data and wrote the manuscript. YZ and QL collected the data. XZ critically revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the National Natural Science Foundation of China (grant number: 81960120) and Gan-Po Talent 555 Project of Jiangxi Province [GCZ (2012)-1].

ACKNOWLEDGMENTS

We would like to thank the National Natural Science Foundation of China and the Gan-Po Talent 555 Project of Jiangxi Province for financial support.

REFERENCES

1. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet.* (2014) 383:1749-61. doi: 10.1016/S0140-6736(14)60121-5
2. Baiges A, Hernández-Gea V, Bosch J. Pharmacologic prevention of variceal bleeding and rebleeding. *Hepatol Int.* (2018) 12(Suppl 1):68-80. doi: 10.1007/s12072-017-9833-y
3. Chalasani N, Kahi C, Francois F, Pinto A, Marathe A, Bini EJ, et al. Improved patient survival after acute variceal bleeding: a multicenter, cohort study. *Am J Gastroenterol.* (2003) 98:653-9. doi: 10.1111/j.1572-0241.2003.07294.x
4. Reverter E, Tandon P, Augustin S, Turon F, Casu S, Bastiampillai R, et al. A MELD-based model to determine risk of mortality among patients with acute variceal bleeding. *Gastroenterology.* (2014) 146:412-9. doi: 10.1053/j.gastro.2013.10.018
5. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: clinical prediction rules and blood-based biomarkers. *J Hepatol.* (2018) 68:305-15. doi: 10.1016/j.jhep.2017.11.013
6. Angulo P, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology.* (2013) 145:782-9.e4. doi: 10.1053/j.gastro.2013.06.057
7. Iwata K, Enomoto H, Nishiguchi S, Aizawa N, Sakai Y, Iwata Y, et al. Serum zinc value in patients with hepatitis virus-related chronic liver disease: association with the histological degree of liver fibrosis and with the severity of varices in compensated cirrhosis. *J Clin Biochem Nutr.* (2014) 55:147-52. doi: 10.3164/jcbn.14-33
8. Pickhardt PJ, Graffy PM, Said A, Jones D, Welsh B, Zea R, et al. Multiparametric CT for noninvasive staging of hepatitis C virus-related liver fibrosis: correlation with the histopathologic fibrosis score. *AJR Am J Roentgenol.* (2019) 212:547-53. doi: 10.2214/AJR.18.20284
9. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut.* (2010) 59:1265-9. doi: 10.1136/gut.2010.216077
10. Dong X-Q, Wu Z, Zhao H, Wang G-Q. Evaluation and comparison of thirty noninvasive models for diagnosing liver fibrosis in Chinese hepatitis B patients. *J Viral Hepat.* (2019) 26:297-307. doi: 10.1111/jvh.13031
11. Boursier J, Vergniol J, Guillet A, Hiriart J-B, Lannes A, Le Bail B, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. *J Hepatol.* (2016) 65:570-8. doi: 10.1016/j.jhep.2016.04.023
12. de Franchis R. Expanding consensus in portal hypertension: report of the Baveno VI Consensus Workshop: stratifying risk and individualizing care for portal hypertension. *J Hepatol.* (2015) 63:743-52. doi: 10.1016/j.jhep.2015.05.022
13. Bertrais S, Boursier J, Ducancelle A, Oberti F, Fouchard-Hubert I, Moal V, et al. Prognostic durability of liver fibrosis tests and improvement in predictive performance for mortality by combining tests. *J Gastroenterol Hepatol.* (2017) 32:1240-9. doi: 10.1111/jgh.13668
14. Liu K, Wong VWS, Liang LY, Lui GCY, Chan HLY, Wong GLH. Clinical outcomes and management of patients with chronic hepatitis B and liver stiffness measurement in the grey zone. *Liver Int.* (2019) 39:494-502. doi: 10.1111/liv.14001
15. Klibansky DA, Mehta SH, Curry M, Nasser I, Challies T, Afdhal NH. Transient elastography for predicting clinical outcomes in patients with chronic liver disease. *J Viral Hepat.* (2012) 19:e184-93. doi: 10.1111/j.1365-2893.2011.01493.x
16. Serenari M, Han K-H, Ravaioli F, Kim S-U, Cucchetti A, Han D-H, et al. A nomogram based on liver stiffness predicts postoperative complications in patients with hepatocellular carcinoma. *J Hepatol.* (2020) 73:855-62. doi: 10.1016/j.jhep.2020.04.032
17. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine.* (2012) 30:2212-9. doi: 10.1016/j.vaccine.2011.12.116
18. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet.* (2017) 390:1151-210. doi: 10.1016/S0140-6736(17)32152-9
19. Simón-Talero M, Roccarina D, Martínez J, Lampichler K, Baiges A, Low G, et al. Association between portosystemic shunts and increased complications and mortality in patients with cirrhosis. *Gastroenterology.* (2018) 154:1694-705. doi: 10.1053/j.gastro.2018.01.028
20. Praktiknjo M, Simón-Talero M, Römer J, Roccarina D, Martínez J, Lampichler K, et al. Total area of spontaneous portosystemic shunts independently predicts hepatic encephalopathy and mortality in liver cirrhosis. *J Hepatol.* (2020) 72:1140-50. doi: 10.1016/j.jhep.2019.12.021
21. Moitinho E, Escorsell A, Bandi JC, Salmerón JM, García-Pagán JC, Rodés J, et al. Prognostic value of early measurements of portal pressure in acute variceal bleeding. *Gastroenterology.* (1999) 117:626-31. doi: 10.1016/S0016-5085(99)70455-5
22. Abraldes JG, Bureau C, Stefanescu H, Augustin S, Ney M, Blasco H, et al. Noninvasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: the "Anticipate" study. *Hepatology.* (2016) 64:2173-84. doi: 10.1002/hep.28824
23. Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology.* (2005) 128:343-50. doi: 10.1053/j.gastro.2004.11.018
24. Petta S, Sebastiani G, Viganò M, Ampuero J, Wai-Sun Wong V, Boursier J, et al. Monitoring occurrence of liver-related events and survival by transient elastography in patients with nonalcoholic fatty liver disease and compensated advanced chronic liver disease. *Clin Gastroenterol Hepatol.* (2020) 19:806–15.e5. doi: 10.1016/j.cgh.2020.06.045
25. Corpechot C, Carrat F, Poujol-Robert A, Gaouar F, Wendum D, Chazouillères O, et al. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. *Hepatology.* (2012) 56:198-208. doi: 10.1002/hep.25599
26. Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology.* (2013) 57:1357-65. doi: 10.1002/hep.26156
27. Sharma S, Khalili K, Nguyen GC. Non-invasive diagnosis of advanced fibrosis and cirrhosis. *World J Gastroenterol.* (2014) 20:16820-30. doi: 10.3748/wjg.v20.i45.16820
28. Staufer K, Halilbasic E, Spindelboeck W, Eilenberg M, Prager G, Stadlbauer V, et al. Evaluation and comparison of six noninvasive tests for prediction of significant or advanced fibrosis in nonalcoholic fatty liver disease. *United Eur Gastroenterol J.* (2019) 7:1113-23. doi: 10.1177/2050640619865133
29. Newsome PN, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan W-K, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol.* (2020) 5:362-73. doi: 10.1016/S2468-1253(19)30383-8
30. Chan W-K, Treeprasertsuk S, Goh GB-B, Fan J-G, Song MJ, Charatcharoenwitthaya P, et al. Optimizing use of nonalcoholic fatty liver disease fibrosis score, fibrosis-4 score, and liver stiffness measurement to identify patients with advanced fibrosis. *Clin Gastroenterol Hepatol.* (2019) 17:2570-80. doi: 10.1016/j.cgh.2019.03.006

31. Trebicka J, Gu W, de Ledinghen V, Aubé C, Krag A, Praktiknjo M, et al. Two-dimensional shear wave elastography predicts survival in advanced chronic liver disease. *Gut.* (2021). doi: 10.1055/s-0040-1721979
32. Jansen C, Bogs C, Verlinden W, Thiele M, Möller P, Görtzen J, et al. Shear-wave elastography of the liver and spleen identifies clinically significant portal hypertension: a prospective multicentre study. *Liver Int.* (2017) 37:396-405. doi: 10.1111/liv.13243
33. Jansen C, Bogs C, Verlinden W, Thiele M, Möller P, Görtzen J, et al. Algorithm to rule out clinically significant portal hypertension combining Shear-wave elastography of liver and spleen: a prospective multicentre study. *Gut.* (2016) 65:1057-8. doi: 10.1136/gutjnl-2016-311536
34. Oikonomou T, Goulis L, Doumtsis P, Tzoumari T, Akriviadis E, Cholongitas E. ALBI and PALBI grades are associated with the outcome of patients with stable decompensated cirrhosis. *Ann Hepatol.* (2019) 18:126-36. doi: 10.5604/01.3001.0012.7904
35. Fujita K, Oura K, Yoneyama H, Shi T, Takuma K, Nakahara M, et al. Albumin-bilirubin score indicates liver fibrosis staging and prognosis in patients with chronic hepatitis C. *Hepatol Res.* (2019) 49:731-42. doi: 10.1111/hepr.13333
36. Hsieh YC, Lee KC, Wang YW, Yang YY, Hou MC, Huo TI, et al. Correlation and prognostic accuracy between noninvasive liver fibrosismarkers and portal pressure in cirrhosis: role of ALBI score. *PLoS ONE.* (2018) 13:e0208903. doi: 10.1371/journal.pone.0208903
37. Cao Z, Liu Y, Wang S, Lu X, Yin S, Jiang S, et al. The impact of HBV flare on the outcome of HBV-related decompensated cirrhosis patients with bacterial infection. *Liver Int.* (2019) 39:1943-53. doi: 10.1111/liv.14176
38. Calvaruso V, Craxì A. Regression of fibrosis after HBV antiviral therapy. Is cirrhosis reversible? *Liver Int.* (2014) 34(Suppl 1):85-90. doi: 10.1111/liv.12395
39. Villanueva C, Albillos A, Genescà J, Garcia-Pagan JC, Calleja JL, Aracil C, et al. β blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet.* (2019) 393:1597-608. doi: 10.1016/S0140-6736(18)31875-0
40. Lv Y, Qi X, He C, Wang Z, Yin Z, Niu J, et al. Covered TIPS versus endoscopic band ligation plus propranolol for the prevention of variceal rebleeding in cirrhotic patients with portal vein thrombosis: a randomised controlled trial. *Gut.* (2018) 67:2156-68. doi: 10.1136/gutjnl-2017-314634
41. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics.* (1988) 44:837–45.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 29 July 2021
doi: 10.3389/fphys.2021.718783

Bile Acids, Liver Cirrhosis, and Extrahepatic Vascular Dysfunction

Tilman Sauerbruch[1], Martin Hennenberg[2], Jonel Trebicka[3] and Ulrich Beuers[4]*

[1]Department of Internal Medicine I, University of Bonn, Bonn, Germany, [2]Department of Urology I, University Hospital, LMU Munich, Munich, Germany, [3]Translational Hepatology, Medical Department, University of Frankfurt, Frankfurt, Germany, [4]Department of Gastroenterology and Hepatology, Amsterdam University Medical Centers, location AMC, Amsterdam, Netherlands

The bile acid pool with its individual bile acids (BA) is modulated in the enterohepatic circulation by the liver as the primary site of synthesis, the motility of the gallbladder and of the intestinal tract, as well as by bacterial enzymes in the intestine. The nuclear receptor farnesoid X receptor (FXR) and Gpbar1 (TGR5) are important set screws in this process. Bile acids have a vasodilatory effect, at least according to *in vitro* studies. The present review examines the question of the extent to which the increase in bile acids in plasma could be responsible for the hyperdynamic circulatory disturbance of liver cirrhosis and whether modulation of the bile acid pool, for example, *via* administration of ursodeoxycholic acid (UDCA) or *via* modulation of the dysbiosis present in liver cirrhosis could influence the hemodynamic disorder of liver cirrhosis. According to our analysis, the evidence for this is limited. Long-term studies on this question are lacking.

Keywords: bile acids, liver cirrhosis, portal hypertension, microbiome, vasodilation

OPEN ACCESS

Edited by:
Steven Dooley,
University of Heidelberg, Germany

Reviewed by:
Honglei Weng,
Heidelberg University, Germany
Matthias J. Bahr,
Sana Kliniken Lübeck, Germany

****Correspondence:***
Tilman Sauerbruch
sauerbruch@uni-bonn.de

Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: *01 June 2021*
Accepted: *29 June 2021*
Published: *29 July 2021*

Citation:
Sauerbruch T, Hennenberg M, Trebicka J and Beuers U (2021) Bile Acids, Liver Cirrhosis, and Extrahepatic Vascular Dysfunction. Front. Physiol. 12:718783. doi: 10.3389/fphys.2021.718783

INTRODUCTION

In liver cirrhosis, there is a marked change in liver perfusion and extrahepatic hemodynamics. The consequences are severe: formation of collaterals bypassing the liver, as well as splanchnic and peripheral systemic vasodilatation with a hyperdynamic circulation and central hypovolemia (Schrier et al., 1988; Maroto et al., 1994; Blendis and Wong, 2001; Moller et al., 2011). These alterations on the one hand increase portal pressure and on the other hand fuel dysfunction of other organs such as of kidneys (Wong et al., 2020) and lungs (Goldberg and Fallon, 2015). The heart may be involved too, in the complex of hyperdynamic circulatory dysfunction or due to cirrhotic cardiomyopathy. To this, the body counter-reacts with activation of the renin-angiotensin system (Bosch et al., 1980) and release of other vasoconstrictors such as catecholamines or vasopressin (Henriksen et al., 1984; **Figure 1**). This may lead to a vicious circle supporting generation of ascites, development of a hepatorenal syndrome, and life-threating variceal bleeding (Sola and Gines, 2015). The hemodynamic changes increase with the degree of liver dysfunction, but are not specific to the particular cause of cirrhosis. They can equally be detected in different animal models (Heller et al., 2005; Hennenberg et al., 2009a).

In addition to initial chronic liver inflammation causing alterations in the intrahepatic blood flow, the intestine has become a focus of pathophysiologic consideration in recent years. Dysbiosis and a disturbed intestinal barrier allow the translocation of molecules, microorganisms, or their products into the interior of the body, where they act as inflammatory stimuli, especially within the liver (Fairfield and Schnabl, 2021).

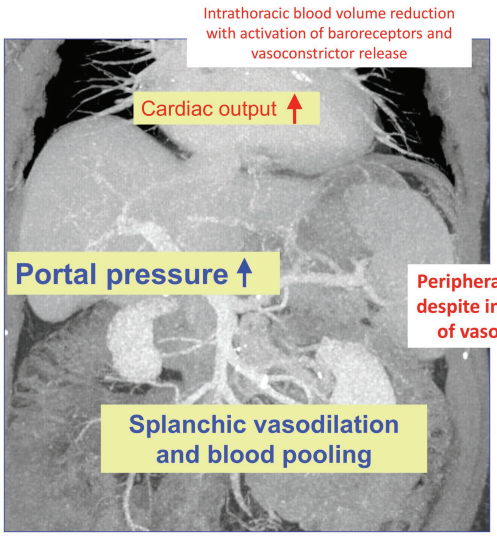


FIGURE 1 | Changes in hemodynamics in liver cirrhosis: As decompensation progresses and portal vein pressure increases, there is initially splanchnic vasodilation with pooling of abdominal blood volume and concomitant reduction in intrathoracic blood volume. Later, peripheral vasodilation is also observed. This results in hyperdynamic circulatory dysfunction with an increase in cardiac output. The compensatory release of vasoconstrictors (partly *via* activation of baroreceptors) cannot completely resolve the hemodynamic dysfunction partly because the vessels have an impaired response to vasoconstrictor stimuli, partly due to formation of vasodilators such as nitric oxide (NO).

Here, we would like to deal mainly with one group of molecules within these complex systems, namely bile acids (BA) and their possible role in the development of changes in hemodynamics in the context of liver cirrhosis with a focus on the extrahepatic vessels. Primary bile acids are formed in the hepatocytes and then underlie an enterohepatic circulation. Thereby, they are subjected to strong intestinal modification. Thus, chronic liver diseases intervene alongside with the intestinal environment in bile acid composition and their pool (Ridlon et al., 2015; Fickert and Wagner, 2017). Unfortunately, we have only incomplete knowledge about the exact distribution of the various bile acids in different organs and compartments. During the course of liver disease, considerable changes occur. Model calculations attempt to approximate this changing process (Voronova et al., 2020). Bile acids have distinct functions. They are breakdown products of cholesterol and they are detergents that play an important role in fat digestion and vitamin intake. Furthermore, it has been shown in recent decades that bile acids are hormones, ligands for receptors, and transcription factors (Hofmann and Hagey, 2014). Bile acids also affect vascular function. This we would like to discuss in the context of liver cirrhosis.

HEMODYNAMIC ALTERATIONS IN LIVER CIRRHOSIS

Roberto Groszman and his group were one of the first to show that there is a paradox in liver cirrhosis: an increased vasoconstriction of the intrahepatic microcirculation with a concomitant vasodilation outside the liver (Iwakiri and Groszmann, 2007) concerning first splanchnic and then peripheral systemic vessels. They and others attributed this quite substantially to decreased formation of the vasodilator nitric oxide (NO) within the liver (Iwakiri et al., 2014) and increased NO generation outside the liver.

Indeed, one finds an increasing excretion of nitrite/nitrate as a surrogate marker for NO formation in humans parallel to worsening of liver function (Heller et al., 1999a). By contrast, stimulation of endothelial NO synthesis using an enhancer of endothelial NO synthase (eNOS) transcription lowers intrahepatic resistance and *in situ* perfusion pressure in cirrhotic rat livers (Biecker et al., 2008). Similarly, the beneficial effect of atorvastatin in liver cirrhosis is associated with increased activity of eNOS (Trebicka et al., 2007). These findings underscore the hypothesis of impaired intrahepatic NO formation as a cause of intrahepatic resistance augmentation in addition to structural remodeling of the liver. Cells involved in the regulation of the intrahepatic vascular bed include endothelial cells, smooth muscle cells, and hepatic stellate cells (HSCs, a form of intrahepatic pericytes). It is assumed that sinusoidal endothelial cells change their phenotype on the way to cirrhosis and produce less NO causing a shortage of vasodilating stimuli on smooth muscle cells and HSC (Iwakiri et al., 2014).

Regarding extrahepatic vasodilation (**Figure 1**), a number of mechanisms are discussed, originating from different structures of the vessels – such as adventitia, smooth muscle, and endothelium – and its neuronal supply. These may influence each other paracrinally or respond to systemic and nerval stimuli.

The essential role of NO-mediated splanchnic and systemic vasodilation and hyporeactivity to vasoconstrictors in liver cirrhosis was highlighted in the 1990s using animal models (Wiest and Groszmann, 2002). NO is produced *via* different synthases (endothelial, eNOS; neuronal NO synthase, nNOS; and inducible NO synthase, iNOS). According to the classical hypothesis of Vallance and Moncada (Bhagat and Vallance, 1996; Vallance et al., 1997), inflammatory stimuli (e.g., endotoxin) were thought to upregulate iNOS in vascular smooth muscle cells in liver cirrhosis – at least for initiation of vasodilatation. Animal experiments, however, pointed to a much more important role of eNOS (Wiest et al., 1999b; Wiest and Groszmann, 2002). According to these findings, NO is formed in the vascular endothelium and causes cGMP-mediated relaxation of the adjacent smooth muscle cell leading to vascular dilatation. The upregulation of eNOS seems to depend more on the degree of portal hypertension and less on the extent of chronic inflammation. Shear stress is hypothesized as a major pathomechanism, but other not fully elucidated molecular mechanisms are also suggested. eNOS may also be upregulated by bacterial translocation and proinflammatory cytokines (Wiest et al., 1999a). Despite these findings, there is indirect evidence, that iNOS also contributes to peripheral vasodilation in humans with decompensated liver cirrhosis (Ferguson et al., 2006). Finally, increased expression of neuronal NOS was also found, suggesting additional nerve-mediated NO formation.

However, findings in this direction are sparse (Moll-Kaufmann et al., 1998). This pathophysiological role of NO in vasodilation unraveled in experimental animals with liver cirrhosis has also been confirmed in humans to a certain degree (Albillos et al., 1995; Battista et al., 1997; Heller et al., 1999a; Helmy et al., 2003).

In liver cirrhosis, a well-characterized activation of the renin-angiotensin-aldosterone system (RAAS) occurs, as mentioned above, in part as response to systemic vasodilation, especially in patients with ascites (Bosch et al., 1980; Helmy et al., 2000; Schepke et al., 2001b). Notably, both in animal models and in humans, an upregulation of ACE2 and of the Mas receptor in the splanchnic vessels result in locally increased angiotensin (1–7) levels – derived from the increased circulating angiotensin II – generating NO mediated *via* the MAS receptor. Thus, the alternate arm of RAAS in patients with liver cirrhosis can cause vasodilatation (Grace et al., 2013).

Nitric oxide is certainly not the only factor driving vasodilation in liver cirrhosis. Many other vasodilating molecules, such as adrenomedullin (mediated by inflammatory stimuli) carbon monoxide (CO), formed by heme oxygenase-1 (endothelial), prostacyclins (PGI_2), endothelial derived epoxyeicosatrienoic (EET) acids , cannabinoids (such as anandamide), or glucagon show elevated plasma levels in liver cirrhosis (Hennenberg et al., 2008; Di Pascoli et al., 2017).

However, increased formation of vasodilators is not the whole explanation for altered hemodynamics in liver cirrhosis. Human hepatic arteries without endothelium from patients with liver cirrhosis obtained during liver transplantation respond significantly worse to vasoconstrictors (α_1- and α_2-adrenergic agonists) compared with corresponding vessels from donors, even after pharmacological blockade of NOS (**Figure 2**). Receptor-independent membrane depolarization with potassium chloride, direct stimulation of the phospholipase C/inositol-1,4,5-trisphosphate (PLC/IP_3) axis, or the G protein pathways led to a similar contraction, so that the contractile proteins were apparently not affected to a major extent by the cirrhotic status (Heller et al., 1999b; Schepke et al., 2001a). Arterial hypocontractility to α_1-adrenergic agonists can also be consistently demonstrated in animal experiments of liver cirrhosis (Hennenberg et al., 2009b), even after removal of the endothelium and blockade of NO synthase. Here, a defective activation of Rho-kinase (ROCK) has been found (Hennenberg et al., 2006), possibly caused by an increased binding of GRK-2/β-arrestin-2 to the AT_1 receptor (Hennenberg et al., 2007). Dysregulation of the neurotransmitter NPY, which enhances α_1-adrenergic vasoconstriction, is also noted in the cirrhotic rat (Moleda et al., 2011). Decreased transcription of vasoconstrictor receptors does not seem to play a role (Neef et al., 2003).

The progressive hepatic impairment with alteration of hemodynamics is additionally accompanied by increasing systemic inflammation (Praktiknjo et al., 2020a), which may be complicated by an acute event leading to rapid failure of one or more organs, a situation now coined acute on chronic liver failure (ACLF; Moreau et al., 2013; Arroyo et al., 2021). During deterioration of disease phenotypic changes of the immune cells occur (Weiss et al., 2020) together with an activation of the inflammasomes (Praktiknjo et al., 2020b; Monteiro et al., 2021) and increases of different cytokines, e.g., Il-6, Il-17, IL-1: a condition we know from subclinical inflammation and sepsis due to other causes (Arina and Singer, 2021). Recent research has increasingly focused on the gut as a major source and trigger of this stimulation of the immune system. Intestinal dysbiosis (a change of the composition of the microorganisms and their diversity) and disturbance of the gut barrier to microorganisms and their metabolites are discussed as major causes (Trebicka et al., 2021). The vasodilatory co-reaction of the vessels in such an inflammatory state has been known for a long

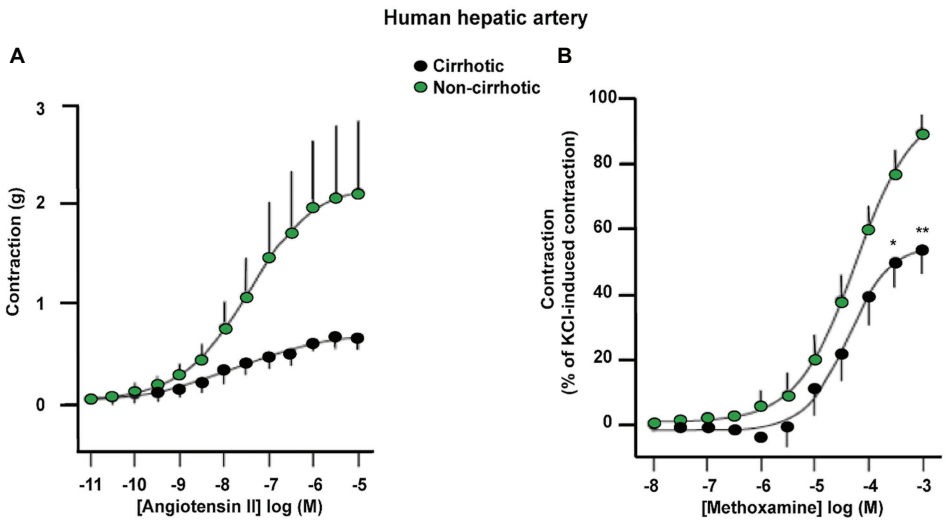


FIGURE 2 | The arterial hepatic vessels of patients who underwent liver transplantation show an impaired vasoconstrictor response compared with the vessels from donors: **(A)** to angiotensin II and **(B)** to methoxamine. Modified according to Heller et al. (1999b) and Schepke et al. (2001a).

time, and the question arises to what extent patient's hemodynamics is altered by inflammatory changes coming from the gut.

Since bile acids (i) are in constant exchange between the hepatic, biliary, intestinal and – to a much lesser degree- plasma compartments *via* the enterohepatic circulation and spill-over into the systematic circulation; (ii) are modified *via* intestinal microorganisms; (iii) interact with immune cells; and (iv) also influence vascular contraction, it is interesting to consider a role of these molecules in the dynamics of the systemic circulation.

PHYSIOLOGY OF VASOCONSTRICTION AND VASODILATATION

Vasocontraction and relaxation essentially contribute to dynamic changes in vessel diameter. It is largely based on dynamic phosphorylation and dephosphorylation of contractile proteins in smooth muscle cells. This in turn depends on calcium homeostasis in the cell, controlled on the one hand by release from intracellular calcium stores, and on the other hand by calcium channels of the plasma membrane, both taking part in a cascade of intracellular signaling toward phosphorylation of myosin light chains (MLC; Hennenberg et al., 2008). This cascade is activated by binding of vasoconstrictors, including catecholamines or vasoactive peptides such as angiotensin II or endothelin-1, to their cognate, G protein coupled receptors. Calcium-independent mechanisms are activated in parallel, which essentially act *via* modulation of MLC phosphorylation as well. Essentials are the RhoA/ROCK pathway and the PLC-DAG/PKC pathway (Hennenberg et al., 2008; Touyz et al., 2018). Thus, ROCK activation blocks MLC phosphatase and therewith facilitates contraction which needs phosphorylation of the MLC. Meanwhile, other non-RhoA GTPases are also discussed in smooth muscle cell regulation, such as Rac1 (Li et al., 2020). Important vasodilatory pathways work *via* formation of the two cyclic nucleotides cGMP and cAMP in smooth muscle cells. Both pathways involve activation of MLC phosphatase and decreases in cytosolic calcium concentrations, with the latter being based on regulation of voltage-gated calcium channels, calcium-dependent (BK_{Ca}), ATP-sensitive (K_{ATP}), and other potassium channels, sarco−/endoplasmic reticulum Ca^{2+} ATPase, as well as other targets. cGMP formation results from paracrine guanylyl cyclase activation by NO, following either acetylcholine- or shear stress-induced eNOS activation in endothelial cells or its release from nitrergic neurotransmisssion and its immediate dissociation into smooth muscle cells. Formation of cAMP and cAMP-mediated vasodilation are induced by prostaglandins and β-adrenoceptors, which activate corresponding receptors located on vascular smooth muscle cells, thereby activating adenylyl cyclase.

Chronic conditions such as subclinical inflammation or stress can probably alter the phenotype and plasticity of smooth muscle cells *via* differential expression of contractile proteins as well as proteins involved in various above mentioned signaling cascades and calcium regulation. However, there are no good studies on the phenotypic change of the vascular smooth muscle cell for the situation of liver cirrhosis.

BILE ACID POOL, BILE ACID COMPOSITION AND THEIR REGULATION

The two primary BA, cholic acid (CA), and chenodeoxycholic acid (CDCA), are formed predominantly in the pericentral hepatocytes over several steps from cholesterol. In this process, cholesterol 7-alpha-hydroxylase (CYP7A1) is the rate-determining enzyme (classical pathway). After conjugation with glycine or taurine in the peroxisomes, the bile acids are actively transported into the bile canaliculus (Fickert and Wagner, 2017). Here, they form micelles with phosphatidylcholine and also cholesterol and enter *via* the bile ducts the duodenum. In the intestine, BA are important for fat digestion. Furthermore, as a degradation product of cholesterol, they have a pivotal function in the cholesterol homeostasis of the body (Hofmann, 2009). Re-uptake of BA occurs *via* apical and basolateral transporters of the ileal enterocytes (**Figure 3**). Around one fifth of BA is not absorbed in the ileum and passes into the colon. Here, deconjugation and dehydroxylation to secondary bile acids occur *via* bacterial intestinal enzymes. Most of these BA are passively reabsorbed by the colon and enter the circulating bile acid pool. Only a small percentage (around 5%) of the whole BA pool leaves the body every day *via* the stool, helping thereby to determine the cholesterol balance (Chiang and Ferrell, 2020). Thus, the intestine with its microbiota plays an important role in the modification and subsequent composition of the bile acid pool. After return to the liver *via* the portal venous blood BA are actively reabsorbed from the sinusoidal blood *via* basolateral transporters on the hepatocytes and, after passage through the liver cell, are reintroduced into the biliary ductal system. In this circuit, tuning of synthesis and transport occurs through nuclear receptors – primarily the nuclear farnesoid X receptor (FXR) – and hormones, mainly through fibroblast growth factor (FGF) 15 (rodent)/19 (human). FXR primarily reduces intestinal bile acid uptake and bile acid synthesis in the liver. Furthermore, it enhances bile acid biotransformation and export into bile (and back into blood when biliary secretion is impaired). Ligands for FXR are, in descending order CDCA > LCA = DCA > CA. FGF 19 is FXR-dependently formed in the ileocyte under physiological conditions and secreted into venous mesenteric blood. Reaching the liver *via* the portal vein, FGF19 binds to the hepatocellular FGF receptor 4 (FGFR4)/ß-klotho complex and suppresses bile acid formation at the level of CYP7A1, the key enzyme for bile acid synthesis. At the same time, FGF19 leads to a dilatation of the gall bladder (Kliewer and Mangelsdorf, 2015). In healthy individuals, the bile acid pool modulated in this way contains predominantly CA (around 40%), CDCA (around 40%), and DCA (around 20%). The size of the pool is about 2 to 5 g.

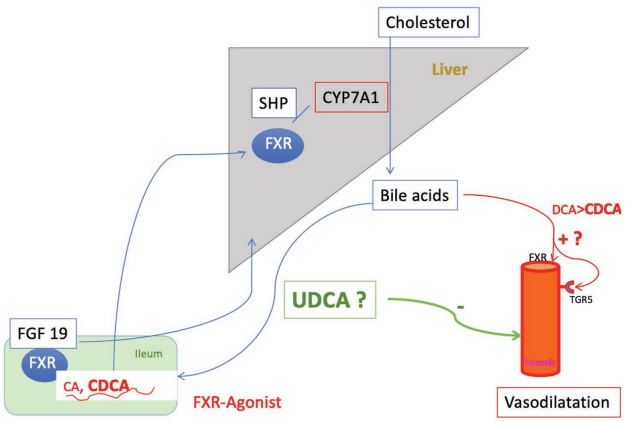


FIGURE 3 | Regulation of the bile acid pool: The primary bile acids (BA) cholic acid (CA) and chenodesoxycholic acid (CDCA), newly formed by the liver and conjugated there are secreted into the intestine and mostly reabsorbed by active transport in the ileum. Around 20% of the primary BA are metabolized in the intestine by bacterial enzyme to secondary bile acids [deoxycholic acid (DCA) and small amounts of lithocholic acid (LCA)], predominantly by loss into the colon. Here, the secondary bile acids are passively absorbed and returned to the liver. Only 5% of the bile acid pool is lost daily *via* the feces. This process of enterohepatic circulation is regulated by gallbladder and intestinal motility and nuclear farnesoid X receptor (FXR). FXR is expressed in the enterocyte of the ileum as well as in the liver. Its action is mediated on the one hand *via* small heterodimer partner (SHP) and on the other hand hormonally *via* Fibroblast growth factor (FGF) 19, which suppresses bile acid formation. Thus, the process of enterohepatic circulation is regulated to a large extent by gallbladder and intestinal motility and FXR. In liver cirrhosis, there is an overall reduction in the bile acid pool with a relative increase in CDCA. At the same time, the concentration of conjugated bile acids in the systemic circulation increases sharply, especially that of CDCA. CDCA is an activating ligand for FXR and also for TGR5 and in this way releases NO, i.e., may cause vasodilation. At the same time, however, activation of FXR has beneficial effects (e.g., downregulation of inflammation). Increased concentrations of bile acids in the plasma compartment found in liver cirrhosis might cause vasodilation either directly or *via* FXR and the G protein-coupled bile acid receptor 1 or TGR5 (see **Figure 6**). Ursodeoxycholic acid (UDCA) is almost inert for FXR and TGR5 and is hepatoprotective. Whether it is useful to modulate the bile acid pool in liver cirrhosis by administration of UDCA for this reason is discussed in the text.

The concentration of total bile acids in the biliary system is 20–40 mmol/L, in the gall bladder 50–200 mmol/L, in the small intestine 20–50 mmol/L, in the portal blood 20–50 μmol/L, and in the liver <50 μmol/L (Kliewer and Mangelsdorf, 2015; Fickert and Wagner, 2017). A spill over into the systemic circulation leads to a plasma concentration of total bile acids of about 2–8 μmol/L in the fasted state (Schalm et al., 1978; Hofmann, 1994). This situation is considerably changed in hepatic disease, especially in liver cirrhosis.

CHANGES OF BILE ACIDS IN LIVER CIRRHOSIS

The bile acid pool of a healthy 70 kg person, which circulates about 5–10 times per day enterohepatically, ranges between 2 and 5 g, as mentioned. Distributed in this pool are the primary bile acids cholic acid (CA, around 40%), CDCA (around 40%), and the secondary bile acid DCA (around 20%), see above. The secondary bile acid lithocholic acid (LCA) and the tertiary bile acid ursodeoxycholic acid (UDCA) account for only a few percent. About one-third of these bile acids are taurine conjugates and two-thirds are glycine conjugates (Carey and Duane, 1994; Hofmann, 1994; Chiang and Ferrell, 2020). In cirrhosis, the bile acid pool decreases as a function of the extent of decompensation, according to one report (Ponz de Leon et al., 1981) from just under 5 g (controls) to 4.6 g [mild cirrhosis (MC)] and just under 2 g in severe decompensated cirrhosis. This decrease affects quite substantially CA and DCA, but hardly CDCA, i.e., there is a relative accumulation of CDCA in liver cirrhosis (Vlahcevic et al., 1981; **Figure 4**). Conjugated CDCA is the major endogenous ligand for FXR. Reduction of CA and DCA may be in part caused *via* increased FXR-mediated formation of FGF 19 in the ileum (Brandl et al., 2018). FGF 19 inhibits bile acid synthesis in the liver *via* binding to the FGFR4 and inhibition of CYP7A1 or directly *via* induction of small heterodimer partner (SHP) expression in the hepatocyte.

The ratio of bile acid concentration of portal venous to peripheral venous blood is about 7–10: 1 in healthy individuals (Angelin et al., 1982), with concentrations increasing by a factor of 2–3 in postprandial portal venous blood (Angelin et al., 1982). The fluctuating concentrations in venous peripheral blood are much less pronounced (LaRusso et al., 1974; Schalm et al., 1978; Angelin et al., 1982). In contrast, in liver cirrhosis, the total bile acid concentration in the peripheral venous blood corresponds to the concentration in the portal vein or may even be higher, partly due to shunting (Ohkubo et al., 1984). In blood, bile acids are tightly bound to serum albumin and lipoproteins (Rudman and Kendall, 1957; Kramer et al., 1979; Roda et al., 1982; Ceryak et al., 1993), hydrophobic bile acids predominantly to albumin and hydrophilic bile acids also to lipoprotein particles, especially HDL and LDL.

The serum concentration of total bile acids increases (especially in alcoholic cirrhosis; Ciocan et al., 2018) with the degree of decompensation of liver cirrhosis and is prognostic for survival (Mannes et al., 1986; Wang et al., 2016). The same holds true for hemodynamic parameters. In parallel, the amount of serum bile acids correlates with the degree of portal hypertension (Horvatits et al., 2017a) and hepatopulmonary syndrome (Horvatits et al., 2017b). These observations raise the question of a causal role of bile acids in the pathogenesis of impaired hemodynamics in liver cirrhosis.

The vast majority of bile acids in peripheral plasma of patients with alcoholic cirrhosis are conjugated with a shift toward taurin-conjugates (nearly 1:1 ratio of glycine- to taurine-conjugates; Brandl et al., 2018; Ciocan et al., 2018; Trefflich et al., 2019). Similar to the bile acid pool, as disease progresses toward liver cirrhosis, the relative proportion of CDCA increases in serum. This is especially true for severe alcoholic cirrhosis with alcoholic hepatitis (Brandl et al., 2018; Ciocan et al., 2018) and less pronounced in hepatitis B-virus induced cirrhosis, primary biliary cholangitis (PBC) or primary sclerosing

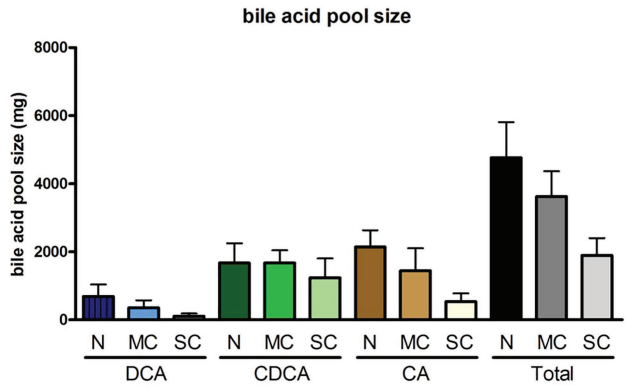


FIGURE 4 | Change in bile acid pool in patients with mild cirrhosis (MC) and severe cirrhosis (SC) compared with normal subjects (N). DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; CA, cholic acid. Modified by data from Ponz de Leon et al. (1981).

cholangitis (PSC; Azer et al., 1996; Dilger et al., 2012; Wang et al., 2016; Ciocan et al., 2018; Liu et al., 2018; Chen et al., 2020).

Driven by the increase of intrahepatic resistance to portal venous outflow with the development of portal hypertension and due to the remodeling of the intrahepatic vascular architecture, intra-, and extrahepatic shunts develop. Recent computed tomography-based studies show that more than half of the patients with liver cirrhosis have spontaneous portosystemic shunts, partially large. In Child-C cirrhosis, the percentage rises to above 70 (Simon-Talero et al., 2018). In patients with liver cirrhosis who underwent shunt surgery, it has been shown that this leads to a permanent increase in serum bile acids (Poupon et al., 1976; Capocaccia et al., 1981). These findings suggest that altered splanchnic hemodynamics is a major cause of the elevated serum bile acid levels. In an older, very elegant paper, Ohkubo et al. (1984) showed in patients with compensated cirrhosis that there is a highly significant correlation between portal venous shunt index as a measure of spontaneous shunting and peripheral venous bile acid concentration. In dogs, the creation of a portocaval end-to-side shunt leads to a marked increase in bile acid, which can be prevented by arterialization of the truncated portal vein stump (Horak et al., 1975). These findings suggest that the increase in serum bile acids of cirrhotics is much more determined by disturbances of the hepatic blood flow, i.e., perfusion of the sinusoids, than by reduced extraction of the hepatocytes. The authors assume that the induction of the partial and permanent bypass of hepatic extraction is not caused by a vasoactive effect of bile acids. However, whether bile acids later contribute to a vicious circle in bypassing the liver is unclear.

To summarize, the circulating bile acid pool decreases in liver cirrhosis, showing a relative increase in CDCA (especially in alcoholic cirrhosis) and a significant spillover into the systemic circulation, depending on the extent of decompensation of cirrhosis, so that serum concentrations of total bile acids approach those in portal blood. Here, the proportion of unconjugated bile acids decreases and the relative proportion of taurine conjugates increases.

DIRECT EFFECTS OF BILE ACIDS ON THE VASCULATURE

There is the clinical – but systematically poorly studied – observation that patients with protracted cholestasis and jaundice have low systemic blood pressure. In rats or mice bile duct ligation leads to systemic vasodilation and hypotension (Bomzon and Ljubuncic, 1995; Heller et al., 2005; Hennenberg et al., 2006, 2007, 2009a). Therefore, some authors speculated that a direct vasodilatory effect of bile acids might be responsible. Indeed, Pak and coworkers (Pak and Lee, 1993) showed that infusion of TCDCA and TDCA in particular increased mesenteric blood flow, decreased systemic blood pressure, and dilated precontracted mesenteric vascular rings in cirrhotic rats and – more so – in controls. In the following, we would like to further discuss the vasodilatory effect of bile acids.

A very careful systematic work (Ljubuncic et al., 2000) investigated the effect of different primary and secondary, taurine- and glycine-conjugated bile acids on phenylephrine- and methoxamine-induced contraction on vascular rings of the abdominal aorta in the rat as well as their possible vasodilatory effect on precontracted aortic rings. Unconjugated CDCA and even more pronounced CDCA but not CA at micromolar to millimolar concentrations were shown to significantly reduce maximal vascular contraction. With the exception of CA, all conjugates (DCA, CDCA, and UDCA) also increased the EC50 value for α_1-adrenergic ligands but not the maximal response (**Figure 5**). There was a linear relationship between the reduction in contractile response and the relative fat solubility of bile acids. Unconjugated DCA and CDCA had the strongest effect, followed by GDCA and TCDCA. The vasorelaxant effect of DCA was not affected by removal of endothelium or blockade of NO formation using L-NAME. The authors speculated that lipophilic bile acids affect the affinity of adrenoceptors by increasing lipid peroxidation (and thus altering the membrane micromilieu at the receptor), possibly also affecting the fluidity of the membrane.

In contrast, another group (Khurana et al., 2005) found in *in vitro* studies using pre-contracted rat and mouse thoracic aortas that the vasodilating effect of taurine conjugated DCA (up to 1 mmol/L) was nearly abolished by blocking NO synthesis (by removing endothelium) or by knockout of the muscarinic M3 receptor. They concluded that the relaxing effect of this bile acid is mediated *via* the endothelial M3 receptor and release of NO. This finding fits with previous *in vitro* studies in endothelial cells, in which DCA and CDCA and their taurine conjugates increased intracellular calcium concentration in a dose-dependent manner and therewith NO formation (Nakajima et al., 2000). However, this group later found in rat mesenteric vessels, that glycine conjugates of DCA inhibited the RhoA/Rho-kinase pathway *via* decreased membranous translocation of RhoA in the smooth muscle cell (Jadeja et al., 2018). That is, the effects were due to a reduction in calcium sensitization and not to NO. Previously, this group had shown in rat mesenteric arteries that the glycine-conjugate of DCA inhibits at physiological concentrations, independent of NO, muscarinic receptors, or potassium channels, which confirmed earlier studies in perfused mesentery of the rat and in arterial rings. In this study TDCA > TCDCA > TUDCA induced NO-independent relaxation mainly due to inhibition of calcium entry into the smooth muscle cell (Pak et al., 1994).

Last but not least, Dopico et al. (2002) showed in a very elegant patch-clamp study on isolated rabbit mesenteric artery and pulmonary artery smooth muscle cells that unconjugated hydrophobic bile acids increase BK_{Ca} (calcium-activated potassium channel) activity and thus counteract cell contraction.

In summary, there are a number of *in vitro* studies showing that predominantly hydrophobic bile acids have vasodilatory effects, mostly NO-independent (**Figure 6**) and at concentrations that can also be found in serum of patients with liver cirrhosis.

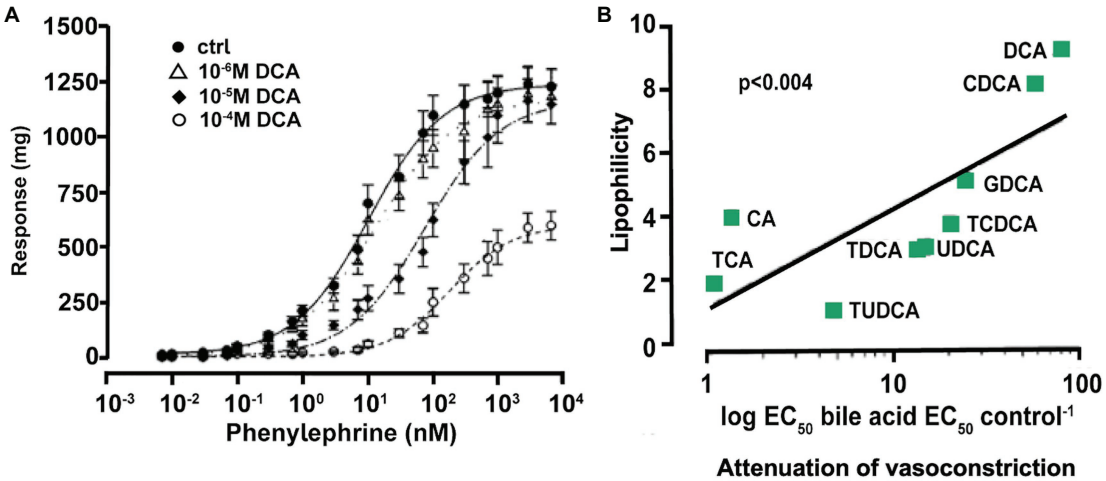


FIGURE 5 | (A) Attenuation of the *in vitro* contractile response to phenylephrine of aortic rings from rats by different concentrations of DCA. **(B)** Relation of the lipophilicity of different conjugated and unconjugated and their attenuation of the vasoconstrictive response of aortic rings to phenylephrine (modified according to Ljubuncic et al., 2000).

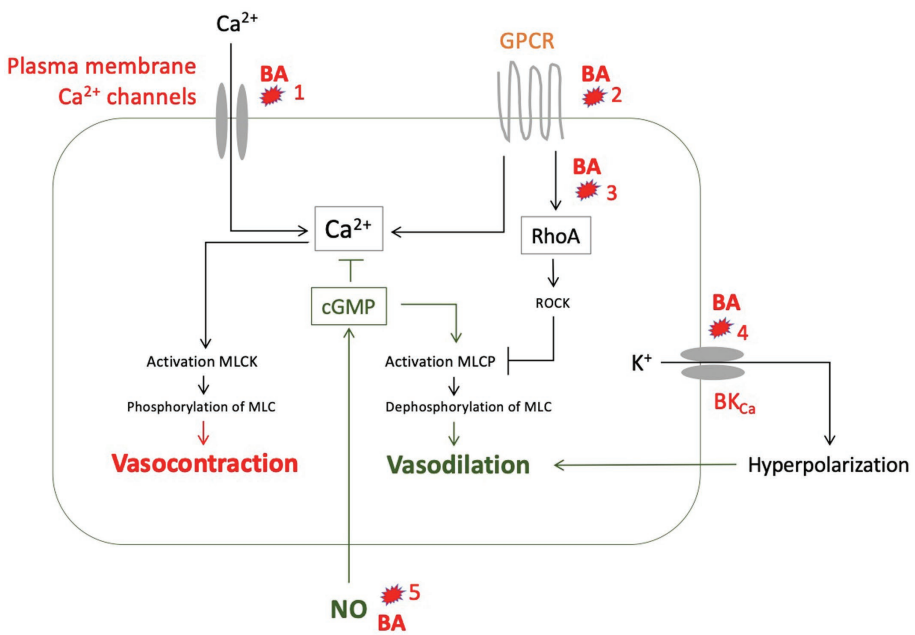


FIGURE 6 | Suggested vasodilatory effects of various bile acids (BA) as to the results of experimental studies: (1) Reduction of calcium influx into smooth muscle cells (Pak et al., 1994). (2) Alteration of G protein-coupled receptors (GPCR), for example by changing membrane fluidity mainly through lipophilic bile acids (Ljubuncic et al., 2000). (3) Inhibition of translocation of RhoA to the cell membrane through glycin conjugates from deoxycholic acid (DCA), thus preventing activation of Rho kinase (ROCK) and ROCK-mediated myosin light chain (MLC) phosphatase inhibition (Jadeja et al., 2018). (4) Activation of calcium-activated potassium channels (BK_{Ca}) by hydrophobic bile acids (Dopico et al., 2002). (5) Increased formation of NO by endothelial cells through taurine-deoxycholic acid (DCA) mediated by M3 receptors (Nakajima et al., 2000; Khurana et al., 2005), mediated by TGR5, especially lithocholic acid (LCA), chenodesoxycholic acid (CDCA), and DCA (Guo et al., 2016a), or by activation of farnesoid X receptor (FXR), especially by CDCA (Li et al., 2008). GPCR, G protein-coupled receptor; MLC, myosin light chain; MLCK, MLC kinase; MLCP, MLC phosphatase; and NO, nitric oxide.

TGR 5 MEDIATED EFFECTS OF BILE ACIDS ON THE VASCULATURE

The G-protein-coupled bile acid receptor Gpbar1 (TGR5) triggers a number of intracellular signaling pathways *via* its natural endogenous ligands, primary and secondary conjugated and unconjugated bile acids (in descending order: LCA > DCA > CDCA > UDCA > CA), including smooth muscle cell relaxation (Guo et al., 2016a,b). For example, bile acids (TLCA and TCDCA) release NO intrahepatically *via* sinusoidal eNOS in the rat *via* TGR5 (Keitel et al., 2007). Expression of TGR5 was also detected in endothelial cells from various human vessels, as was activation/phosphorylation of eNOS by TLCA (Kida et al., 2013). In dogs, TGR 5 agonists cause peripheral vasodilation and reduction of mean arterial blood pressure (NO-independent), most likely due to a cross-talk to BKca, but the mechanism is still unclear (Fryer et al., 2014).

FXR-MEDIATED EFFECTS OF BILE ACIDS ON THE VASCULATURE

The FXR belongs to the ligand-activated transcription factors. Important activating ligands are conjugated bile acids, especially CDCA > LCA = DCA > CA. In addition, synthetic ligands such as obeticholic acid (OCA) are used for the treatment of cholestatic and metabolic liver diseases. FXR is expressed in the liver and also in the intestine and regulates bile acid uptake and neosynthesis in the liver-gut axis, partly in interaction with TGR5 and FGF19. FXR is also expressed in endothelial cells. Here, activating ligands – such as CDCA – lead to increased expression of eNOS and increased formation of NO (Li et al., 2008) and also to a decrease in endothelin-1 expression (He et al., 2006). Both phenomena suggest the possibility that bile acids, especially CDCA, may also produce vasodilation *via* FXR. Finally, activation of FXR on rat aortic smooth muscle cell leads to increased expression of angiotensin II type 2 receptor, which is also thought to have a vasodilatory function (Zhang et al., 2016).

INTESTINE

In healthy individuals, the bile acid pool in the body is distributed mainly through the liver (<1%), intestine (85–90%) and the gallbladder (10–15%; Carey and Duane, 1994; Chiang and Ferrell, 2020). The proportion that reaches the systemic circulation in healthy individuals through spill over is low. As mentioned above, about 20% of bile acids are not actively absorbed from the ileum into the portal blood and enter the colon (Chiang and Ferrell, 2020) where they are deconjugated by bile salt hydrolases (BSH) and then passively absorbed (Begley et al., 2005). Only 5% of total bile acids leave the body *via* feces. In the resident microbiome of the colon, BSH are redundantly available even with a change in the microflora (Ridlon et al., 2015). By contrast, the 7-alpha-dehydroxylation of primary bile acids (CA to DCA and CDCA to LCA) is restricted to the genera *Eubacterium* and *Clostridium* (Firmicutes), *via* the bile acid inducible (*bai*) operon. This means that the conversion to secondary bile acids is more subject to a change in the microbiota (Begley et al., 2005) than deconjugation. In liver cirrhosis, bacterial overgrowth of the upper intestine has been demonstrated in many studies (Gurusamy et al., 2021), i.e., bacterial biotransfomation of bile acids can already take place in the small intestine. In cirrhosis of the liver, it is not only the bacterial overgrowth of the upper intestinal tract that accompanies disease, but also a dysbiosis (Qin et al., 2014; Arab et al., 2018; Fairfield and Schnabl, 2021) that takes place mainly in the large bowel. The major phyla in the colon in healthy individuals are Firmicutes and Bacteroidetes as well as Proteobacteria (Bajaj et al., 2018; Haran and McCormick, 2021). In liver cirrhosis occurs a shift in favor of the Bacteroidetes. The essential taxa containing enzyme for 7-alpha-dehydroxylation, however, belong to the Firmicutes (Ridlon et al., 2015), a possible explanation for the decrease of secondary bile acids in liver cirrhosis. Furthermore, the diversity of the microbiota decreases – also reflected by a reduction in the number and diversity of fecal bacterial genes (Qin et al., 2014). This shift appears to occur earlier and more severely in alcoholic liver damage, particularly in alcoholic hepatitis with underlying cirrhosis. In alcoholic cirrhosis, candida is also found more frequently in the stool, microorganisms increasingly considered to have pathogenic influence (Yang et al., 2017).

Taken together, dysbiosis in cirrhosis favors the accumulation of primary bile acids in the stool. The development of the dysbiosis is often initially caused by food and beverages and is then reinforced or perpetuated by the liver disease. Comprehensive studies to what extent change of intestinal microbiota influences the plasma BA pattern and hemodynamics are still missing. But a link must exist.

INDIRECT EFFECT *VIA* INFLAMMATION

With the deterioration of liver function, inflammatory markers (Trebicka et al., 2019), bile acid concentration (Mannes et al., 1986), and bilirubin (Kamath et al., 2001) increase in blood with simultaneous alteration of hemodynamics (Maroto et al., 1994; Trebicka et al., 2011). Unfortunately, the mere association of the quantitative changes of these different parameters does not help us to establish a possible pathogenetic role of bile acids and their interaction with inflammatory markers. Release of cytokines (e.g., TNFa, IL6, and IL8) can lead to vasodilation *via* the endothelium by activation of iNOS and formation of NO. It is also possible that chronic inflammation induces a change in the phenotype of the smooth muscle cell with a decreased response to vasoconstrictors. Finally, there is an indirect link to bile acids, e.g., through their effect on the immune system (Chen et al., 2019). According to *in vitro* studies, bile acids may activate the NLRP3 inflammasome and Il-1ß secretion in macrophages by TGR5/EGFR signaling (Gong et al., 2016). By contrast, others showed that TGR5 activating bile acids reduce the formation of proinflammatory cytokines by monocytes and macrophages *via* the corresponding receptor

on these immune cells (Leonhardt et al., 2021). Such effects would on the one hand inhibit an adequate immune response, but on the other hand could attenuate vasodilation *via* cytokines. Furthermore, activation of FXR (which may directly cause vasodilation) supports intestinal barrier function and thus may prevent vasodilating inflammatory stimuli coming from the gut (Inagaki et al., 2006; Verbeke et al., 2015; Fiorucci et al., 2021).

In summary, one could speculate that different bile acids indirectly modulate vascular tone *via* their differential immunomodulatory effects on TGR5 and FXR, respectively. However, we do not know how such effects play out across the body (lymphoid tissue, blood compartment, and liver). Thus, it remains elusive whether and in which direction the change in bile acid concentration and composition in liver cirrhosis indirectly drives vasodilation *via* inflammation.

HEART

Although not the focus of this paper, the influence of different bile acids on cardiac function and cardiomyocytes cannot be ignored. We refer here to summaries (Bernardi, 2013; Voiosu et al., 2017; Vasavan et al., 2018). Cirrhotic cardiomyopathy is characterized by systolic and diastolic dysfunction as well as by electrophysiological changes (Bernardi, 2013; Voiosu et al., 2017). Cardiac impairment increases with the degree of liver dysfunction. Latent heart failure can rapidly progress to decompensation due to infections, volume loading (e.g. via insertion of TIPS), or surgery. The role played here by various bile acids is unclear. As in the smooth muscle cell, an influence on transmembrane ion channels and on adrenoceptors by altering the fluidity of the cell membrane cannot be ruled out. Cardiomyocytes express TGR5 and respond to FXR ligands. Mice models with elevated bile acids show an alteration of cardiac metabolism and function (Desai et al., 2017). According to *in vitro* studies, analogous to vascular smooth muscle cells, lipophilic bile acids mainly impair cell function, among others by damaging mitochondria. Impairment of cell contraction, ß-adrenoceptor activation, and induction of arrythmias by hydrophobic bile acids were observed in these mostly animal studies (Zavecz and Battarbee, 2010).

POSSIBLE INTERVENTIONS

Finally, the question that remains is whether it might be useful to modulate the bile acid pool in liver cirrhosis in order to counteract the hyperdynamic circulatory disturbance, a question that has been raised before and by others. The rationale behind is quite evident:

- Cirrhosis of the liver causes hyperdynamic circulatory disturbance with significant extrahepatic consequences.
- In liver cirrhosis, there is a marked change in bile acid metabolism associated with a significant increase in the concentration of bile acids in the systemic circulation.
- The vasodilatory effect of especially hydrophobic bile acids is well-established.

Therefore, it would be interesting to examine whether a modulation of the bile acid pool might counteract the pathophysiology described above. Such an approach could have implications not only for hemodynamics but also for hepatic inflammation and intestinal dysbiosis in patients with liver cirrhosis, given the interaction between bile acids and intestinal bacteria. However, such an attempt deals with extremely complex systems. For example, we do not really know the distribution of individual bile acids (free, taurine or glycine-conjugated) and their concentration in the different organs, the systemic arterial or splanchnic vascular compartments, the amount of binding to albumin and lipoprotein or even their concentration and distribution along the intestine. One could even go so far as to assume, that bile acids act on the cardiovascular system *via* the central nervous system (Kiriyama and Nochi, 2019). All this makes it very difficult to predict the effect of such an intervention. This means that – as Gregorian creatures – we would have to adopt a trial and error approach on the basis of an educated guess (Dennett, 2017).

What could be considered?

- Early modulation of the pool towards individual bile acids that do not lead to vasodilation.
- Influencing the bile acid metabolism *via* TGR 5 or FXR.
- Influencing the bile acids *via* the intestinal flora.

Altering the Pool by Application of Bile Acids or Other TGR5/FXR Modulators

Two bile acids, even as conjugates, have little vasoactive effect: cholic acid and UDCA (**Figure 5**). As explained, the cholic acid pool decreases with increasing decompensation of liver cirrhosis. Cholic acid is available as orphan drug for expansion through exogenous supply. But there are no long-term observations in cirrhosis, and one would have to expect that the loss of CA into the intestine would lead to an increase in the occurrence of DCA. DCA, a lipophylic bile acid, is hepatotoxic and has a vasodilatory effect. In contrast, UDCA has been used for many years in liver diseases without harm and even has a proven life-prolonging effect in PBC.

UDCA Application

Ursodeoxycholic acid is a secondary/tertiary bile acid formed in humans by intestinal microorganisms from CDCA in small amounts. It is contribution to the human bile acid pool is normally very low (1–3%). We have known for about 40 years that UDCA has a "hepatoprotective" effect. There is good evidence for the beneficial effect of UDCA in PBC (Poupon, 2014). Yet, the proof of a beneficial influence for other chronic liver diseases such as PSC or metabolic liver diseases is much lower or nonexisting (Reardon et al., 2016; de Vries and Beuers, 2017). Nevertheless, no studies showed a deterioration of serum liver tests under UDCA; on the contrary, a decrease in serum aminotransferases, alkaline phosphatase, and y-GT was observed in the majority of trials. The accessible data on hemodynamic parameters in these studies are sparse or nonexistent. Noteworthy, in icteric

patients with decompensated alcoholic cirrhosis, UDCA administration had an unfavorable effect (Pelletier et al., 2003) as when administered at very high doses (28–30 mg/kg/d) in patients with PSC (Lindor et al., 2009).

Ursodeoxycholic acid is hydrophilic and, as explained above, has hardly any vasoactive properties. According to recent studies its hepatoprotection is caused by influencing intracellular calcium-dependent signals, improvement of the transporter function of the apical membrane of hepatocytes, anti-apototic effects and the establishment of a "bicarbonate umbrella" at the apical cholangiocyte membrane (Beuers et al., 2015). The displacement of hepatotoxic bile acids during long-term, but not short-term treatment could also play a role. However, treatment with UDCA (1 month) in five patients with PBC or PSC did not lead to a reduction in the pool of hydrophobic bile acids DCA or CDCA (Beuers et al., 1992). When looking at the serum levels of patients with PBC before and after 2 years of therapy with UDCA, there is a slight increase in total bile acid concentration in plasma (29 to 31 μM) with a significant decrease in CA and CDCA, constant serum levels of DCA, a slight increase in LCA, and a very significant increase in UDCA (Poupon et al., 1993; Chen et al., 2020). Thus, UDCA treatment increases the ration of hydrophilic/hydrophobic bile acids in serum, which should be favorable concerning vasodilation. Could this – by displacing hydrophobic bile acids in the systemic circulation – have an effect on hemodynamics in the chronic liver disease patient? We do not know for sure. The available data are not rousing.

In healthy subjects, 4 weeks of UDCA vs. placebo does not affect basal or postprandial portal flow or cardiac parameters, such as cardiac output, ejection fraction and QT time. Only diastolic blood pressure was slightly but significantly reduced (Schiedermaier et al., 2000). As known from other studies, UDCA leads to an immediate (Sailer et al., 1996; Schiedermaier et al., 2000; Neubrand et al., 2004) increase in gallbladder volume. The mechanism of this effect on a smooth muscle organ is still unclear; one explanation could be the inhibition of cholinergic pathways (Neubrand et al., 2004). To what extent this effect on the gallbladder influences intestinal metabolism of BA is unclear.

Two decades ago, clinical researchers already tested the hypothesis of influencing the unfavorable hemodynamics in liver cirrhosis by modulating the bile acid pool. In patients who had received TIPS for refractory ascites, treatment with UDCA (15 mg/kg) for 1 month did not affect hemodynamic parameters (systemic, renal, and forearm blood flow), but (even in cirrhotics with ascites without TIPS) resulted in significant sodium retention, which was interpreted as an effect on the proximal tubule (Wong et al., 1999). To our knowledge, such an effect of UDCA on the sodium balance in patients with liver cirrhosis has never been further systematically investigated.

In a small cohort of patients with compensated cirrhosis (PBC, posthepatitic, Child A), 1 month of treatment with UDCA (13 mg/kg/d) did not significantly affect cardiovascular parameters except for a slight reduction in diastolic volume in the PBC patients and a slight reduction in cardiac output in the patients with PBC cirrhosis (Baruch et al., 1999).

There is only rudimentary research on the effect of UDCA on human portal pressure. In the rat model (bile duct ligation), administration of UDCA for 1 month resulted in a decrease in portal venous blood pressure *via* a decrease in intrahepatic resistance, which was interpreted as an intrahepatic antioxidant effect (Yang et al., 2009). In a small series of patients with PBC and discretely elevated portal pressure, portal pressure was unchanged after 2 years of UDCA treatment, while it slightly increased in the placebo patients. Information on other hemodynamic parameters is not available (Huet et al., 2008).

There is evidence from animal experiments – and also in humans (Rainer et al., 2013) – that hydrophobic bile acids have an arrhythmogenic effect and influence cardiac function (see above); on the other hand, UDCA may be cardioprotective (Zavecz and Battarbee, 2010). Small pilot studies in humans additionally indicate that administration of UDCA (and its taurine conjugate) at a dose of 1,000 mg/d for 1 month favorably affect endothelial dysfunction under glucose challenge (Walsh et al., 2016) as well as in coronary artery disease (Sinisalo et al., 1999), and in heart failure (von Haehling et al., 2012). It is unclear how this is mediated. According to animal studies, this effect of UDCA could be induced in a completely different way, namely *via* a reduction of cholesterol crystals in vascular macrophages, where these – *via* stimulation of the inflammasome – have an IL-1-mediated proatherogenic effect and can also cause endothelial dysfunction (Bode et al., 2016).

INFLUENCING TGR 5 AND FXR

Little evidence emerges for TGR5 modulation to affect vasodilation of liver cirrhosis. It may be that free or conjugated CDCA also causes vasodilation in cirrhosis *via* TGR5 and NO liberation (see above and **Figure 3**) and in the addition *via* inhibition of RhoA/ROCK signaling (Zhu et al., 2020). But the effect of antagonizing TGR5 might counteract favorable antiinflammatory influences mediated *via* TGR5 stimulation (Duboc et al., 2014; Guo et al., 2016b; Deutschmann et al., 2018).

As stated above, FXR stimulation exhibits vasodilator effects *via* several mechanisms. Thus, taurine-conjugated BA mediate NO-fomation *via* the endothelial FXR (Guizoni et al., 2020). Since various steroidal and nonsteroidal (Schwabl et al., 2017) agonists are available, it would be interesting to analyze their effect on hemodynamics in chronic liver disease. In the cirrhotic animal models (rats and mice), feeding with non-steroidal FXR agonists lowered portal pressure by lowering intrahepatic resistance and simultaneously reducing inflammatory stimuli from the gut (Verbeke et al., 2014, 2016). Systemic hemodynamics showed a slight reduction of systemic arterial pressure in rats but not in the mouse model (Schwabl et al., 2017). Similarly, in an animal model of colitis, FXR activation led to downregulation of the expression of pro-inflammatory cytokines and to an improvement of the intestinal barrier (Gadaleta et al., 2011).

The large placebo-controlled study (Nevens et al., 2016) on the effect of the FXR agonist OCA in addition to UDCA in

non-cirrhotic patients with PBC incompletely responding to UDCA showed a positive effect on the prognostic surrogate parameters alkaline phosphatase and bilirubin, as well as a reduction in inflammatory serum markers. Detailed analysis of hemodynamic parameters had not been performed in this trial. An increase in cardiovascular adverse events or ECG changes did not occur. This is reassuring considering that cardiotoxicity in animal models is partially FXR mediated (Pu et al., 2013) and that FXR agonists lead to an increase in LDL-cholesterol in serum (Nevens et al., 2016).

In liver cirrhosis – alcoholic and non-alcoholic – and also in alcohol exposure of the pre-cirrhotic patient, there is dysbiosis and concomitant increase of CDCA in serum with reduction of DCA (Brandl et al., 2018; Ciocan et al., 2018). Conjugated and unconjugated CDCA are among the strongest endogenous activators of FXR. Therefore, one could consider blocking – not antagonizing – FXR in patients with liver cirrhosis to counteract putative FXR mediated vasodilation in advanced liver damage. However, this is a double-edged sword, as one would then simultaneously weaken the described anti-inflammatory and hepatoprotective effect of FXR activation. We do not know whether FXR agonists exacerbate hemodynamic circulatory dysfunction in patients with liver cirrhosis.

INFLUENCING THE INTESTINE

Bile acids influence the microflora in the intestine and, vice versa, bacterial enzymes metabolize bile acids and thus alter the bile acid pool. Interestingly, almost complete reduction of the serum bile acid concentration in animal models (portal vein ligation) with cholestyramine gavage leads neither to change in splanchnic and systemic hemodynamics (Genecin et al., 1990), nor to a reduction of portal pressure. Also, from a theoretical point of view, binding of intestinal bile acids by sequestrants (cholestyramine or colestipol) makes no sense. Total bile acids are already reduced in liver cirrhosis and it would be more important to modulate the composition of individual bile acids with their different direct and indirect effects on intestinal microflora, receptors, vascular smooth muscle cells, or on the heart. Can this be achieved by antibiotics? A small randomized, placebo-controlled trial found improvement in hyperdynamic circulatory dysfunction (Rasaratnam et al., 2003) with 4 weeks of norfloxacin. Rifaximin is a non-absorbable antibiotic with proven effect on hepatic encephalopathy (Bass et al., 2010). While its effect in the intestine has not been fully elucidated, it has been found to reduce the production and absorption of gut-derived toxins and inflammatory stimuli, such as ammonia and endotoxin (Bajaj, 2016). Overall, its effects in the intestine may be more eubiotic than antibiotic (Ponziani et al., 2017). According to uncontrolled trials, rifaximin reduced plasma endotoxin levels and HVPG in alcohol-related decompensated liver cirrhosis (Vlachogiannakos et al., 2009). Furthermore, it lowered the 5-year cumulative probability of decompensation of cirrhosis, including bleeding and encephalopathy, and resulted in better survival (Vlachogiannakos et al., 2013). However, although the data are promising, they are from only one center and as yet remain uncontrolled. A randomized double-blind placebo-controlled trial consisting of a 4-week treatment with rifaximin found no effect on bacterial translocation, HVPG, systemic hemodynamics, kidney function, or vasoactive hormones, including plasma renin (Kimer et al., 2017), and a further study found that there was no short-term effect of rifaximin on systemic inflammatory markers or intestinal bacterial composition (Kimer et al., 2018). This may be due to the technique of sequencing used. A recent study demonstrates significant and profound changes in the microbiota in liver cirrhosis – using metagenomics and not only 16S sequencing – with a favorable effect of rifaximin unlike absorbable antibiotics (Shamsaddini et al., 2021). The extent to which such antibiotic therapies shift individual bile acids in a favorable direction in humans is unclear. Recent studies have demonstrated a reduction of secondary fecal bile acids by rifaximin in mice with humanized stools (Kang et al., 2016). The result of further clinical trials on the role rifaximin on the progress of liver cirrhosis have to be awaited (Caraceni et al., 2021), hopefully with a concomitant focus on bile acid metabolism.

In the intestine – contrary to the vascular system – an increased activation of FXR would be desirable (Flynn et al., 2015) because of its anti-inflammatory effects. But CDCA, the most potent FXR agonist, is already elevated in liver cirrhosis, at least in serum, where it might have vasodilatory effects. Portal pressure reduction might be taken as an indirect indication of a favorable intervention on splanchnic hemodynamics. A meta-analysis found no convincing effect of antibiotics acting in the intestine on portal pressure (Mendoza et al., 2020). Again, it is difficult to distinguish extrahepatic from intrahepatic effects. Relatively specific dysbiosis in therapy-naive patients with PBC was reversed by 6 months of treatment with UDCA (Tang et al., 2018). For all these studies, there are no findings on the change in the bile acid pool, its distribution and associated hemodynamic changes.

Another approach is the modulation of the intestinal microbiota with probiotics, prebiotics or synbiotics (Sarin et al., 2019). Most studies are related to the influence on hepatic encephalopathy in liver cirrhosis of different etiologies. Another study with a mixture of eight probiotic strains found a reduction in hospitalization in predominantly alcoholic cirrhosis, but again, no analyses of the individual bile acids or hemodynamics are available. Plasma renin, aldosterone, and BNP levels decreased significantly in the probiotic group. In the placebo group, there was no change in plasma renin and BNP levels, but a significant increase in aldosterone levels (Dhiman et al., 2014). Any connections to the bile acid pattern and its change as well as relationship to hemodynamic findings remain elusive.

Last but not least, we need more information about diet, liver cirrhosis and its influence on the bile acid pool. Tea, coffee fermented milk products, vegetable and chocolate were associated with a higher diversity with the microbiota in patients with liver cirrhosis, but again there is no data on the relationship of food to bile acids and hemodynamics in these patients (Bajaj et al., 2018).

Albumin

Albumin with its oncotic properties is an effective plasma expander. By this, it acts on the baroreceptors and reduces the augmented neurohumoral response in liver cirrhosis. But albumin has further pleiotropic non-oncotic features. Among others, it can bind particles and molecules important for inflammation and it has antioxidant function (Fernandez et al., 2019, 2020; Bernardi et al., 2020; Tufoni et al., 2020). Metabolomic analyses show an increase of a number of molecules – some of intestinal origin – associated with decompensation in liver cirrhosis (Bajaj et al., 2020). This leads to the question whether vasoactive molecules such as bile acids could be bound by the administration of fresh albumin in order to compensate for a reduced albumin concentration and its impaired structural integrity in liver cirrhosis. Unfortunately, there are no studies on the complex question of the exchange of bile acid molecules between exogenously applied and endogenous albumin (**Figure 7**). There is also a lack of studies on how the various bile acids – conjugated and unconjugated – dissociate from the albumin molecule or the lipoproteins and then exert their effect on the endothelium or the smooth muscle cell. The question could be roughly approached in a first step by determining only the plasma concentration of bile acids before and after album administration.

SUMMARY AND CONCLUSION

The present work analyzed here on the role of bile acids in the pathogenesis of extrahepatic vasodilation and cardiovascular alteration is not conclusive. There is good evidence that hydrophobic bile acids in particular can lead directly and indirectly to vasodilation. But in liver cirrhosis, the pool of DA, the bile acid with the highest lipophilicity (Roda et al., 1990) actually decreases. On the other hand, the plasma concentration of serum bile acids increases markedly in the systemic vascular compartment, mainly in favor of CDCA and its conjugates. Here, concentrations are theoretically reached for which *in vitro* studies have shown that CDCA can induce vasodilation. But we have no data at all on the exchange of bile acids between albumin or lipoproteins and the vascular endothelium or vascular smooth muscle cell where they could have a direct or indirect vasodilatory effect. Even assuming that CDCA would induce vasodilation in liver cirrhosis on the one hand there remains its beneficial anti-inflammatory effect as an FXR agonist on the other hand. UDCA is hydrophilic, hardly vasoactive and furthermore hardly stimulates TGR5 or FXR. Thus, this bile acid would be the most convenient candidate, an idea suggested early on by other clinical investigators. But the evidence that administration of UDCA could be effective is most limited. Nevertheless, with the exception of PBC, the studies are too short and there is too little experience in the stage of cirrhosis. We lack long-term randomized placebo-controlled long-term trials (including hemodynamic endpoints) in early stage cirrhosis, regardless of etiology.

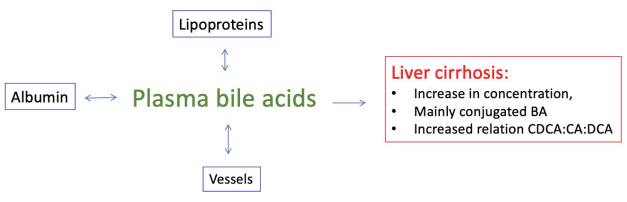


FIGURE 7 | In liver cirrhosis, there are markedly elevated plasma levels of conjugated bile acids in the peripheral circulation, and in alcoholics, especially of CDCA. We know too little about the exchange of these molecules between circulating lipoproteins, plasma albumin and the vascular wall.

SUGGESTIONS FOR FUTURE RESEARCH

- Studies on the modulation of the microbiome and its effect on the bile acid pool, serum bile acid pattern and hemodynamic parameters.
- Studies on the distribution of different individual bile acids between albumin and lipoproteins in serum and their dissociation into vascular cells or to their receptors and membranes.
- Controlled studies of early and long-term administration of UDCA – independent of etiology – on liver function and hemodynamic parameters such as cardiac output, heart rhythm, blood pressure and portal pressure in patients with advanced fibrosis or early compensated cirrhosis.
- Studies on the alteration of the serum bile acid pattern after TIPS insertion and including this into parameters for multivariate analysis in relation to hemodynamic changes.

For Gustav Paumgartner in memory of the Pichlschloss transport meeting in October 2018.

AUTHOR CONTRIBUTIONS

TS wrote the first draft of the manuscript. MH, JT, and UB corrected and reformulated sections of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

JT is supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18, CRC 1382AO9), European Union's Horizon 2020 Research and Innovation Programme (Galaxy, No. 668031, MICROB-PREDICT, No. 825694, and DECISION, No.847949), and Societal Challenges - Health, Demographic Change and Wellbeing (No. 731875), and Cellex Foundation (PREDICT). Wenyi Gu is supported by the China Scholarships Council (CSC: #201906230332). The funders had no influence on study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Albillos, A., Rossi, I., Cacho, G., Martinez, M. V., Millan, I., Abreu, L., et al. (1995). Enhanced endothelium-dependent vasodilation in patients with cirrhosis. *Am. J. Phys.* 268, G459–G464. doi: 10.1152/ajpgi.1995.268.3.G459

Angelin, B., Bjorkhem, I., Einarsson, K., and Ewerth, S. (1982). Hepatic uptake of bile acids in man. Fasting and postprandial concentrations of individual bile acids in portal venous and systemic blood serum. *J. Clin. Invest.* 70, 724–731. doi: 10.1172/JCI110668

Arab, J. P., Martin-Mateos, R. M., and Shah, V. H. (2018). Gut-liver axis, cirrhosis and portal hypertension: the chicken and the egg. *Hepatol. Int.* 12(Suppl. 1), 24–33. doi: 10.1007/s12072-017-9798-x

Arina, P., and Singer, M. (2021). Pathophysiology of sepsis. *Curr. Opin. Anaesthesiol.* 34, 77–84. doi: 10.1097/ACO.0000000000000963

Arroyo, V., Angeli, P., Moreau, R., Jalan, R., Claria, J., Trebicka, J., et al. (2021). The systemic inflammation hypothesis: towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. *J. Hepatol.* 74, 670–685. doi: 10.1016/j.jhep.2020.11.048

Azer, S. A., Coverdale, S. A., Byth, K., Farrell, G. C., and Stacey, N. H. (1996). Sequential changes in serum levels of individual bile acids in patients with chronic cholestatic liver disease. *J. Gastroenterol. Hepatol.* 11, 208–215. doi: 10.1111/j.1440-1746.1996.tb00064.x

Bajaj, J. S. (2016). Review article: potential mechanisms of action of rifaximin in the management of hepatic encephalopathy and other complications of cirrhosis. *Aliment. Pharmacol. Ther.* 43(Suppl. 1), 11–26. doi: 10.1111/apt.13435

Bajaj, J. S., Idilman, R., Mabudian, L., Hood, M., Fagan, A., Turan, D., et al. (2018). Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology* 68, 234–247. doi: 10.1002/hep.29791

Bajaj, J. S., Reddy, K. R., O'Leary, J. G., Vargas, H. E., Lai, J. C., Kamath, P. S., et al. (2020). Serum levels of metabolites produced by intestinal microbes and lipid moieties independently associated with acute-on-chronic liver failure and death in patients with cirrhosis. *Gastroenterology* 159, 1715–1730.e1712. doi: 10.1053/j.gastro.2020.07.019

Baruch, Y., Assy, N., Weisbruch, F., Reisner, S. A., Rinkevich, D., Enat, R., et al. (1999). A pilot study on the hemodynamic effect of short-term ursodeoxycholic acid therapy in patients with stable liver cirrhosis. *Am. J. Gastroenterol.* 94, 3000–3004. doi: 10.1111/j.1572-0241.1999.01450.x

Bass, N. M., Mullen, K. D., Sanyal, A., Poordad, F., Neff, G., Leevy, C. B., et al. (2010). Rifaximin treatment in hepatic encephalopathy. *N. Engl. J. Med.* 362, 1071–1081. doi: 10.1056/NEJMoa0907893

Battista, S., Bar, F., Mengozzi, G., Zanon, E., Grosso, M., and Molino, G. (1997). Hyperdynamic circulation in patients with cirrhosis: direct measurement of nitric oxide levels in hepatic and portal veins. *J. Hepatol.* 26, 75–80. doi: 10.1016/S0168-8278(97)80012-8

Begley, M., Gahan, C. G., and Hill, C. (2005). The interaction between bacteria and bile. *FEMS Microbiol. Rev.* 29, 625–651. doi: 10.1016/j.femsre.2004.09.003

Bernardi, M. (2013). Cirrhotic cardiomyopathy. *Clin. Liver Dis. (Hoboken)* 2, 99–101. doi: 10.1002/cld.141

Bernardi, M., Angeli, P., Claria, J., Moreau, R., Gines, P., Jalan, R., et al. (2020). Albumin in decompensated cirrhosis: new concepts and perspectives. *Gut* 69, 1127–1138. doi: 10.1136/gutjnl-2019-318843

Beuers, U., Spengler, U., Zwiebel, F. M., Pauletzki, J., Fischer, S., and Paumgartner, G. (1992). Effect of ursodeoxycholic acid on the kinetics of the major hydrophobic bile acids in health and in chronic cholestatic liver disease. *Hepatology* 15, 603–608. doi: 10.1002/hep.1840150409

Beuers, U., Trauner, M., Jansen, P., and Poupon, R. (2015). New paradigms in the treatment of hepatic cholestasis: from UDCA to FXR, PXR and beyond. *J. Hepatol.* 62(Suppl. 1), S25–S37. doi: 10.1016/j.jhep.2015.02.023

Bhagat, K., and Vallance, P. (1996). Nitric oxide 9 years on. *J. R. Soc. Med.* 89, 667–673. doi: 10.1177/014107689608901204

Biecker, E., Trebicka, J., Kang, A., Hennenberg, M., Sauerbruch, T., and Heller, J. (2008). Treatment of bile duct-ligated rats with the nitric oxide synthase transcription enhancer AVE 9488 ameliorates portal hypertension. *Liver Int.* 28, 331–338. doi: 10.1111/j.1478-3231.2008.01664.x

Blendis, L., and Wong, F. (2001). The hyperdynamic circulation in cirrhosis: an overview. *Pharmacol. Ther.* 89, 221–231. doi: 10.1016/S0163-7258(01)00124-3

Bode, N., Grebe, A., Kerksiek, A., Lutjohann, D., Werner, N., Nickenig, G., et al. (2016). Ursodeoxycholic acid impairs atherogenesis and promotes plaque regression by cholesterol crystal dissolution in mice. *Biochem. Biophys. Res. Commun.* 478, 356–362. doi: 10.1016/j.bbrc.2016.07.047

Bomzon, A., and Ljubuncic, P. (1995). Bile acids as endogenous vasodilators? *Biochem. Pharmacol.* 49, 581–589. doi: 10.1016/0006-2952(94)00428-O

Bosch, J., Arroyo, V., Betriu, A., Mas, A., Carrilho, F., Rivera, F., et al. (1980). Hepatic hemodynamics and the renin-angiotensin-aldosterone system in cirrhosis. *Gastroenterology* 78, 92–99. doi: 10.1016/0016-5085(80)90197-3

Brandl, K., Hartmann, P., Jih, L. J., Pizzo, D. P., Argemi, J., Ventura-Cots, M., et al. (2018). Dysregulation of serum bile acids and FGF19 in alcoholic hepatitis. *J. Hepatol.* 69, 396–405. doi: 10.1016/j.jhep.2018.03.031

Capocaccia, L., Attili, A. F., Cantafora, A., Bracci, F., Paciscopi, L., Puoti, C., et al. (1981). Sulfated bile acids in serum, bile, and urine of cirrhotic patients before and after portacaval anastomosis. *Dig. Dis. Sci.* 26, 513–517. doi: 10.1007/BF01308099

Caraceni, P., Vargas, V., Sola, E., Alessandria, C., de Wit, K., Trebicka, J., et al. (2021). The use of Rifaximin in patients with cirrhosis. *Hepatology* doi: 10.1002/hep.31708 [Epub ahead of print]

Carey, M. C., and Duane, W. C. (1994). *Enterohepatic Circulation. 3rd Edn*. New York: Raven Press, 719–767.

Ceryak, S., Bouscarel, B., and Fromm, H. (1993). Comparative binding of bile acids to serum lipoproteins and albumin. *J. Lipid Res.* 34, 1661–1674. doi: 10.1016/S0022-2275(20)35729-1

Chen, M. L., Takeda, K., and Sundrud, M. S. (2019). Emerging roles of bile acids in mucosal immunity and inflammation. *Mucosal Immunol.* 12, 851–861. doi: 10.1038/s41385-019-0162-4

Chen, W., Wei, Y., Xiong, A., Li, Y., Guan, H., Wang, Q., et al. (2020). Comprehensive analysis of serum and fecal bile acid profiles and interaction with gut microbiota in primary biliary cholangitis. *Clin. Rev. Allergy Immunol.* 58, 25–38. doi: 10.1007/s12016-019-08731-2

Chiang, J. Y. L., and Ferrell, J. M. (2020). Bile acid biology, pathophysiology, and therapeutics. *Clin. Liver. Dis. (Hoboken)* 15, 91–94. doi: 10.1002/cld.861

Ciocan, D., Voican, C. S., Wrzosek, L., Hugot, C., Rainteau, D., Humbert, L., et al. (2018). Bile acid homeostasis and intestinal dysbiosis in alcoholic hepatitis. *Aliment. Pharmacol. Ther.* 48, 961–974. doi: 10.1111/apt.14949

de Vries, E., and Beuers, U. (2017). Management of cholestatic disease in 2017. *Liver Int.* 37(Suppl. 1), 123–129. doi: 10.1111/liv.13306

Dennett, D. C. (2017). *From Bacteria to BACH and Back: The Evolution of Minds*. New York: WW Norton & Company.

Desai, M. S., Mathur, B., Eblimit, Z., Vasquez, H., Taegtmeyer, H., Karpen, S. J., et al. (2017). Bile acid excess induces cardiomyopathy and metabolic dysfunctions in the heart. *Hepatology* 65, 189–201. doi: 10.1002/hep.28890

Deutschmann, K., Reich, M., Klindt, C., Droge, C., Spomer, L., Haussinger, D., et al. (2018). Bile acid receptors in the biliary tree: TGR5 in physiology and disease. *Biochim. Biophys. Acta Mol. basis Dis.* 1864, 1319–1325. doi: 10.1016/j.bbadis.2017.08.021

Dhiman, R. K., Rana, B., Agrawal, S., Garg, A., Chopra, M., Thumburu, K. K., et al. (2014). Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* 147, 1327–1337.e1323. doi: 10.1053/j.gastro.2014.08.031

Di Pascoli, M., Sacerdoti, D., Pontisso, P., Angeli, P., and Bolognesi, M. (2017). Molecular mechanisms leading to splanchnic vasodilation in liver cirrhosis. *J. Vasc. Res.* 54, 92–99. doi: 10.1159/000462974

Dilger, K., Hohenester, S., Winkler-Budenhofer, U., Bastiaansen, B. A., Schaap, F. G., Rust, C., et al. (2012). Effect of ursodeoxycholic acid on bile acid profiles and intestinal detoxification machinery in primary biliary cirrhosis and health. *J. Hepatol.* 57, 133–140. doi: 10.1016/j.jhep.2012.02.014

Dopico, A. M., Walsh, J. V. Jr., and Singer, J. J. (2002). Natural bile acids and synthetic analogues modulate large conductance Ca^{2+}-activated K^+ (BKCa) channel activity in smooth muscle cells. *J. Gen. Physiol.* 119, 251–273. doi: 10.1085/jgp.20028537

Duboc, H., Tache, Y., and Hofmann, A. F. (2014). The bile acid TGR5 membrane receptor: from basic research to clinical application. *Dig. Liver Dis.* 46, 302–312. doi: 10.1016/j.dld.2013.10.021

Fairfield, B., and Schnabl, B. (2021). Gut dysbiosis as a driver in alcohol-induced liver injury. *JHEP Rep.* 3:100220. doi: 10.1016/j.jhepr.2020.100220

Ferguson, J. W., Dover, A. R., Chia, S., Cruden, N. L., Hayes, P. C., and Newby, D. E. (2006). Inducible nitric oxide synthase activity contributes to the regulation of peripheral vascular tone in patients with cirrhosis and ascites. *Gut* 55, 542–546. doi: 10.1136/gut.2005.076562

Fernandez, J., Angeli, P., Trebicka, J., Merli, M., Gustot, T., Alessandria, C., et al. (2020). Efficacy of albumin treatment for patients with cirrhosis and infections unrelated to spontaneous bacterial peritonitis. *Clin. Gastroenterol. Hepatol.* 18, 963–973.e914. doi: 10.1016/j.cgh.2019.07.055

Fernandez, J., Claria, J., Amoros, A., Aguilar, F., Castro, M., Casulleras, M., et al. (2019). Effects of albumin treatment on systemic and portal hemodynamics and systemic inflammation in patients with decompensated cirrhosis. *Gastroenterology* 157, 149–162. doi: 10.1053/j.gastro.2019.03.021

Fickert, P., and Wagner, M. (2017). Biliary bile acids in hepatobiliary injury-what is the link? *J. Hepatol.* 67, 619–631. doi: 10.1016/j.jhep.2017.04.026

Fiorucci, S., Distrutti, E., Carino, A., Zampella, A., and Biagioli, M. (2021). Bile acids and their receptors in metabolic disorders. *Prog. Lipid Res.* 82:101094. doi: 10.1016/j.plipres.2021.101094

Flynn, C. R., Albaugh, V. L., Cai, S., Cheung-Flynn, J., Williams, P. E., Brucker, R. M., et al. (2015). Bile diversion to the distal small intestine has comparable metabolic benefits to bariatric surgery. *Nat. Commun.* 6:7715. doi: 10.1038/ncomms8715

Fryer, R. M., Ng, K. J., Nodop Mazurek, S. G., Patnaude, L., Skow, D. J., Muthukumarana, A., et al. (2014). G protein-coupled bile acid receptor 1 stimulation mediates arterial vasodilation through a K(Ca)1.1 (BK(Ca))-dependent mechanism. *J. Pharmacol. Exp. Ther.* 348, 421–431. doi: 10.1124/jpet.113.210005

Gadaleta, R. M., van Erpecum, K. J., Oldenburg, B., Willemsen, E. C., Renooij, W., Murzilli, S., et al. (2011). Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* 60, 463–472. doi: 10.1136/gut.2010.212159

Genecin, P., Polio, J., Colombato, L. A., Ferraioli, G., Reuben, A., and Groszmann, R. J. (1990). Bile acids do not mediate the hyperdynamic circulation in portal hypertensive rats. *Am. J. Phys.* 259, G21–G25. doi: 10.1152/ajpgi.1990.259.1.G21

Goldberg, D. S., and Fallon, M. B. (2015). The art and science of diagnosing and treating lung and heart disease secondary to liver disease. *Clin. Gastroenterol. Hepatol.* 13, 2118–2127. doi: 10.1016/j.cgh.2015.04.024

Gong, Z., Zhou, J., Zhao, S., Tian, C., Wang, P., Xu, C., et al. (2016). Chenodeoxycholic acid activates NLRP3 inflammasome and contributes to cholestatic liver fibrosis. *Oncotarget* 7, 83951–83963. doi: 10.18632/oncotarget.13796

Grace, J. A., Klein, S., Herath, C. B., Granzow, M., Schierwagen, R., Masing, N., et al. (2013). Activation of the MAS receptor by angiotensin-(1-7) in the renin-angiotensin system mediates mesenteric vasodilatation in cirrhosis. *Gastroenterology* 145, 874–884.e875. doi: 10.1053/j.gastro.2013.06.036

Guizoni, D. M., Vettorazzi, J. F., Carneiro, E. M., and Davel, A. P. (2020). Modulation of endothelium-derived nitric oxide production and activity by taurine and taurine-conjugated bile acids. *Nitric Oxide* 94, 48–53. doi: 10.1016/j.niox.2019.10.008

Guo, C., Chen, W. D., and Wang, Y. D. (2016a). TGR5, not only a metabolic regulator. *Front. Physiol.* 7:646. doi: 10.3389/fphys.2016.00646

Guo, C., Xie, S., Chi, Z., Zhang, J., Liu, Y., Zhang, L., et al. (2016b). Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. *Immunity* 45, 802–816. doi: 10.1016/j.immuni.2016.09.008

Gurusamy, S. R., Shah, A., Talley, N. J., Koloski, N., Jones, M. P., Walker, M. M., et al. (2021). Small intestinal bacterial overgrowth in functional dyspepsia: a systematic review and meta-analysis. *Am. J. Gastroenterol.* 116, 935–942. doi: 10.14309/ajg.0000000000001197

Haran, J. P., and McCormick, B. A. (2021). Aging, frailty, and the microbiome-how dysbiosis influences human aging and disease. *Gastroenterology* 160, 507–523. doi: 10.1053/j.gastro.2020.09.060

He, F., Li, J., Mu, Y., Kuruba, R., Ma, Z., Wilson, A., et al. (2006). Downregulation of endothelin-1 by farnesoid X receptor in vascular endothelial cells. *Circ. Res.* 98, 192–199. doi: 10.1161/01.RES.0000200400.55539.85

Heller, J., Kristeleit, H., Brensing, K. A., Woitas, R. P., Spengler, U., and Sauerbruch, T. (1999a). Nitrite and nitrate levels in patients with cirrhosis of the liver: influence of kidney function and fasting state. *Scand. J. Gastroenterol.* 34, 297–302. doi: 10.1080/00365529950173726

Heller, J., Schepke, M., Gehnen, N., Molderings, G. J., Muller, A., Erhard, J., et al. (1999b). Altered adrenergic responsiveness of endothelium-denuded hepatic arteries and portal veins in patients with cirrhosis. *Gastroenterology* 116, 387–393. doi: 10.1016/s0016-5085(99)70136-8

Heller, J., Trebicka, J., Shiozawa, T., Schepke, M., Neef, M., Hennenberg, M., et al. (2005). Vascular, hemodynamic and renal effects of low-dose losartan in rats with secondary biliary cirrhosis. *Liver Int.* 25, 657–666. doi: 10.1111/j.1478-3231.2005.01053.x

Helmy, A., Jalan, R., Newby, D. E., Hayes, P. C., and Webb, D. J. (2000). Role of angiotensin II in regulation of basal and sympathetically stimulated vascular tone in early and advanced cirrhosis. *Gastroenterology* 118, 565–572. doi: 10.1016/S0016-5085(00)70263-0

Helmy, A., Newby, D. E., Jalan, R., Johnston, N. R., Hayes, P. C., and Webb, D. J. (2003). Nitric oxide mediates the reduced vasoconstrictor response to angiotensin II in patients with preascitic cirrhosis. *J. Hepatol.* 38, 44–50. doi: 10.1016/S0168-8278(02)00319-7

Hennenberg, M., Biecker, E., Trebicka, J., Jochem, K., Zhou, Q., Schmidt, M., et al. (2006). Defective RhoA/Rho-kinase signaling contributes to vascular hypocontractility and vasodilation in cirrhotic rats. *Gastroenterology* 130, 838–854. doi: 10.1053/j.gastro.2005.11.029

Hennenberg, M., Trebicka, J., Biecker, E., Schepke, M., Sauerbruch, T., and Heller, J. (2007). Vascular dysfunction in human and rat cirrhosis: role of receptor-desensitizing and calcium-sensitizing proteins. *Hepatology* 45, 495–506. doi: 10.1002/hep.21502

Hennenberg, M., Trebicka, J., Buecher, D., Heller, J., and Sauerbruch, T. (2009a). Lack of effect of norfloxacin on hyperdynamic circulation in bile duct-ligated rats despite reduction of endothelial nitric oxide synthase function: result of unchanged vascular Rho-kinase? *Liver Int.* 29, 933–941. doi: 10.1111/j.1478-3231.2009.02010.x

Hennenberg, M., Trebicka, J., Kohistani, A. Z., Heller, J., and Sauerbruch, T. (2009b). Vascular hyporesponsiveness to angiotensin II in rats with CCl(4)-induced liver cirrhosis. *Eur. J. Clin. Investig.* 39, 906–913. doi: 10.1111/j.1365-2362.2009.02181.x

Hennenberg, M., Trebicka, J., Sauerbruch, T., and Heller, J. (2008). Mechanisms of extrahepatic vasodilation in portal hypertension. *Gut* 57, 1300–1314. doi: 10.1136/gut.2007.144584

Henriksen, J. H., Ring-Larsen, H., Kanstrup, I. L., and Christensen, N. J. (1984). Splanchnic and renal elimination and release of catecholamines in cirrhosis. Evidence of enhanced sympathetic nervous activity in patients with decompensated cirrhosis. *Gut* 25, 1034–1043. doi: 10.1136/gut.25.10.1034

Hofmann, A. F. (1994). "Bile Acids," in *The Liver: Biology and Pathobiology. 3rd Edn*. eds. I. M. Arias, J. L. Boyer, N. Fausto, W. B. Jakoby, D. Schachter and D. A. Shafritz (New York: Raven Press), 677–718.

Hofmann, A. F. (2009). Bile acids: trying to understand their chemistry and biology with the hope of helping patients. *Hepatology* 49, 1403–1418. doi: 10.1002/hep.22789

Hofmann, A. F., and Hagey, L. R. (2014). Key discoveries in bile acid chemistry and biology and their clinical applications: history of the last eight decades. *J. Lipid Res.* 55, 1553–1595. doi: 10.1194/jlr.R049437

Horak, W., Gangl, A., Funovics, J., and Grabner, G. (1975). Effect of portacaval shunt and arterialization of the liver on bile acid metabolism. *Gastroenterology* 69, 338–341. doi: 10.1016/S0016-5085(19)32574-0

Horvatits, T., Drolz, A., Roedl, K., Rutter, K., Ferlitsch, A., Fauler, G., et al. (2017a). Serum bile acids as marker for acute decompensation and acute-on-chronic liver failure in patients with non-cholestatic cirrhosis. *Liver Int.* 37, 224–231. doi: 10.1111/liv.13201

Horvatits, T., Drolz, A., Rutter, K., Roedl, K., Fauler, G., Muller, C., et al. (2017b). Serum bile acids in patients with hepatopulmonary syndrome. *Z. Gastroenterol.* 55, 361–367. doi: 10.1055/s-0042-121268

Huet, P. M., Vincent, C., Deslaurier, J., Cote, J., Matsutami, S., Boileau, R., et al. (2008). Portal hypertension and primary biliary cirrhosis: effect of long-term ursodeoxycholic acid treatment. *Gastroenterology* 135, 1552–1560. doi: 10.1053/j.gastro.2008.07.019

Inagaki, T., Moschetta, A., Lee, Y. K., Peng, L., Zhao, G., Downes, M., et al. (2006). Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3920–3925. doi: 10.1073/pnas.0509592103

Iwakiri, Y., and Groszmann, R. J. (2007). Vascular endothelial dysfunction in cirrhosis. *J. Hepatol.* 46, 927–934. doi: 10.1016/j.jhep.2007.02.006

Iwakiri, Y., Shah, V., and Rockey, D. C. (2014). Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. *J. Hepatol.* 61, 912–924. doi: 10.1016/j.jhep.2014.05.047

Jadeja, R. N., Thounaojam, M. C., Bartoli, M., and Khurana, S. (2018). Deoxycholylglycine, a conjugated secondary bile acid, reduces vascular tone by attenuating Ca(2+) sensitivity via rho kinase pathway. *Toxicol. Appl. Pharmacol.* 348, 14–21. doi: 10.1016/j.taap.2018.04.012

Kamath, P. S., Wiesner, R. H., Malinchoc, M., Kremers, W., Therneau, T. M., Kosberg, C. L., et al. (2001). A model to predict survival in patients with end-stage liver disease. *Hepatology* 33, 464–470. doi: 10.1053/jhep.2001.22172

Kang, D. J., Kakiyama, G., Betrapally, N. S., Herzog, J., Nittono, H., Hylemon, P. B., et al. (2016). Rifaximin exerts beneficial effects independent of its ability to alter microbiota composition. *Clin. Transl. Gastroenterol.* 7:e187. doi: 10.1038/ctg.2016.44

Keitel, V., Reinehr, R., Gatsios, P., Rupprecht, C., Gorg, B., Selbach, O., et al. (2007). The G-protein coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells. *Hepatology* 45, 695–704. doi: 10.1002/hep.21458

Khurana, S., Yamada, M., Wess, J., Kennedy, R. H., and Raufman, J. P. (2005). Deoxycholyltaurine-induced vasodilation of rodent aorta is nitric oxide- and muscarinic M(3) receptor-dependent. *Eur. J. Pharmacol.* 517, 103–110. doi: 10.1016/j.ejphar.2005.05.037

Kida, T., Tsubosaka, Y., Hori, M., Ozaki, H., and Murata, T. (2013). Bile acid receptor TGR5 agonism induces NO production and reduces monocyte adhesion in vascular endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 33, 1663–1669. doi: 10.1161/ATVBAHA.113.301565

Kimer, N., Pedersen, J. S., Busk, T. M., Gluud, L. L., Hobolth, L., Krag, A., et al. (2017). Rifaximin has no effect on hemodynamics in decompensated cirrhosis: a randomized, double-blind, placebo-controlled trial. *Hepatology* 65, 592–603. doi: 10.1002/hep.28898

Kimer, N., Pedersen, J. S., Tavenier, J., Christensen, J. E., Busk, T. M., Hobolth, L., et al. (2018). Rifaximin has minor effects on bacterial composition, inflammation, and bacterial translocation in cirrhosis: a randomized trial. *J. Gastroenterol. Hepatol.* 33, 307–314. doi: 10.1111/jgh.13852

Kiriyama, Y., and Nochi, H. (2019). The biosynthesis, signaling, and neurological functions of bile acids. *Biomol. Ther.* 9:232. doi: 10.3390/biom9060232

Kliewer, S. A., and Mangelsdorf, D. J. (2015). Bile acids as hormones: the FXR-FGF15/19 pathway. *Dig. Dis.* 33, 327–331. doi: 10.1159/000371670

Kramer, W., Buscher, H. P., Gerok, W., and Kurz, G. (1979). Bile salt binding to serum components. Taurocholate incorporation into high-density lipoprotein revealed by photoaffinity labelling. *Eur. J. Biochem.* 102, 1–9. doi: 10.1111/j.1432-1033.1979.tb06257.x

LaRusso, N. F., Korman, M. G., Hoffman, N. E., and Hofmann, A. F. (1974). Dynamics of the enterohepatic circulation of bile acids. Postprandial serum concentrations of conjugates of cholic acid in health, cholecystectomized patients, and patients with bile acid malabsorption. *N. Engl. J. Med.* 291, 689–692. doi: 10.1056/NEJM197410032911401

Leonhardt, J., Haider, R. S., Sponholz, C., Leonhardt, S., Drube, J., Spengler, K., et al. (2021). Circulating bile acids in liver failure activate TGR5 and induce monocyte dysfunction. *Cell. Mol. Gastroenterol. Hepatol.* 12, 25–40. doi: 10.1016/j.jcmgh.2021.01.011

Li, B., Wang, R., Wang, Y., Stief, C. G., and Hennenberg, M. (2020). Regulation of smooth muscle contraction by monomeric non-RhoA GTPases. *Br. J. Pharmacol.* 177, 3865–3877. doi: 10.1111/bph.15172

Li, J., Wilson, A., Kuruba, R., Zhang, Q., Gao, X., He, F., et al. (2008). FXR-mediated regulation of eNOS expression in vascular endothelial cells. *Cardiovasc. Res.* 77, 169–177. doi: 10.1093/cvr/cvm016

Lindor, K. D., Kowdley, K. V., Luketic, V. A., Harrison, M. E., McCashland, T., Befeler, A. S., et al. (2009). High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 50, 808–814. doi: 10.1002/hep.23082

Liu, Z., Zhang, Z., Huang, M., Sun, X., Liu, B., Guo, Q., et al. (2018). Taurocholic acid is an active promoting factor, not just a biomarker of progression of liver cirrhosis: evidence from a human metabolomic study and in vitro experiments. *BMC Gastroenterol.* 18:112. doi: 10.1186/s12876-018-0842-7

Ljubuncic, P., Said, O., Ehrlich, Y., Meddings, J. B., Shaffer, E. A., and Bomzon, A. (2000). On the in vitro vasoactivity of bile acids. *Br. J. Pharmacol.* 131, 387–398. doi: 10.1038/sj.bjp.0703554

Mannes, G. A., Thieme, C., Stellaard, F., Wang, T., Sauerbruch, T., and Paumgartner, G. (1986). Prognostic significance of serum bile acids in cirrhosis. *Hepatology* 6, 50–53. doi: 10.1002/hep.1840060110

Maroto, A., Gines, A., Salo, J., Claria, J., Gines, P., Anibarro, L., et al. (1994). Diagnosis of functional kidney failure of cirrhosis with Doppler sonography: prognostic value of resistive index. *Hepatology* 20, 839–844. doi: 10.1002/hep.1840200411

Mendoza, Y. P., Rodrigues, S. G., Bosch, J., and Berzigotti, A. (2020). Effect of poorly absorbable antibiotics on hepatic venous pressure gradient in cirrhosis: A systematic review and meta-analysis. *Dig. Liver Dis.* 52, 958–965. doi: 10.1016/j.dld.2020.06.048

Moleda, L., Trebicka, J., Dietrich, P., Gabele, E., Hellerbrand, C., Straub, R. H., et al. (2011). Amelioration of portal hypertension and the hyperdynamic circulatory syndrome in cirrhotic rats by neuropeptide Y via pronounced splanchnic vasoaction. *Gut* 60, 1122–1132. doi: 10.1136/gut.2010.226407

Moll-Kaufmann, C., Sumanovski, L. T., and Sieber, C. C. (1998). Neurally-mediated vasodilatation in normal and portal hypertensive rats: role of nitric oxide and calcitonin gene-related peptide. *J. Hepatol.* 28, 1031–1036. doi: 10.1016/S0168-8278(98)80353-X

Moller, S., Hobolth, L., Winkler, C., Bendtsen, F., and Christensen, E. (2011). Determinants of the hyperdynamic circulation and central hypovolaemia in cirrhosis. *Gut* 60, 1254–1259. doi: 10.1136/gut.2010.235473

Monteiro, S., Grandt, J., Uschner, F. E., Kimer, N., Madsen, J. L., Schierwagen, R., et al. (2021). Differential inflammasome activation predisposes to acute-on-chronic liver failure in human and experimental cirrhosis with and without previous decompensation. *Gut* 70, 379–387. doi: 10.1136/gutjnl-2019-320170

Moreau, R., Jalan, R., Gines, P., Pavesi, M., Angeli, P., Cordoba, J., et al. (2013). Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 144, 1426–1437. e1–9. doi: 10.1053/j.gastro.2013.02.042

Nakajima, T., Okuda, Y., Chisaki, K., Shin, W. S., Iwasawa, K., Morita, T., et al. (2000). Bile acids increase intracellular Ca(2+) concentration and nitric oxide production in vascular endothelial cells. *Br. J. Pharmacol.* 130, 1457–1467. doi: 10.1038/sj.bjp.0703471

Neef, M., Biecker, E., Heller, J., Schepke, M., Nischalke, H. D., Wolff, M., et al. (2003). Portal hypertension is associated with increased mRNA levels of vasopressor G-protein-coupled receptors in human hepatic arteries. *Eur. J. Clin. Investig.* 33, 249–255. doi: 10.1046/j.1365-2362.2003.01131.x

Neubrand, M. W., Dominguez-Munoz, J. E., Reichel, C., Kampmann, S., Eschmann, K., von Falkenhausen, M., et al. (2004). Effect of intraduodenal administration of ursodeoxycholic acid on interdigestive interaction between gallbladder motility, pancreatic secretion and endocrine activity. *Digestion* 69, 149–157. doi: 10.1159/000078153

Nevens, F., Andreone, P., Mazzella, G., Strasser, S. I., Bowlus, C., Invernizzi, P., et al. (2016). A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. *N. Engl. J. Med.* 375, 631–643. doi: 10.1056/NEJMoa1509840

Ohkubo, H., Okuda, K., Iida, S., Ohnishi, K., Ikawa, S., and Makino, I. (1984). Role of portal and splenic vein shunts and impaired hepatic extraction in the elevated serum bile acids in liver cirrhosis. *Gastroenterology* 86, 514–520. doi: 10.1016/S0016-5085(84)80022-0

Pak, J. M., Adeagbo, A. S., Triggle, C. R., Shaffer, E. A., and Lee, S. S. (1994). Mechanism of bile salt vasoactivity: dependence on calcium channels in vascular smooth muscle. *Br. J. Pharmacol.* 112, 1209–1215. doi: 10.1111/j.1476-5381.1994.tb13212.x

Pak, J. M., and Lee, S. S. (1993). Vasoactive effects of bile salts in cirrhotic rats: in vivo and in vitro studies. *Hepatology* 18, 1175–1181.

Pelletier, G., Roulot, D., Davion, T., Masliah, C., Causse, X., Oberti, F., et al. (2003). A randomized controlled trial of ursodeoxycholic acid in patients with alcohol-induced cirrhosis and jaundice. *Hepatology* 37, 887–892. doi: 10.1053/jhep.2003.50118

Ponz de Leon, M., Loria, P., Iori, R., and Carulli, N. (1981). Cholesterol absorption in cirrhosis: the role of total and individual bile acid pool size. *Gastroenterology* 80, 1428–1437. doi: 10.1016/0016-5085(81)90253-5

Ponziani, F. R., Zocco, M. A., D'Aversa, F., Pompili, M., and Gasbarrini, A. (2017). Eubiotic properties of rifaximin: disruption of the traditional concepts in gut microbiota modulation. *World J. Gastroenterol.* 23, 4491–4499. doi: 10.3748/wjg.v23.i25.4491

Poupon, R. (2014). Evidence-based treatment of primary biliary cirrhosis. *Dig. Dis.* 32, 626–630. doi: 10.1159/000360516

Poupon, R., Poupon, R., Grosdemouge, M. L., Dumont, M., and Erlinger, S. (1976). Influence of bile acids upon biliary cholesterol and phospholipid

secretion in the dog. *Eur. J. Clin. Investig.* 6, 279–284. doi: 10.1111/j.1365-2362.1976.tb00522.x

Poupon, R. E., Chretien, Y., Poupon, R., and Paumgartner, G. (1993). Serum bile acids in primary biliary cirrhosis: effect of ursodeoxycholic acid therapy. *Hepatology* 17, 599–604. doi: 10.1002/hep.1840170412

Praktiknjo, M., Monteiro, S., Grandt, J., Kimer, N., Madsen, J. L., Werge, M. P., et al. (2020a). Cardiodynamic state is associated with systemic inflammation and fatal acute-on-chronic liver failure. *Liver Int.* 40, 1457–1466. doi: 10.1111/liv.14433

Praktiknjo, M., Schierwagen, R., Monteiro, S., Ortiz, C., Uschner, F. E., Jansen, C., et al. (2020b). Hepatic inflammasome activation as origin of interleukin-1alpha and interleukin-1beta in liver cirrhosis. *Gut* doi: 10.1136/gutjnl-2020-322621 [Epub ahead of print]

Pu, J., Yuan, A., Shan, P., Gao, E., Wang, X., Wang, Y., et al. (2013). Cardiomyocyte-expressed farnesoid-X-receptor is a novel apoptosis mediator and contributes to myocardial ischaemia/reperfusion injury. *Eur. Heart J.* 34, 1834–1845. doi: 10.1093/eurheartj/ehs011

Qin, N., Yang, F., Li, A., Prifti, E., Chen, Y., Shao, L., et al. (2014). Alterations of the human gut microbiome in liver cirrhosis. *Nature* 513, 59–64. doi: 10.1038/nature13568

Rainer, P. P., Primessnig, U., Harenkamp, S., Doleschal, B., Wallner, M., Fauler, G., et al. (2013). Bile acids induce arrhythmias in human atrial myocardium--implications for altered serum bile acid composition in patients with atrial fibrillation. *Heart* 99, 1685–1692. doi: 10.1136/heartjnl-2013-304163

Rasaratnam, B., Kaye, D., Jennings, G., Dudley, F., and Chin-Dusting, J. (2003). The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann. Intern. Med.* 139, 186–193. doi: 10.7326/0003-4819-139-3-200308050-00008

Reardon, J., Hussaini, T., Alsahafi, M., Azalgara, V. M., Erb, S. R., Partovi, N., et al. (2016). Ursodeoxycholic acid in treatment of non-cholestatic liver diseases: a systematic review. *J. Clin. Transl. Hepatol.* 4, 192–205. doi: 10.14218/JCTH.2016.00023

Ridlon, J. M., Kang, D. J., Hylemon, P. B., and Bajaj, J. S. (2015). Gut microbiota, cirrhosis, and alcohol regulate bile acid metabolism in the gut. *Dig. Dis.* 33, 338–345. doi: 10.1159/000371678

Roda, A., Cappelleri, G., Aldini, R., Roda, E., and Barbara, L. (1982). Quantitative aspects of the interaction of bile acids with human serum albumin. *J. Lipid Res.* 23, 490–495. doi: 10.1016/S0022-2275(20)38147-5

Roda, A., Minutello, A., Angellotti, M. A., and Fini, A. (1990). Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partition coefficient and reverse phase HPLC. *J. Lipid Res.* 31, 1433–1443. doi: 10.1016/S0022-2275(20)42614-8

Rudman, D., and Kendall, F. E. (1957). Bile acid content of human serum. II. The binding of cholanic acids by human plasma proteins. *J. Clin. Invest.* 36, 538–542. doi: 10.1172/JCI103451

Sailer, C., Pauletzki, J., Klueppelberg, U. G., Fischer, S., Sackmann, M., and Paumgartner, G. (1996). Acute effect of ursodeoxycholic acid on gallbladder volume in healthy subjects. *Scand. J. Gastroenterol.* 31, 512–516. doi: 10.3109/00365529609006774

Sarin, S. K., Pande, A., and Schnabl, B. (2019). Microbiome as a therapeutic target in alcohol-related liver disease. *J. Hepatol.* 70, 260–272. doi: 10.1016/j.jhep.2018.10.019

Schalm, S. W., LaRusso, N. F., Hofmann, A. F., Hoffman, N. E., van Berge-Henegouwen, G. P., and Korman, M. G. (1978). Diurnal serum levels of primary conjugated bile acids. Assessment by specific radioimmunoassays for conjugates of cholic and chenodeoxycholic acid. *Gut* 19, 1006–1014. doi: 10.1136/gut.19.11.1006

Schepke, M., Heller, J., Paschke, S., Thomas, J., Wolff, M., Neef, M., et al. (2001a). Contractile hyporesponsiveness of hepatic arteries in humans with cirrhosis: evidence for a receptor-specific mechanism. *Hepatology* 34, 884–888. doi: 10.1053/jhep.2001.28794

Schepke, M., Werner, E., Biecker, E., Schiedermaier, P., Heller, J., Neef, M., et al. (2001b). Hemodynamic effects of the angiotensin II receptor antagonist irbesartan in patients with cirrhosis and portal hypertension. *Gastroenterology* 121, 389–395. doi: 10.1053/gast.2001.26295

Schiedermaier, P., Hansen, S., Asdonk, D., Brensing, K., and Sauerbruch, T. (2000). Effects of ursodeoxycholic acid on splanchnic and systemic hemodynamics. A double-blind, cross-over, placebo-controlled study in healthy volunteers. *Digestion* 61, 107–112. doi: 10.1159/000007742

Schrier, R. W., Arroyo, V., Bernardi, M., Epstein, M., Henriksen, J. H., and Rodes, J. (1988). Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 8, 1151–1157. doi: 10.1002/hep.1840080532

Schwabl, P., Hambruch, E., Seeland, B. A., Hayden, H., Wagner, M., Garnys, L., et al. (2017). The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. *J. Hepatol.* 66, 724–733. doi: 10.1016/j.jhep.2016.12.005

Shamsaddini, A., Gillevet, P. M., Acharya, C., Fagan, A., Gavis, E., Sikaroodi, M., et al. (2021). Impact of antibiotic resistance genes in gut microbiome of patients with cirrhosis. *Gastroenterology* doi: 10.1053/j.gastro.2021.04.013 [Epub ahead of print]

Simon-Talero, M., Roccarina, D., Martinez, J., Lampichler, K., Baiges, A., Low, G., et al. (2018). Association between portosystemic shunts and increased complications and mortality in patients with cirrhosis. *Gastroenterology* 154, 1694–1705.e4. doi: 10.1053/j.gastro.2018.01.028

Sinisalo, J., Vanhanen, H., Pajunen, P., Vapaatalo, H., and Nieminen, M. S. (1999). Ursodeoxycholic acid and endothelial-dependent, nitric oxide-independent vasodilatation of forearm resistance arteries in patients with coronary heart disease. *Br. J. Clin. Pharmacol.* 47, 661–665. doi: 10.1046/j.1365-2125.1999.00940.x

Sola, E., and Gines, P. (2015). Challenges and management of liver cirrhosis: pathophysiology of renal dysfunction in cirrhosis. *Dig. Dis.* 33, 534–538. doi: 10.1159/000375344

Tang, R., Wei, Y., Li, Y., Chen, W., Chen, H., Wang, Q., et al. (2018). Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut* 67, 534–541. doi: 10.1136/gutjnl-2016-313332

Touyz, R. M., Alves-Lopes, R., Rios, F. J., Camargo, L. L., Anagnostopoulou, A., Arner, A., et al. (2018). Vascular smooth muscle contraction in hypertension. *Cardiovasc. Res.* 114, 529–539. doi: 10.1093/cvr/cvy023

Trebicka, J., Amoros, A., Pitarch, C., Titos, E., Alcaraz-Quiles, J., Schierwagen, R., et al. (2019). Addressing profiles of systemic inflammation across the different clinical phenotypes of acutely decompensated cirrhosis. *Front. Immunol.* 10:476. doi: 10.3389/fimmu.2019.00476

Trebicka, J., Bork, P., Krag, A., and Arumugam, M. (2021). Utilizing the gut microbiome in decompensated cirrhosis and acute-on-chronic liver failure. *Nat. Rev. Gastroenterol. Hepatol.* 18, 167–180. doi: 10.1038/s41575-020-00376-3

Trebicka, J., Hennenberg, M., Laleman, W., Shelest, N., Biecker, E., Schepke, M., et al. (2007). Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. *Hepatology* 46, 242–253. doi: 10.1002/hep.21673

Trebicka, J., Krag, A., Gansweid, S., Appenrodt, B., Schiedermaier, P., Sauerbruch, T., et al. (2011). Endotoxin and tumor necrosis factor-receptor levels in portal and hepatic vein of patients with alcoholic liver cirrhosis receiving elective transjugular intrahepatic portosystemic shunt. *Eur. J. Gastroenterol. Hepatol.* 23, 1218–1225. doi: 10.1097/MEG.0b013e32834a75dc

Trefflich, I., Marschall, H. U., Giuseppe, R. D., Stahlman, M., Michalsen, A., Lampen, A., et al. (2019). Associations between dietary patterns and bile acids-results from a cross-sectional study in vegans and omnivores. *Nutrients* 12:47. doi: 10.3390/nu12010047

Tufoni, M., Baldassarre, M., Zaccherini, G., Antognoli, A., and Caraceni, P. (2020). Hemodynamic and systemic effects of albumin in patients with advanced liver disease. *Curr. Hepatol. Rep.* 1–12. doi: 10.1007/s11901-020-00521-1 [Epub ahead of print]

Vallance, P., Collier, J., and Bhagat, K. (1997). Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? *Lancet* 349, 1391–1392. doi: 10.1016/S0140-6736(96)09424-X

Vasavan, T., Ferraro, E., Ibrahim, E., Dixon, P., Gorelik, J., and Williamson, C. (2018). Heart and bile acids - clinical consequences of altered bile acid metabolism. *Biochim. Biophys. Acta Mol. basis Dis.* 1864, 1345–1355. doi: 10.1016/j.bbadis.2017.12.039

Verbeke, L., Farre, R., Trebicka, J., Komuta, M., Roskams, T., Klein, S., et al. (2014). Obeticholic acid, a farnesoid X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats. *Hepatology* 59, 2286–2298. doi: 10.1002/hep.26939

Verbeke, L., Farre, R., Verbinnen, B., Covens, K., Vanuytsel, T., Verhaegen, J., et al. (2015). The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am. J. Pathol.* 185, 409–419. doi: 10.1016/j.ajpath.2014.10.009

Verbeke, L., Mannaerts, I., Schierwagen, R., Govaere, O., Klein, S., Vander Elst, I., et al. (2016). FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. *Sci. Rep.* 6:33453. doi: 10.1038/srep33453

Vlachogiannakos, J., Saveriadis, A. S., Viazis, N., Theodoropoulos, I., Foudoulis, K., Manolakopoulos, S., et al. (2009). Intestinal decontamination improves liver haemodynamics in patients with alcohol-related decompensated cirrhosis. *Aliment. Pharmacol. Ther.* 29, 992–999. doi: 10.1111/j.1365-2036.2009.03958.x

Vlachogiannakos, J., Viazis, N., Vasianopoulou, P., Vafiadis, I., Karamanolis, D. G., and Ladas, S. D. (2013). Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. *J. Gastroenterol. Hepatol.* 28, 450–455. doi: 10.1111/jgh.12070

Vlahcevic, Z. R., Goldman, M., Schwartz, C. C., Gustafsson, J., and Swell, L. (1981). Bile acid metabolism in cirrhosis. VII. Evidence for defective feedback control of bile acid synthesis. *Hepatology* 1, 146–150. doi: 10.1002/hep.1840010210

Voiosu, A., Wiese, S., Voiosu, T., Bendtsen, F., and Moller, S. (2017). Bile acids and cardiovascular function in cirrhosis. *Liver Int.* 37, 1420–1430. doi: 10.1111/liv.13394

von Haehling, S., Schefold, J. C., Jankowska, E. A., Springer, J., Vazir, A., Kalra, P. R., et al. (2012). Ursodeoxycholic acid in patients with chronic heart failure: a double-blind, randomized, placebo-controlled, crossover trial. *J. Am. Coll. Cardiol.* 59, 585–592. doi: 10.1016/j.jacc.2011.10.880

Voronova, V., Sokolov, V., Al-Khaifi, A., Straniero, S., Kumar, C., Peskov, K., et al. (2020). A physiology-based model of bile acid distribution and metabolism under healthy and pathologic conditions in human beings. *Cell. Mol. Gastroenterol. Hepatol.* 10, 149–170. doi: 10.1016/j.jcmgh.2020.02.005

Walsh, L. K., Restaino, R. M., Neuringer, M., Manrique, C., and Padilla, J. (2016). Administration of tauroursodeoxycholic acid prevents endothelial dysfunction caused by an oral glucose load. *Clin. Sci. (Lond.)* 130, 1881–1888. doi: 10.1042/CS20160501

Wang, X., Xie, G., Zhao, A., Zheng, X., Huang, F., Wang, Y., et al. (2016). Serum bile acids are associated with pathological progression of hepatitis B-induced cirrhosis. *J. Proteome Res.* 15, 1126–1134. doi: 10.1021/acs.jproteome.5b00217

Weiss, E., de la Grange, P., Defaye, M., Lozano, J. J., Aguilar, F., Hegde, P., et al. (2020). Characterization of blood immune cells in patients with decompensated cirrhosis including ACLF. *Front. Immunol.* 11:619039. doi: 10.3389/fimmu.2020.619039

Wiest, R., Das, S., Cadelina, G., Garcia-Tsao, G., Milstien, S., and Groszmann, R. J. (1999a). Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. *J. Clin. Invest.* 104, 1223–1233. doi: 10.1172/JCI7458

Wiest, R., and Groszmann, R. J. (2002). The paradox of nitric oxide in cirrhosis and portal hypertension: too much, not enough. *Hepatology* 35, 478–491. doi: 10.1053/jhep.2002.31432

Wiest, R., Shah, V., Sessa, W. C., and Groszmann, R. J. (1999b). NO overproduction by eNOS precedes hyperdynamic splanchnic circulation in portal hypertensive rats. *Am. J. Phys.* 276, G1043–G1051. doi: 10.1152/ajpgi.1999.276.4.G1043

Wong, F., Bomzon, A., Allard, J., Liu, P., and Blendis, L. (1999). Effects of ursodeoxycholic acid on systemic, renal and forearm haemodynamics and sodium homoeostasis in cirrhotic patients with refractory ascites. *Clin. Sci. (Lond.)* 96, 467–474. doi: 10.1042/CS19980275

Wong, F., Reddy, K. R., Tandon, P., O'Leary, J. G., Garcia-Tsao, G., Vargas, H. E., et al. (2020). Progression of stage 2 and 3 acute kidney injury in patients with decompensated cirrhosis and ascites. *Clin. Gastroenterol. Hepatol.* doi: 10.1016/j.cgh.2020.08.025 [Epub ahead of print]

Yang, A. M., Inamine, T., Hochrath, K., Chen, P., Wang, L., Llorente, C., et al. (2017). Intestinal fungi contribute to development of alcoholic liver disease. *J. Clin. Invest.* 127, 2829–2841. doi: 10.1172/JCI90562

Yang, Y. Y., Huang, Y. T., Lee, K. C., Lee, F. Y., Lee, T. Y., Hou, M. C., et al. (2009). Chronic administration of ursodeoxycholic acid decreases portal pressure in rats with biliary cirrhosis. *Clin. Sci. (Lond.)* 116, 71–79. doi: 10.1042/CS20080075

Zavecz, J. H., and Battarbee, H. D. (2010). The role of lipophilic bile acids in the development of cirrhotic cardiomyopathy. *Cardiovasc. Toxicol.* 10, 117–129. doi: 10.1007/s12012-010-9069-8

Zhang, R., Ran, H., Peng, L., Zhang, Y., Shen, W., Sun, T., et al. (2016). Farnesoid X receptor regulates vasoreactivity via angiotensin II type 2 receptor and the kallikrein-kinin system in vascular endothelial cells. *Clin. Exp. Pharmacol. Physiol.* 43, 327–334. doi: 10.1111/1440-1681.12535

Zhu, L., Wang, W., Xie, T. H., Zou, J., Nie, X., Wang, X., et al. (2020). TGR5 receptor activation attenuates diabetic retinopathy through suppression of RhoA/ROCK signaling. *FASEB J.* 34, 4189–4203. doi: 10.1096/fj.201902496RR

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

GLOSSARY

Terms	Definitions
ACE2	Angiotensin-converting enzyme 2
ACLF	Acute on chronic liver failure
BA	Bile acids
BNP	Brain natriuretic peptide
BSH	Bile salt hydrolases
CA	Cholic acid
cAMP	cyclic adenosine monophosphate
CDCA	Chenodeoxycholic acid
cGMP	cyclic guanosine monophosphate
CO	Carbon monoxide
CYP7A1	Cholesterol 7-alpha-hydroxylase
DAG	Diacyl glycerol
DCA	Deoxycholic acid
eNOS	Endothelial nitric oxide synthase
FGF	Fibroblast growth factor
FXR	Nuclear farnesoid X receptor
GDCA	Glycine conjugated DCA
GPBAR1	G protein-coupled bile acid receptor 1 or TGR5
GRK	G protein-coupled receptor kinase
GTP	Guanosine triphosphate
GTPases	Enzymes that hydrolise GTP to GDP (guanosine diphosphate)
HSC	Hepatic stellate cell
HVPG	Hepatic venous pressure gradient (surrogate for portal pressure)
iNOS	Inducible nitric oxide synthase
IP3	Inositol 1,4,5-triphosphate
LCA	Lithocholic acid
L-NAME	N(ω)-nitro-L-arginine methyl ester
MLC	Myosin light chains
NO	Nitiric oxide
NPY	Neuropeptide Y
NOS	Nitric oxide synthase
nNOS	Neuronal nitric oxide synthase
PBC	Primary biliary cholangitis
PSC	Primary sclerosing cholangitis
PLC	Phospholipase C
PKC	Protein kinase C
RAAS	Renin-angiotensin-aldosterone system
RhoA	Ras homolog family member A
ROCK	Rho-kinase
SHP	Small heterodimer partner
TCDCA	Taurine conjugated CDCA
TDCA	Taurine conjugated DCA
TGR5	See GPBAR1
TIPS	Transjugular intrahepatic portosystemic shunt
TUDCA	Taurine conjugate of UDCA
UDCA	Ursodeoxycholic acid
VSCM	Vascular smooth muscle cell

frontiers in Physiology

ORIGINAL RESEARCH
published: 20 August 2021
doi: 10.3389/fphys.2021.720898

Extrahepatic Surgery in Cirrhosis Significantly Increases Portal Pressure in Preclinical Animal Models

Johannes Chang[1†], Jonathan Meinke[1†], Moritz Geck[1], Marc Hebest[1], Nina Böhling[1], Ramona Dolscheid-Pommerich[2], Birgit Stoffel-Wagner[2], Glen Kristiansen[3], Marcus Overhaus[4], Leon O. Peyman[1], Sabine Klein[5], Frank E. Uschner[5], Maximilian J. Brol[5], Tim O. Vilz[6], Philipp Lingohr[6], Jörg C. Kalff[6], Christian Jansen[1], Christian P. Strassburg[1], Sven Wehner[6‡], Jonel Trebicka[5,7‡] and Michael Praktiknjo[1‡]*

[1] Department of Internal Medicine 1, Center for Cirrhosis and Portal Hypertension Bonn (CCB), University Hospital Bonn, Bonn, Germany, [2] Department of Clinical Pharmacology, University Hospital Bonn, Bonn, Germany, [3] Institute of Pathology, University Hospital Bonn, Bonn, Germany, [4] Department of Visceral Surgery, Malteser Hospital Sankt Hildegardis, Cologne, Germany, [5] Translational Hepatology, Department of Internal Medicine 1, University of Frankfurt, Frankfurt, Germany, [6] Department of Surgery, University of Bonn, Bonn, Germany, [7] European Foundation for the Study of Chronic Liver Failure, Barcelona, Spain

Background: Liver cirrhosis is a relevant comorbidity with increasing prevalence. Postoperative decompensation and development of complications in patients with cirrhosis remains a frequent clinical problem. Surgery has been discussed as a precipitating event for decompensation and complications of cirrhosis, but the underlying pathomechanisms are still obscure. The aim of this study was to analyze the role of abdominal extrahepatic surgery in cirrhosis on portal pressure and fibrosis in a preclinical model.

Methods: Compensated liver cirrhosis was induced using tetrachlormethane (CCL4) inhalation and bile duct ligation (BDL) models in rats, non-cirrhotic portal hypertension by partial portal vein ligation (PPVL). Intestinal manipulation (IM) as a model of extrahepatic abdominal surgery was performed. 2 and 7 days after IM, portal pressure was measured *in-vivo*. Hydroxyproline measurements, Sirius Red staining and qPCR measurements of the liver were performed for evaluation of fibrosis development and hepatic inflammation. Laboratory parameters of liver function in serum were analyzed.

Results: Portal pressure was significantly elevated 2 and 7 days after IM in both models of cirrhosis. In the non-cirrhotic model the trend was the same, while not statistically significant. In both cirrhotic models, IM shows strong effects of decompensation, with significant weight loss, elevation of liver enzymes and hypoalbuminemia. 7 days after IM in the BDL group, Sirius red staining and hydroxyproline levels showed significant progression of fibrosis and significantly elevated mRNA levels of hepatic inflammation compared to the respective control group. A progression of fibrosis was not observed in the CCL4 model.

Conclusion: In animal models of cirrhosis with continuous liver injury (BDL), IM increases portal pressure, and development of fibrosis. Perioperative portal pressure and hence inflammation processes may be therapeutic targets to prevent post-operative decompensation in cirrhosis.

Keywords: surgery, acute decompensation, cirrhosis, ACLF, portal pressure, HVPG, intestinal manipulation

OPEN ACCESS

Edited by:
Pavel Strnad, University Hospital RWTH Aachen, Germany

Reviewed by:
Oriol Juanola, University of Italian Switzerland, Switzerland
Savio George Barreto, Flinders Medical Centre, Australia

***Correspondence:**
Michael Praktiknjo
michael.praktiknjo@ukbonn.de

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: *05 June 2021*
Accepted: *27 July 2021*
Published: *20 August 2021*

Citation:
Chang J, Meinke J, Geck M, Hebest M, Böhling N, Dolscheid-Pommerich R, Stoffel-Wagner B, Kristiansen G, Overhaus M, Peyman LO, Klein S, Uschner FE, Brol MJ, Vilz TO, Lingohr P, Kalff JC, Jansen C, Strassburg CP, Wehner S, Trebicka J and Praktiknjo M (2021) Extrahepatic Surgery in Cirrhosis Significantly Increases Portal Pressure in Preclinical Animal Models. Front. Physiol. 12:720898. doi: 10.3389/fphys.2021.720898

INTRODUCTION

Liver cirrhosis is the common end-stage of chronic liver diseases. Acute decompensation (AD) such as variceal bleeding, refractory ascites, hepatorenal syndrome, or hepatic encephalopathy can develop and define advanced stages (Angeli et al., 2018). AD may also precipitate acute-on-chronic liver failure (ACLF), a distinct syndrome recently characterized in the CANONIC- and PREDICT-study (Moreau et al., 2013; Gustot et al., 2015; Trebicka et al., 2019, 2020a,b). ACLF is defined by the development of multiorgan failure resulting in high short-term mortality.

Postoperative decompensation of cirrhosis is a well-known but still unsolved problem in surgery. Even though there has been substantial progress in the fields of hepatology and surgery in managing patients with cirrhosis, surgery-associated AD and mortality remains high and correlates with severity of liver disease (Friedman, 2010; de Goede et al., 2012). Recently, the role of surgery as a precipitating event for ACLF development has been characterized, resulting in high rates of ACLF development even after electively performed surgical procedures (Klein et al., 2020; Chang et al., 2021). Therefore, in many hospitals, with the presence of cirrhosis especially in advanced stages is considered a contraindication for all kinds of surgery.

Clinically significant portal hypertension has been associated with increased numbers of episodes of acute decompensation after hepatic surgery (Bruix et al., 1996). In a recent prospective study, hepatic venous pressure gradient (HVPG) has also been described as a predictor for mortality after extrahepatic surgery, indicating that optimization of portal hypertension might be the key to improve postoperative outcome (Reverter et al., 2019). However, data about underlying mechanisms of post-operative decompensation and characterization of portal pressure in the pre- and post-operative period are at best scarce and thus need to be studied more to shed light on the pathophysiology involved in the post-operative development of AD and ACLF.

In this context, preclinical models to characterize proinflammatory downstream signaling and portal hemodynamics that help to understand the pathophysiology of post-operative decompensation of cirrhosis are needed. This study aimed to establish a preclinical model of extrahepatic abdominal surgery in animal models of portal hypertension and to study consecutive changes of portal pressure and liver fibrosis.

Abbreviations: 18s rRNA, Eukaryotic 18S ribosomal ribonucleic acid; ACLF, acute-on-chronic liver failure; AD, acute decompensation; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile-duct ligation; CCL2, chemokine (C-C motif) ligand 2; CCL4, tetrachlormethane; cDNA, complementary desoxyribonucleic acid; CTP, Child-Turcotte-Pugh; EMR-1, EGF-like module containing mucin-like hormone receptor-like 1; HE, hematoxylin and eosin; HVPG, hepatic venous pressure gradient; i.p., intraperitoneal; ICU, intensive care unit; IL-6, interleukin 6; IL1b, interleukin 1 beta; IM, intestinal manipulation; LAP, median laparotomy; mRNA, messenger ribonucleic acid; PE, Polyethylen; qPCR, real-time polymerase chain reaction; TGF-beta, transforming growth factor beta 1; TIPS, transjugular portosystemic shunt; TLR-4, toll-like receptor 4; TNF-alpha, tumor necrosis factor alpha; TP, total protein.

MATERIALS AND METHODS

Animal Experiments

Specific pathogen-free male Sprague Dawley rats were used for this study. Animals were acquired from Charles-River (Sulzfeld, Germany) and maintained in the animal facility at the University Clinic of Bonn, Department for Experimental Therapy in individually ventilated cages with a 12:12-h day-night cycle at 22 °C. Water and chow were provided *ad libitum*. Animal studies were performed in accordance with the German Animal Welfare Act and standard operation procedures of the Laboratory of Liver Fibrosis and Portal Hypertension and the animal care facility. Studies were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV, Reference: 81-02-04.2018.A348). Animals were sufficiently handled before all operations and received sufficient pain medication after all operations. When reaching human endpoint the experiment was stopped and animals were euthanized.

Establishing a Preclinical Model of Extrahepatic Abdominal Surgery in Cirrhosis and Non-cirrhotic Portal Hypertension

A two-step animal model of extrahepatic abdominal surgery was established. Cirrhosis was induced via bile duct ligation (BDL) and CCL4-intoxication via inhalation, non-cirrhotic portal hypertension via partial portal vein ligation (PPVL) as previously described (Uschner et al., 2015; Klein et al., 2017). 3 weeks after BDL or PPVL and 14 weeks after CCL4-intoxication (stage of compensated cirrhosis) intestinal manipulation (IM) was performed as previously described (Bortscher et al., 2012; Chang et al., 2012). 2 and 7 days after IM *in-vivo* portal pressure measurement was performed according to established protocol. Animals were then sacrificed and harvested. The experimental design is shown in **Figure 1**.

Bile Duct Ligation (BDL)

BDL was performed as previously described in a sterile environment (Uschner et al., 2015; Klein et al., 2017). In short, the common bile duct was ligated twice and dissected between the two ligatures to induce cholestatic cirrhosis. Sham animals received a median laparotomy (group name: Sham). All BDL procedures were performed by the same individual.

CCL4-Inhalation

Inhalation with CCL4 (abcr, Karlsruhe, Germany) was started at the age of 4 weeks (80–100 g body weight) and performed as previously described (Klein et al., 2017; Brol et al., 2019). Inhalation was done twice a week in growing intervals of 30 s. Reaching 5 min, animals inhaled CCL4 until week 14 (stage of compensated cirrhosis). All animals received phenobarbital (0.33 g/l) via drinking water for induction of cytochrome P-450 metabolic activity starting from 1 week before CCL4 inhalation until animal sacrifice. Inhalation was stopped 3 days before IM as a model of toxic cirrhosis with removal of the injuring agent. Age-matched animals without CCL4 inhalation served as controls (group name: AIR).

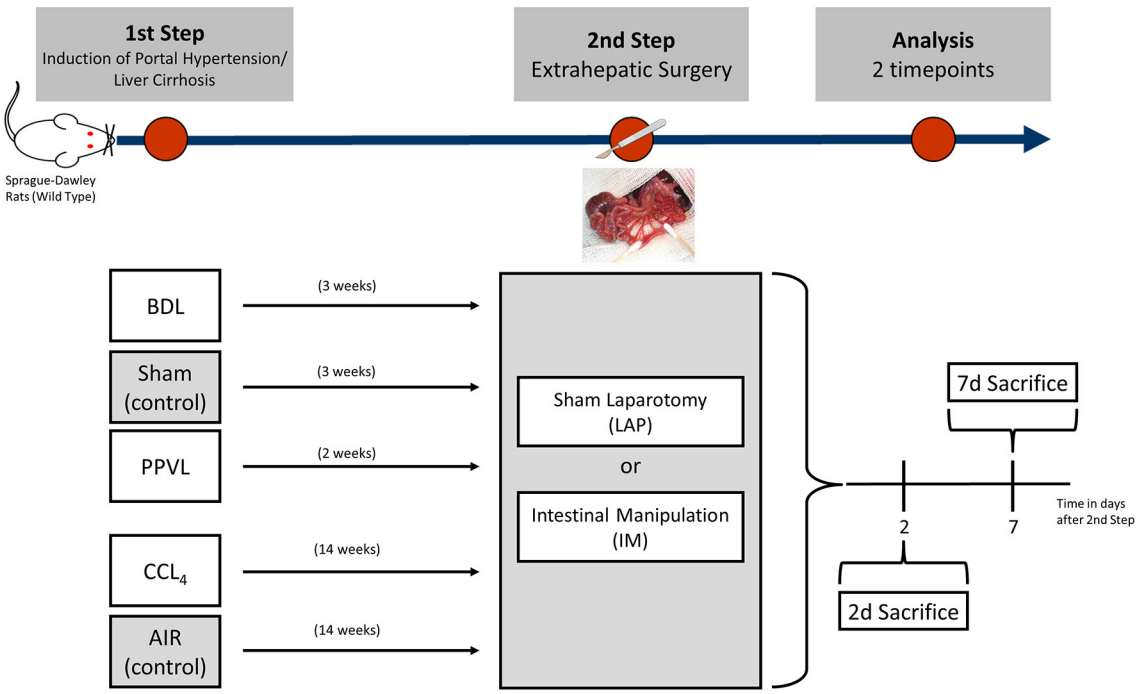


FIGURE 1 | Diagram showing how a two-step pre-clinical model of extrahepatic abdominal surgery (intestinal manipulation) in Sprague-Dawley rats with cirrhosis induced through bile duct ligation (BDL) or inhalation of tetrachlormethan (CCL4) and non-cirrhotic portal hypertension via partial portal vein ligation (PPVL) was established with two timepoints of final analysis.

Partial Portal Vein Ligation (PPVL)

To induce non-cirrhotic portal hypertension via PPVL, the portal vein was ligated around a 22 G needle. After ligation, the 22 G needle was removed immediately, resulting in a smaller diameter of the portal vein with consecutive development of non-cirrhotic portal hypertension. The same sham group used for the BDL group served as controls (group name: Sham). All PPVL procedures were performed by the same individual.

Intestinal Manipulation (Model of Extrahepatic Abdominal Surgery)

Intestinal manipulation (IM) was performed as previously described (Chang et al., 2012). IM was chosen as an established standardized model associated with postsurgical local inflammation and breakup of extracellular matrix in the gut wall (Chang et al., 2012). After median laparotomy, cecum and small bowel were placed on moist gauze outside the abdominal cavity. Then the entire small bowel and colon were manipulated between two sterile cotton swabs twice in a standardized fashion. The intestine was kept moist with saline at all times. After IM the intestine was placed back into the abdominal cavity, the abdomen was then closed with two layers of sutures. Age-matched animals only receiving a median laparotomy without IM served as sham controls (group name: LAP). All IM procedures were performed by the same individual.

Portal Pressure Measurement and Animal Sacrifice

Before sacrifice animals were put into anaethesia with an intraperitoneal injection of ketamine/xylazin (dose: ketamin 100 mg/kg/body weight (bw)/xylazin: 20 mg/kg/bw). After median laparotomy, for *in-vivo* portal pressure measurements the portal vein was dissected and punctured with a polyethylen catheter (B. Braun, Melsungen, Germany). The catheter was fixated with a vascular clamp. Portal pressure was then recorded over a time of 5 min under echocardiogram monitoring using PowerLab 8/35 and LabChart Software (ADInstruments, Dunedin, New Zealand). The used value of portal pressure and heart frequency of one biological replicate for further analysis, was determined as mean of three randomly chosen values in the recorded phase after calibrated recording. After portal pressure measurement and aquiring blood samples from the caval vein, the animal was sacrificed by dissecting the inferior caval vein. Liver samples were snap frozen in liquid nitrogen at −80 °C, fixated in 4% paraformaldehyde and embedded in paraffin or in Tissue Tek OCT (Sakura Finetek, Staufen, Germany).

Measurement of Hydroxproline Content in Liver Samples

Hydroxyproline content measurement was performed as described previously (Brol et al., 2019). Snap-frozen liver samples were weighed and dissolved in 12 N hydrochloric acid at 110 °C, then homogenized and incubated for another 16 h

at 110 °C. After filtering, samples were dissolved in methanol and oxidized in a chloramine T buffer. Finally, Ehrlich's reagent was added, the photometric product was measured at 558 nm wave length.

Parameters of Hepatic Inflammation and Circulating Endotoxin Levels

Hepatic inflammation was assessed by mRNA gene expression. RNA isolation was done using the ReliaPrep RNA Miniprep Systems (Promega, Madison, WI). For cDNA synthesis ImProm-II Reverse Transcription System (Promega, Madison, WI) was used. For every sample, two DNase digestion steps were done for genomic DNA to be disposed. Quantitative PCR (qPCR) was carried out using TaqMan gene expression assays (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. qPCR amplification was performed on the 7300 Real-Time PCR Cycler (Applied Biosystems, Foster City, CA). qPCR analyses were done using duplicates. Gene expression was calculated with the Delta-Delta CT method. 18s rRNA was used as housekeeping gene. Levels of gene expression are shown as x-fold compared to the respective control group. A list of gene expression assays is shown in **Supplementary Table 1**.

Circulating endotoxin levels were measured using the Pierce Limulus Amebocyte Lysate (LAL) Chromogenic Endotoxin Quantitation Kit (Thermo Fisher Scientific, Oberhausen, Germany) according to the manufacturer's protocol. In short, all samples were diluted and adjusted to a pH between 6 and 8. After pipetting standards and samples on a 96-well plate and incubating at 37 °C for 4 h, activation in the modified LAL was stopped. Endotoxin concentration was then photometrically measured at 405 nm wavelength. Levels of endotoxin are expressed as EU/ml. Only endotoxin-free plastic ware or sterile glass ware was used for the experiment.

Histological Staining and Quantification

Sirius red and Hematoxylin and eosin (HE) stainings were performed on paraffin slides (2–3 μm) of the liver as previously described (Trebicka et al., 2011; Schierwagen et al., 2015; Brol et al., 2019). Stainings were captured with a Nikon Digital Sight DS-Vi1 microscope (Chiyoda, Tokyo, Japan) and quantified via ImageJ software (V.1.51q; National Institutes of Health, Bethesda, USA) using macros for automatized quantification and color detection. Individual samples were controlled for correct analysis, if color analysis was not executed properly, threshold was adjusted manually. Images were taken in 10-fold magnification and a minimum of 10 representative fields per biological replicate were taken into analysis.

Analysis of Laboratory Parameters of Liver Function

Electrolytes (sodium, potassium) and parameters of liver function (alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total protein, and ammonia were analyzed in serum using the Cobas 8000 (Roche Diagnostics, Rotkreuz, Switzerland), modules 8000 ISE, c502, and c702 according to the manufacturer's protocol.

Statistical Analyses

Statistical analyses were performed using Prism V.5.0 (GraphPad, San Diego, CA). Data are expressed as means ± SEM. For comparisons between two groups, student's *t*-test was used. *P*-values ≤ 0.05 were considered significant.

RESULTS

Establishing a Preclinical Model of Extrahepatic Intestinal Surgery in Different Animal Models of Cirrhotic or Non-cirrhotic Portal Hypertension

Only animals that recovered completely from the first operation (BDL, PPVL, Sham) were included in the final analysis. Animals were then randomized into a group that underwent intestinal manipulation (IM) or median laparotomy (LAP). Animals presenting with ascites as a clinical sign of AD prior to IM or LAP were excluded from the experiment.

Due to the more aggressive nature of cirrhosis and expected higher postoperative mortality rate in BDL, a preliminary study had to be performed to determine the optimal time for IM after induction of cirrhosis. This preliminary study was approved within the applied project. When IM was performed 28 days after BDL, postoperative mortality was high, as expected (30%, data not shown). Therefore, the time point of 21 days (3 weeks) after BDL with a mortality of 10% after IM was established for the experiment.

Postoperative Mortality and Development of Ascites After Intestinal Manipulation

Combined postoperative mortality for BDL, PPVL, and CCL4 groups after IM for both timepoints (2 and 7 days after IM) was 10, 0, and 6%, respectively. Due to a low number of animal deaths without significant distribution concerning time after IM or operation type (IM/LAP), survival analysis was not performed. No deaths were recorded in all control groups (Sham (controls for BDL and PPVL), AIR (controls for CCL4) (**Figure 1**) after IM or LAP. In the BDL group that underwent IM and were sacrificed 7 days after IM, 3 (43%) developed ascites, vs. 1 (13%) animal in the control group ($p = 0.3$). The rate of development of ascites in the CCL4 group was similar 7 days after IM (IM 3 (38%) vs. LAP 1 (14%), $p = 0.5$). No animals in in BDL and CCL4 groups developed ascites 2 days after IM, also none of the animals belonging to the PPVL groups developed ascites.

Weight Development

Significant weight differences were observed in all models of cirrhosis or non-cirrhotic portal hypertension at time of IM, compared to the respective control groups (Sham, Air), with a significantly lower body weight in the PPVL, BDL, and CCL4 groups at the time of IM vs. LAP (**Supplementary Figure 1**).

Moreover, significant weight loss was observed in some groups after IM vs. LAP. In the BDL model, 7 days after IM animals lost significantly more weight compared to LAP, but not at 2 days (**Table 1B**). The same effect was observed in the CCL4 groups

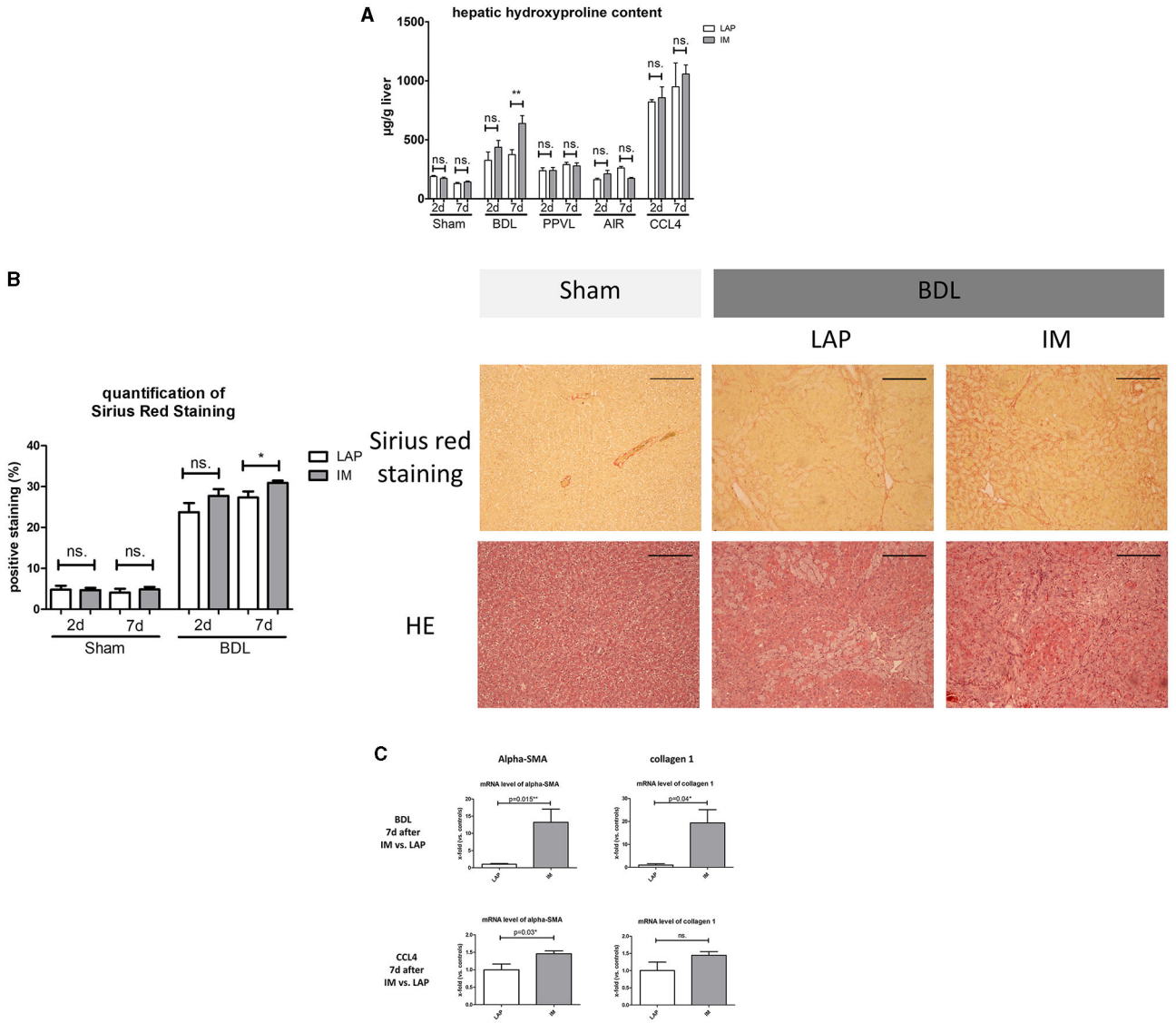


FIGURE 2 | (A) Hepatic hydroxyproline content measurements 2 or 7 days after IM vs. LAP, showing progression of fibrosis through a highly significant elevation of hydroxyproline concentration (expressed in µg/g liver) of the liver in the BDL group 7 days after IM vs. LAP. No differences are seen within the Sham, PPVL, and CCL4 groups. **(B)** Representative images of liver samples with Sirius-red staining for assessment of fibrosis with observed significant differences of fibrosis (expressed as percentage of positive stained area) 7 days after intestinal manipulation vs. the respective control group (LAP). Images of Hematoxylin Eosin staining are shown for morphologic comparison. The scale bar is 200 µm. **(C)** mRNA gene expression for alpha-SMA and collagen 1, showing a significant increase of both parameters of fibrosis progression in the BDL group 7 days after IM and for alpha-SMA in the CCL4 group 7 days after IM. Levels of gene expression are shown as x-fold compared to the respective control group. *$p \leq 0.05$; **$p \leq 0.01$; ns. ($p > 0.05$).

(**Table 1D**). In the control and PPVL groups, weight loss before and after IM, was not statistically significant (**Tables 1B**,**D**,**F**).

Laboratory Parameters of Liver Function at the Time of Sacrifice

Among laboratory parameters representing liver function in the Sham and AIR groups, no significant changes could be observed between IM and LAP groups (**Table 1A**).

In the BDL groups, Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) were significantly elevated 7 days after IM vs. LAP (**Table 1A**). Albumin levels were significantly lower, 2 and 7 days after IM vs. LAP (**Table 1A**). Interestingly, ammonia serum levels were elevated 2 days after IM vs. LAP (**Table 1B**).

In the CCL4 group, AST and ALT were significantly elevated 7 days after IM vs. LAP (**Table 1D**), while albumin levels were significantly lower (**Table 1D**). No significant differences in laboratory parameters were observed 2 days after IM vs. LAP.

In the PPVL group 2 days after IM, AST was significantly elevated vs. LAP (**Table 1E**). No other significant differences were observed 2 and 7 days after IM vs. LAP.

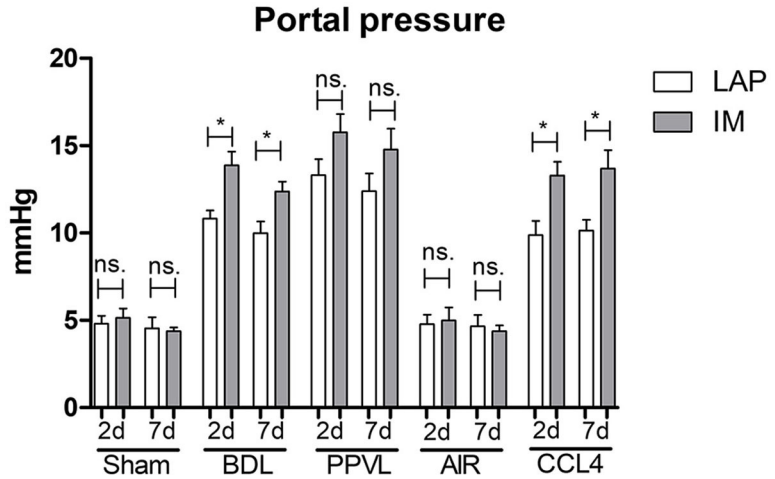


FIGURE 3 | *In-vivo* portal pressure development 2 or 7 days after IM vs. LAP in the different animal models (BDL and PPVL vs. Sham and CCL4 vs. AIR), showing significantly elevated portal pressure after IM in all BDL and CCL4 groups. *$p \leq 0.05$; ns. ($p > 0.05$).

Development of Hepatic Fibrosis After Intestinal Manipulation

Hydroxyproline measurement showed significant increase of hydroxyproline levels and significantly larger Sirius-Red stained areas in the BDL group 7 days after IM vs. LAP (**Figures 2A,B**). In the BDL group 7 days after IM, mRNA expression of alpha-SMA (a marker of profibrogenic hepatic stellate cells) and collagen 1 were significantly upregulated compared to LAP (13- and 19-fold, $p = 0.02$ and $p = 0.04$, respectively) (**Figure 2C**). In the other cirrhotic and non-cirrhotic groups (PPVL, Sham, Air, CCL4), no significant differences in fibrosis development between animals receiving IM vs. LAP could be observed, except a slight, but significant upregulation of alpha-SMA gene expression in the CCL4 group 7 days after IM compared to LAP (**Figures 2A,C**; **Supplementary Figure 2**).

Portal Pressure After Intestinal Manipulation

In the cirrhotic groups (BDL and CCL4) portal pressure was significantly elevated 2 and 7 days after IM compared to LAP (BDL 2 d: IM 13.9 ± 0.8 mmHg vs. LAP 10.8 ± 0.5 mmHg, $p = 0.01$*; BDL 7 d: IM 12.4 ± 0.6 mmHg vs. LAP 10.0 ± 0.7 mmHg, $p = 0.02$; CCL4 2 d: IM 13.3 ± 0.8 mmHg vs. LAP 10.7 ± 0.3 mmHg, $p = 0.03$; CCL4 7 d: IM 13.7 ± 1.1 mmHg vs. LAP 10.2 ± 0.6 mmHg, $p = 0.02$) (**Figure 3**; **Tables 1A–D**). The control groups (Sham, AIR) did not show any significant increase of portal pressure after IM or LAP (**Figure 3**; **Tables 1A–D**). While there was a trend of higher portal pressure after IM in the PPVL group, results were not significantly different compared to LAP (**Figure 3**; **Tables 1E,F**).

Parameters of Hepatic Inflammation and Circulatory Level of Endotoxins

Transforming growth factor beta 1 (TGF-beta), interleukin 6 (IL-6), interleukin 1 beta (IL-1b), tumor necrosis factor alpha (TNF-alpha), chemokine (C-C motif) ligand 2 (CCL2), EGF-like module containing mucin-like hormone receptor-like 1 (EMR-1), toll-like receptor 4 (TLR-4) were measured in liver samples via mRNA gene expression as parameters of hepatic inflammation. These inflammatory parameters were elevated in the BDL group 7 days after IM but not 2 days after IM vs. LAP, especially IL-6 (25-fold), TNF-alpha (14-fold) and CCL2 (8-fold) ($p = 0.05$, $p = 0.006$, $p = 0.02$, respectively) (**Figure 4**). In the CCL4 model IL-6 gene expression was significantly upregulated after IM vs. LAP among the measured inflammatory parameters (**Supplementary Figure 3**).

Endotoxin levels were measured in the different models 2 and 7 days after IM/LAP as a marker of bacterial translocation as possible trigger for upregulation of hepatic inflammation. No significant differences could be detected 2 and 7 days after IM/LAP between operation type (IM vs. LAP) or blood compartment (portal vein vs. caval vein). However, in both cirrhosis models circulatory level of endotoxins was significantly higher than in the non-cirrhotic groups (**Supplementary Figure 4**).

DISCUSSION

This study is the first to characterize portal pressure after abdominal extrahepatic surgery in preclinical models of cirrhosis with ongoing hepatic injury (BDL) and discontinued hepatic injury (CCL4) prior to surgery. It shows that an abdominal extrahepatic surgical procedure significantly increases portal pressure, rendering our model suitable for studying pathomechanisms of post-operative acute decompensation (AD).

Postoperative AD in patients with cirrhosis is a long existing clinical problem which to date limits surgical procedures in cirrhosis. Moreover, AD can precipitate ACLF, resulting in multiorgan-failure and high short-term mortality (Moreau et al., 2013; Trebicka et al., 2020a). In previous studies, our group

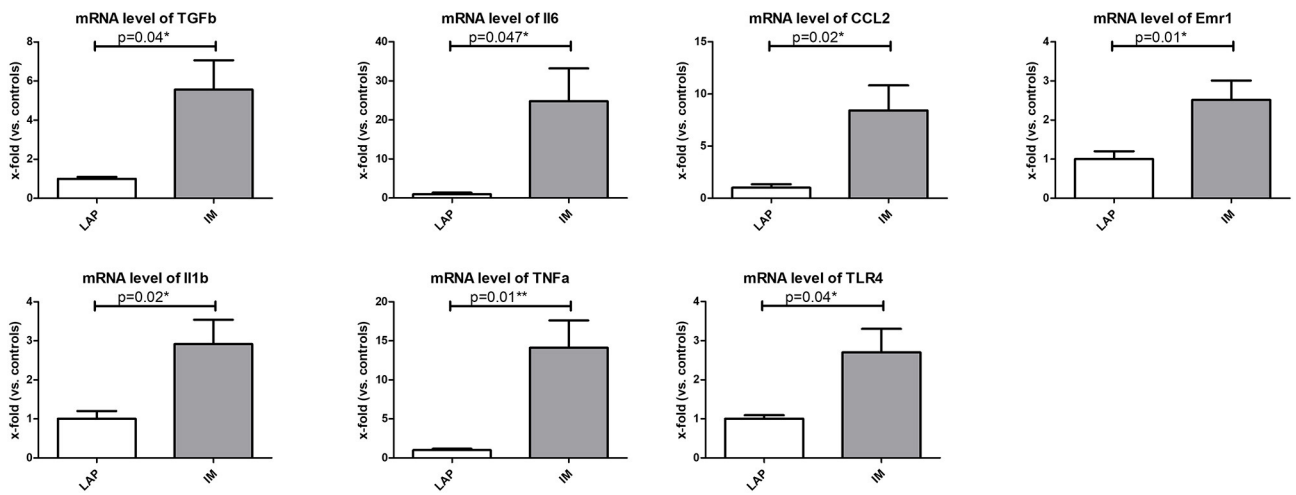


FIGURE 4 | Analysis of parameters of hepatic inflammation (TGFb, IL-6, IL-1b, TNF-alpha, Emr1, TLR-4) in the BDL group 7 days after IM vs. LAP, all significantly elevated. Levels of gene expression are shown as x-fold compared to the respective control group. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; ns. ($p > 0.05$).

TABLE 1A | General characteristics and clinical data of rats undergoing Sham/BDL at sacrifice 2 days after IM/LAP.

Parameter		Sham + LAP $n = 8$	Sham + IM $n = 8$	*p*	BDL + LAP $n = 7$	BDL + IM $n = 7$	*p*
Weight data	Liver weight [g]	15 ± 0.6	14.4 ± 0.8	0.41	13.7 ± 0.8	13.3 ± 0.8	0.7
	Body weight at sacrifice [g]	394.6 ± 8.5	393.2 ± 17.9	0.94	330.9 ± 18.2$^{\#}$	329 ± 15.3$^{\#}$	0.94
	Weight development surgery - sacrifice [%]	−0.25 ± 1.3	−2.7 ± 3.2	0.52	−5.3 ± 2.2$^{\$}$	−5.4 ± 0.9$^{\$}$	0.94
Baseline laboratory	Sodium [mmol/l]	134.8 ± 2.6	137.0 ± 1.2	0.41	137.4 ± 1.2	139 ± 1.1	0.39
	ALP [U/L]	148.2 ± 21.3	141.3 ± 10.4	0.76	131.3 ± 8.3$^{\$}$	151.8 ± 29.3$^{\$}$	0.48
	AST [U/L]	88.8 ± 15.7	81.3 ± 6.3	0.63	96 ± 4.7$^{\$}$	110.8 ± 17.3$^{\$}$	0.39
	ALT [U/L]	47.3 ± 4	38.7 ± 3.4	0.15	47.3 ± 3.5$^{\$}$	47.3 ± 3.5$^{\$}$	0.47
	ALB [g/L]	30.4 ± 0.5	31.7 ± 1.3	0.41	30.6 ± 0.5$^{\$}$	27.3 ± 0.9$^{\#}$	0.009**
	TP [g/L]	50.3 ± 0.6	51.9 ± 1.8	0.49	50 ± 0.6$^{\$}$	47.3 ± 1.3$^{\$}$	0.07
	Urea [mg/dl]	38.0 ± 2.4	44.3 ± 6.0	0.41	37.8 ± 2.5$^{\$}$	42.0 ± 4.9$^{\$}$	0.44
	Ammonia [μmol/L]	121.3 ± 6.4	106.7 ± 8.1	0.2	101.6 ± 18.5$^{\$}$	191.5 ± 17.6$^{\#}$	0.007**
Hemodynamics	Portal Pressure [mmHg]	4.8 ± 0.5	5.1 ± 0.5	0.64	10.8 ± 0.5$^{\#\#}$	13.9 ± 0.8$^{\#\#\#}$	0.011*
	Heart frequency [bpm]	195 ± 12.8	200 ± 12.8	0.77	202.2 ± 22.0	208.9 ± 13.9	0.82

Sham vs. BDL: $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$; $^{\$}$ns. ($p > 0.05$). BDL + LAP vs. BDL + IM: $^{*}p \leq 0.05$.

showed that about 25% of patients with cirrhosis develop post-operative ACLF, including emergency surgery (Klein et al., 2020). Similar rates of post-operative ACLF are demonstrated even in elective surgical procedures, thus establishing surgical procedures as a precipitant of ACLF (Chang et al., 2021). In clinical practice, Child-Turcotte-Pugh-Score is commonly used for pre-operative risk stratification. Higher Child-Turcotte-Pugh-Score at the time of surgery is associated with higher mortality (Friedman, 2010). However, more biomarkers and clinical parameters are needed for better risk stratification for patients with liver cirrhosis in need of a surgical procedure.

Portal pressure seems to play an important role in post-operative outcome of patients with cirrhosis. In a recent prospective study, it was shown that preoperative HVPG below 16 mmHg before a surgical procedure is associated with a better postoperative outcome (Reverter et al., 2019). In a series of smaller studies it was shown that preoperative decompression of portal pressure via transjugular portosystemic shunt (TIPS) improves post-operative outcome, thus the concept of preoperative TIPS has been discussed as well (García-Pagán et al., 2020). However, no studies investigated the evolution of portal pressure after surgery, limiting the investigation

TABLE 1B | General characteristics and clinical data of rats undergoing Sham/BDL at sacrifice 7 days after IM/LAP.

Parameter		Sham + LAP $n = 8$	Sham + IM $n = 8$	p	BDL + LAP $n = 8$	BDL + IM $n = 7$	p
Weight data	Liver weight [g]	17.2 ± 0.4	15.9 ± 0.8	0.17	12.8 ± 1.3	15.3 ± 1.4	0.24
	Body weight at sacrifice [g]	408.5 ± 7.3	396.3 ± 12.6	0.42	345.0 ± 34.4$^{\$}$	312.6 ± 16.6$^{\#\#}$	0.42
	Weight development surgery - sacrifice [%]	−1.1 ± 1	−1.4 ± 1.8	0.9	4.5 ± 4$^{\$}$	−8.4 ± 3$^{\$}$	0.03*
Baseline laboratory	Sodium [mmol/l]	135.9 ± 1.1	137.4 ± 0.6	0.31	141.4 ± 1.4	141.8 ± 0.5	0.83
	ALP [U/L]	160.0 ± 7.1	147.8 ± 11.6	0.36	154 ± 19.7$^{\$}$	340.8 ± 57.4$^{\#}$	0.01*
	AST [U/L]	76.3 ± 6.4	81.5 ± 4	0.48	116.2 ± 22.7$^{\$}$	288.3 ± 80.4$^{\#}$	0.05*
	ALT [U/L]	49.4 ± 3.5	45.2 ± 1.6	0.36	50.2 ± 11.7$^{\$}$	69.8 ± 13.7$^{\$}$	0.31
	ALB [g/L]	32.6 ± 0.6	32.7 ± 0.9	0.92	32.7 ± 1.6$^{\$}$	22.3 ± 2.1$^{\#\#}$	0.002**
	TP [g/L]	52.1 ± 0.6	50.3 ± 1.4	0.22	50.5 ± 1.7$^{\$}$	42.5 ± 2.7$^{\#\#}$	0.01*
	Urea [mg/dl]	36.7 ± 3.6	31.3 ± 1.8	0.25	29.5 ± 2.4$^{\$}$	44.1 ± 5.7$^{\$}$	0.07
	Ammonia [μmol/L]	92.2 ± 14.4	80.3 ± 19.11	0.63	100 ± 12.9$^{\$}$	223.6 ± 65$^{\$}$	0.07
Hemodynamics	Portal Pressure [mmHg]	4.5 ± 0.6	4.4 ± 0.2	0.82	10.0 ± 0.7$^{\#\#\#}$	12.4 ± 0.6$^{\#\#\#}$	0.02*
	Heart frequency [bpm]	205.8 ± 16.7	196.6 ± 14.2	0.73	199.1 ± 20.34	197.2 ± 15.37	0.94

Sham vs. BDL: $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$; $^{\$}$ns. ($p > 0.05$). BDL + LAP vs. BDL + IM: $^{*}p \leq 0.05$.

TABLE 1C | General characteristics and clinical data of rats receiving AIR/CCL4 at sacrifice 2 days after IM/LAP.

Parameter		Air + LAP $n = 8$	Air + IM $n = 8$	p	CCL4 + LAP $n = 7$	CCL4 + IM $n = 8$	p
Weight data	Liver weight [g]	19.6 ± 0.6	18.9 ± 0.7	0.45	19.2 ± 0.7	17.6 ± 0.6	0.1
	Body weight at sacrifice [g]	546.5 ± 11.7	546.8 ± 11.4	0.99	438.4 ± 15.12$^{\#\#\#}$	422.8 ± 22.58$^{\#\#}$	0.57
	Weight development surgery - sacrifice [%]	−3.8 ± 0.6	−6.1 ± 1.0	0.1	−1.6 ± 2.0$^{\$}$	−7.1 ± 1.3$^{\$}$	0.07
Baseline laboratory	Sodium [mmol/l]	139.4 ± 0.2	138.2 ± 1.9	0.6	142.2 ± 0.3	142.5 ± 1.0	0.8
	ALP [U/L]	95.7 ± 10.7	79.6 ± 7.2	0.3	177.4 ± 20$^{\#}$	150 ± 27.4$^{\#}$	0.4
	AST [U/L]	94.8 ± 10.9	102.4 ± 7.0	0.6	338.3 ± 29.2$^{\#\#\#}$	405 ± 77.7$^{\#}$	0.5
	ALT [U/L]	42.8 ± 1.2	36.6 ± 3.5	0.13	132.4 ± 16.7$^{\#\#\#}$	131.2 ± 22.7$^{\#\#}$	0.96
	ALB [g/L]	33.5 ± 0.9	35.0 ± 1.2	0.4	33.3 ± 0.9$^{\$}$	32.5 ± 0.8$^{\$}$	0.6
	TP [g/L]	51.4 ± 1.8	53.4 ± 2.0	0.5	49.5 ± 1.3$^{\$}$	50.2 ± 1.4$^{\$}$	0.7
	Urea [mg/dl]	36.4 ± 1.2	39.9 ± 2.0	0.2	25.2 ± 2.6$^{\#\#}$	29.6 ± 3.3$^{\#}$	0.3
	Ammonia [μmol/L]	73.8 ± 5.3	106.8 ± 26	0.24	107.6 ± 17.9$^{\#\#}$	85.8 ± 6.0$^{\#\#\#}$	0.3
Hemodynamics	Portal Pressure [mmHg]	4.8 ± 0.6	5.0 ± 0.7	0.81	10.7 ± 0.3$^{\#\#\#}$	13.3 ± 0.8$^{\#\#\#}$	0.03*
	Heart frequency [bpm]	205.9 ± 11.03	201.0 ± 14.6	0.8	203.7 ± 15.0	197.5 ± 34.8	0.86

AIR vs. CCL4: $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$; $^{\$}$ns. ($p > 0.05$). BDL + LAP vs. BDL + IM: $^{*}p \leq 0.05$.

of pathophysiological pathways driving post-operative hepatic decompensation. Prospective clinical studies to characterize post-operative measurements of portal pressure in patients with cirrhosis are ethically difficult to perform, given that patients are mostly under postoperative care in the intensive care unit and in danger of AD or ACLF development. Therefore, animal models are needed to explore the mechanisms of postoperative AD or ACLF, and to study the evolution of portal pressure after surgery and its association with potential underlying inflammatory processes.

In our study, we show in two different animal models of cirrhosis (BDL and CCL4), that portal pressure is significantly elevated 2 and 7 days after IM vs. LAP (median laparotomy). Accordingly, in both models, 7 days after IM, there were more clinical events of decompensation such as the development of ascites or significant weight loss. The BDL model seems to mimic the clinical situation after surgery in patients more accurately, showing progression of fibrosis and significant elevation of parameters of hepatic inflammation 7 days after IM and elevation of liver enzymes and ammonia acutely within 2 days after IM. In the BDL model, IM was performed in an earlier stage of fibrosis (3 weeks after BDL), leading to progression of fibrosis and earlier decompensation events after IM vs. LAP. In the CCL4 group however, no significant changes of the fibrosis parameters could be observed after IM compared to LAP. A possible reason might be that IM was performed at more advanced stages of cirrhosis. However, a regression of fibrosis after withdrawal of the injuring agent has been described in the CCL4 model (Nevzorova

TABLE 1D | General characteristics and clinical data of rats receiving AIR/CCL4 at sacrifice 7 days after IM/LAP.

Parameter		Air + LAP $n = 8$	Air + IM $n = 8$	*p*	CCL4 + LAP $n = 8$	CCL4 + IM $n = 7$	*p*
Weight data	Liver weight [g]	18.1 ± 0.9	18.8 ± 0.6	0.7	17.7 ± 1.1	16.2 ± 0.9	0.3
	Body weight at sacrifice [g]	540.7 ± 14.8	553.0 ± 14.1	0.6	465.6 ± 4.1$^{\#\#}$	404.6 ± 9.7$^{\#\#\#}$	<0.001***
	Weight development surgery - sacrifice [%]	−4.0 ± 0.5	−3.9 ± 0.7	0.9	−1.2 ± 0.3$^{\$}$	−10.7 ± 2.2$^{\$}$	0.005**
Baseline laboratory	Sodium [mmol/l]	138.8 ± 0.3	139.8 ± 0.6	0.2	141.6 ± 0.8	141.5 ± 1.3	1
	ALP [U/L]	79.2± 11.6	77.4 ± 2.8	0.9	146 ± 23$^{\#}$	194.1 ± 22.6$^{\#\#}$	0.17
	AST [U/L]	75.8 ± 5.7	103. ± 12.1	0.08	171.0 ± 29.0$^{\#}$	306.7 ± 30.7$^{\#\#\#}$	0.02*
	ALT [U/L]	38.0 ± 2.1	43.8 ± 3.7	0.21	77.4 ± 5.4$^{\#\#\#}$	118.7 ± 6.0$^{\#\#\#}$	<0.001***
	ALB [g/L]	34.2 ± 1.0	33.2 ± 0.6	0.4	34.6 ± 1.2$^{\$}$	28.0 ± 1.0$^{\#\#}$	0.0017**
	TP [g/L]	47.7 ± 1.4	48.0 ± 1.5	0.9	49.1 ± 1.1$^{\$}$	45.4 ± 1.3$^{\$}$	0.1
	Urea [mg/dl]	31.9 ± 0.9	40.3 ± 3.3	0.02*	25.8 ± 2.1$^{\#}$	30.7 ± 1.9$^{\#}$	0.1
	Ammonia [μmol/L]	100.2 ± 18.7	109.4 ± 38.4	0.8	86.8 ± 18.3$^{\#}$	111.75 ± 27$^{\$}$	0.5
Hemodynamics	Portal Pressure [mmHg]	4.7 ± 0.7	4.4 ± 0.3	0.71	10.2 ± 0.6$^{\#\#\#}$	13.7 ± 1.1$^{\#\#\#}$	0.02*
	Heart frequency [bpm]	210.2 ± 27.94	194.0 ± 0.5	0.59	190.8 ± 8.3	191.2 ± 8.6	0.43

AIR vs. CCL4: $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$; $^{\$}$ns. ($p > 0.05$). CCL4 + LAP vs. CCL4 + IM: $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

TABLE 1E | General characteristics and clinical data of rats undergoing Sham/PPVL at sacrifice 2 days after IM/LAP.

Parameter		Sham + LAP n =8	Sham + IM $n = 8$	*p*	PPVL + LAP $n = 8$	PPVL + IM $n = 8$	*p*
Weight data	Liver weight [g]	15 ± 0.6	14.4 ± 0.8	0.41	14.1 ± 0.7	15.4 ± 1.0	0.34
	Body weight at sacrifice [g]	394.6 ± 8.5	393.2 ± 17.9	0.94	388.1 ± 11.1$^{\$}$	385.7 ± 9.1$^{\$}$	0.87
	Weight development surgery - sacrifice [%]	−0.25 ± 1.3	−2.7 ± 3.2	0.52	0.8 ± 1.0$^{\$}$	−1.8 ± 0.5$^{\$}$	0.16
Baseline laboratory	Sodium [mmol/l]	134.8 ± 2.6	137.0 ± 1.2	0.41	140.1 ± 1.0	139.9 ± 0.6	0.85
	ALP [U/L]	148.2 ± 21.3	141.3 ± 10.4	0.76	132.1 ± 8.3$^{\$}$	135.5 ± 5.6$^{\$}$	0.73
	AST [U/L]	88.8 ± 15.7	81.3 ± 6.3	0.63	82.0 ± 6.0$^{\$}$	123 ± 16.6$^{\$}$	0.04*
	ALT [U/L]	47.3 ± 4	38.7 ± 3.4	0.15	57.4 ± 3.1$^{\$}$	60.2 ± 4.0$^{\$}$	0.6
	ALB [g/L]	30.4 ± 0.5	31.7 ± 1.3	0.41	31.7 ± 0.8$^{\$}$	31.6 ± 0.6$^{\$}$	0.96
	TP [g/L]	50.3 ± 0.6	51.9 ± 1.8	0.49	48.6 ± 0.9$^{\$}$	48.8 ± 1.0$^{\$}$	0.85
	Urea [mg/dl]	38.0 ± 2.4	44.3 ± 6.0	0.41	41 ± 2.4$^{\$}$	36.3 ± 1.4$^{\$}$	0.1
	Ammonia [μmol/L]	121.3 ± 6.4	106.7 ± 8.1	0.2	138.8 ± 15.1$^{\$}$	136.2 ± 13.8$^{\$}$	0.91
Hemodynamics	Portal Pressure [mmHg]	4.8 ± 0.5	5.1 ± 0.5	0.64	13.3 ± 0.9$^{\#\#\#}$	15.8 ± 1.0$^{\#\#\#}$	0.11
	Heart frequency [bpm]	195 ± 12.8	200 ± 12.8	0.77	211.1 ± 12.2	207.3 ± 14.6	0.84

Sham vs. PPVL: $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$; $^{\#\#\#}p \leq 0.001$; $^{\$}$ns. ($p > 0.05$). CCL4 + LAP vs. CCL4 + IM: $^{*}p \leq 0.05$.

et al., 2020). In our model CCL4 inhalation was stopped 3 days before IM, which might be masking progression of fibrosis in this model by IM. However, the perioperative discontinuation of the hepatotoxic agents such as alcohol reflects clinical reality. Still, in our CCL4 model IM leads to significantly elevated portal pressure, elevated levels of liver enzymes and alpha-SMA and IL-6 gene expression after IM compared to LAP as relevant surrogate parameters for hepatic inflammation and decompensation.

An elevated portal pressure after surgery may be the expression of increased systemic inflammation. Our data shows significantly upregulated parameters of inflammation after IM, suggesting an association between inflammation and the development of elevated portal pressure after surgery. A close association of HVPG and systemic inflammation has been shown recently (Praktiknjo et al., 2020). A hyperinflammatory state is also a key element of ACLF (Trebicka et al., 2019).

Inflammatory pathways driven by bacterial translocation and mechanisms of sterile inflammation may play a role in post-operative portal pressure elevation. In a recent retrospective study, bowel-related surgery was associated with a poor outcome in patients with cirrhosis, especially in those presenting with ascites and thrombocytopenia (Wetterkamp et al., 2020). Our study supports the role of bacterial translocation, indicated by the significant increase of hepatic TLR-4 expression in the BDL model 7 d after IM and significantly higher levels of endotoxin in the cirrhosis models. However, our data do not show differences of endotoxin levels between IM and LAP groups at 2 and 7 days. It has been shown that major abdominal surgery is associated

TABLE 1F | General characteristics and clinical data of rats undergoing Sham/PPVL at sacrifice 7 days after IM/LAP.

Parameter		Sham + LAP $n = 8$	Sham + IM $n = 8$	p	PPVL + LAP $n = 8$	PPVL + IM $n = 8$	p
Weight data	Liver weight [g]	17.2 ± 0.4	15.9 ± 0.8	0.17	14.2 ± 0.3	13.9 ± 0.4	0.58
	Body weight at sacrifice [g]	408.5 ± 7.3	396.3 ± 12.6	0.42	378.6 ± 8.5$^{\$}$	369.3 ± 7.7$^{\$}$	0.42
	Weight development surgery - sacrifice [%]	−1.1 ± 1	−1.4 ± 1.8	0.9	−0.3 ± 1.71$^{\$}$	−2.2 ± 1.5$^{\$}$	0.41
Baseline laboratory	Sodium [mmol/l]	135.9 ± 1.1	137.4 ± 0.6	0.31	139.0 ± 1.1$^{\$}$	140.7 ± 0.5$^{\$}$	0.2
	ALP [U/L]	160.0 ± 7.1	147.8 ± 11.6	0.36	127.3 ± 7.8$^{\#}$	139.7 ± 11.4$^{\$}$	0.42
	AST [U/L]	76.3 ± 6.4	81.5 ± 4	0.48	71.9 ± 4.8$^{\$}$	79.5 ± 7.3$^{\$}$	0.42
	ALT [U/L]	49.4 ± 3.5	45.2 ± 1.6	0.36	48.7 ± 1.4$^{\$}$	40.7 ± 2.7$^{\#}$	0.11
	ALB [g/L]	32.6 ± 0.6	32.7 ± 0.9	0.92	31.7 ± 0.6$^{\$}$	30.0 ± 1.0$^{\$}$	0.17
	TP [g/L]	52.1 ± 0.6	50.3 ± 1.4	0.22	50.2 ± 0.7$^{\#}$	46.6 ± 2.2$^{\$}$	0.15
	Urea [mg/dl]	36.7 ± 3.6	31.3 ± 1.8	0.25	37.8 ± 2.1$^{\$}$	34.2 ± 2.1$^{\$}$	0.23
	Ammonia [μmol/L]	92.2 ± 14.4	80.3 ± 19.11	0.63	89.5 ± 16$^{\$}$	151.7 ± 46.3$^{\#}$	0.18
Hemodynamics	Portal Pressure [mmHg]	4.5 ± 0.6	4.4 ± 0.2	0.82	12.4 ± 1.0$^{\#\#\#}$	14.8 ± 1.2$^{\#\#\#}$	0.16
	Heart frequency [bpm]	205.8 ± 16.7	196.6 ± 14.2	0.73	222.2 ± 16.9	200.9 ± 2.9	0.1

Sham vs. PPVL: $^{\#}p \leq 0.05$; $^{\#\#\#}p \leq 0.001$; $^{\$}$ns. ($p > 0.05$).

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile-duct ligation; bpm, beats per minutes; CCL4, tetrachlormethane; IM, intestinal manipulation; LAP, median laparotomy; ns, no significance; PPVL, partial portal vein ligation; TP, total protein.

with transient endotoxemia that peak between 1 and 24 h after surgery (Buttenschoen et al., 2001, 2009). Endotoxin levels might be significantly higher immediately after IM and then quickly decrease after increase of systemic and inflammation markers and upregulation of hepatic inflammation. This hypothesis needs to be further investigated in the future.

It has also been shown that IM leads to a disruption of the gut wall with the release of sterile proinflammatory agents, e.g., extracellular matrix components that lead to local and systemic inflammation (Bortscher et al., 2012; Chang et al., 2012; Nielsen et al., 2015; Lehmann et al., 2019). Our data show significant upregulation of collagen type 1 and elevated hepatic hydroxyproline levels in the BDL model 7 days after IM as expression of fibrosis progression and clinical events of decompensation. A boost of collagens and fragments or neoepitopes of extracellular matrix systemically and in the portal vein have been shown to be significantly associated with outcome in patients with advanced stages of cirrhosis (Leeming et al., 2013, 2015; Nielsen et al., 2015; Praktiknjo et al., 2018a; Lehmann et al., 2019). Our data indicate that extrahepatic bowel surgery, especially in the model of continuous liver injury, may have the same effect, but needs to be confirmed in further studies using this model. We believe that our model is well-suited to study different pathways of inflammation and thus to investigate pathomechanisms of postoperative hepatic decompensation.

Interestingly in the PPVL groups, portal pressure was not significantly elevated after IM but showed the same trend as the cirrhotic groups. No deaths or signs of decompensation after IM in this model were recorded, at best, only transient changes were seen in the expression of liver enzymes. IM in this important non-cirrhotic control group was performed relatively early after PPVL. Patients with portal hypertension without cirrhosis, e.g., with vascular disorders of the liver have better postsurgical prognosis, if they are treated early before the presence of liver decompensation (Elkrief et al., 2019). Our data show distinct post-surgical differences of inflammatory pathways between cirrhosis and non-cirrhotic portal hypertension, which can be further evaluated using this model.

Sarcopenia seems to play a role in these animal models of cirrhotic and non-cirrhotic portal hypertension. Our data show significant weight differences during the time of development of cirrhosis or non-cirrhotic portal hypertension. Weight loss seems to be more significant after IM in models of cirrhosis than in sham and PPVL animals. While weight loss in cirrhosis is a well-known fact, molecular mechanisms are still not fully understood, since obtaining muscle biopsies in patients might be ethically difficult. In recent studies it has been shown that muscle mass in patients with cirrhosis is associated with outcome and ACLF (Praktiknjo et al., 2018b, 2019). Pathophysiological investigation of the role of sarcopenia in the development of AD and ACLF especially after surgery in this model should be performed in the future, but is beyond the scope of this study.

There are several limitations to the study. Different surgical models, especially IM, might be dependent on the animal surgeon. However, to remove bias, all surgical procedures were performed by the same and trained individual for each surgery type (BDL, Sham, IM, LAP). Surgical procedures were performed in a block design, and animals were randomized into the different groups. Clear-cut criteria of AD in animals are missing, but our data includes relevant surrogate parameters of systemic inflammation and important laboratory parameters as well as clinical features of decompensation. Whether progression of fibrosis and inflammation can be seen more clearly in the CCL4 model without the removal of the hepatotoxic agent CCL4 remains to be investigated. Finally, the role and relevance of bacterial translocation and integrity of intestinal barrier need

to be assessed in more detail in further experiments including groups treated with antibiotics.

In conclusion, this study showed significantly elevated portal pressure and systemic inflammation in preclinical models of cirrhosis after IM. It also shows progression of fibrosis especially in models of continuous liver injury. These models may be useful to investigate pathophysiological mechanisms of post-operative decompensation. Lowering the risk of postoperative portal pressure elevation may be a therapeutic target.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because restrictions according to GDPR and LANUV (local animal authority) apply. Requests to access the datasets should be directed to Dr. Michael Praktiknjo, michael.praktiknjo@ukbonn.de.

ETHICS STATEMENT

The animal study was reviewed and approved by Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (81-02-04.2018.A348).

AUTHOR CONTRIBUTIONS

JC, JM, MG, and MH: acquisition of data, analysis, interpretation of data, drafting of the manuscript, and statistical analysis. NB, RD-P, BS-W, and GK: acquisition of data, analysis, and interpretation of data and critical revision of the manuscript regarding important intellectual content. MO, LP, SK, FU, MB, TV, PL, and JK: interpretation of data and critical revision of the manuscript regarding important intellectual content. CJ and CS: administrative, technical and material support, and critical revision of the manuscript regarding important intellectual content. SW, JT, and MP: study concept and design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript regarding important intellectual content, final approval of the version to be published, administrative, technical and material support, and study supervision. All authors contributed to the article and approved the submitted version.

FUNDING

JC was funded by grants from the Else-Kroener Fresenius Foundation (2014_Kolleg.05) and BONFOR research program of the University of Bonn (grant ID 2019-2-08). JT was supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18, CRC 1382 A09), European Union's Horizon 2020 Research and Innovation Programme (Galaxy, No. 668031; MICROB-PREDICT, No. 825694; DECISION, No. 847949), Societal Challenges – Health, Demographic Change and Wellbeing (No. 731875), and Cellex Foundation (PREDICT). MP was funded by the Ernst-und-Berta Grimmke Foundation (No. 5/19) and BONFOR research program of the University of Bonn (grant ID 2020-2A-07 and 2021-2A-07). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

We thank Jennifer Söhne, Franziska Füllmann, Christiane Esch, and Christine Peigney for their excellent technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.720898/full#supplementary-material

REFERENCES

Angeli, P., Bernardi, M., Villanueva, C., Francoz, C., Mookerjee, R. P., Trebicka, J., et al. (2018). EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J. Hepatol.* 69, 406–460. doi: 10.1016/j.jhep.2018.03.024

Bortscher, S., Chang, J., Vilz, T. O., Schäfer, N., Sommer, N., Wehner, S., et al. (2012). Hemin induction of HO-1 protects against LPS-induced septic ileus. *J. Surg. Res.* 178, 866–873. doi: 10.1016/j.jss.2012.07.064

Brol, M. J., Rösch, F., Schierwagen, R., Magdaleno, F., Uschner, F. E., Manekeller, S., et al. (2019). Combination of CCl_4 with alcoholic and metabolic injuries mimics human liver fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 317, G182–G194. doi: 10.1152/ajpgi.00361.2018

Bruix, J., Castells, A., Bosch, J., Feu, F., Fuster, J., Garcia-Pagan, J., et al. (1996). Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 111, 1018–1022. doi: 10.1016/S0016-5085(96)70070-7

Buttenschoen, K., Buttenschoen, D. C., Berger, D., Vasilescu, C., Schafheutle, S., Goeltenboth, B., et al. (2001). Endotoxemia and acute-phase proteins in major abdominal surgery. *Am. J. Surg.* 181, 36–43. doi: 10.1016/S0002-9610(00)00534-1

Buttenschoen, K., Schneider, M. E., Utz, K., Kornmann, M., Beger, H. G., and Carli Buttenschoen, D. (2009). Effect of major abdominal surgery on endotoxin release and expression of Toll-like receptors 2/4. *Langenbecks. Arch. Surg.* 394, 293–302. doi: 10.1007/s00423-008-0357-8

Chang, J., Bamarni, A., Böhling, N., Zhou, X., Klein, L.-M., Meinke, J., et al. (2021). Elective surgery but not transjugular intrahepatic portosystemic shunt precipitates acute-on-chronic liver failure. *Hepatol. Commun.* 5, 1265–1277. doi: 10.1002/hep4.1712

Chang, J., Wehner, S., Schäfer, N., Sioutis, M., Bortscher, S., Hirner, A., et al. (2012). Iatrogenic extracellular matrix disruption as a local trigger for postoperative ileus. *J. Surg. Res.* 178, 632–639. doi: 10.1016/j.jss.2012.05.044

de Goede, B., Klitsie, P. J., Lange, J. F., Metselaar, H. J., and Kazemier, G. (2012). Morbidity and mortality related to non-hepatic surgery in patients with liver cirrhosis; a systematic review. *Best Pract. Res. Clin. Gastroenterol.* 26, 47–59. doi: 10.1016/j.bpg.2012.01.010

Elkrief, L., Ferrusquia-Acosta, J., Payancé, A., Moga, L., Tellez, L., Praktiknjo, M., et al. (2019). Abdominal surgery in patients with idiopathic noncirrhotic portal hypertension: a multicenter retrospective study. *Hepatology* 70, 911–924. doi: 10.1002/hep.30628

Friedman, L. S. (2010). Surgery in the patient with liver disease. *Trans. Am. Clin. Climatol. Assoc.* 121, 192–204; discussion 205.

García-Pagán, J. C., Saffo, S., Mandorfer, M., and Garcia-Tsao, G. (2020). Where does TIPS fit in the management of patients with cirrhosis? *JHEP Rep*. 2:100122. doi: 10.1016/j.jhepr.2020.100122

Gustot, T., Fernandez, J., Garcia, E., Morando, F., Caraceni, P., Alessandria, C., et al. (2015). Clinical Course of acute-on-chronic liver failure syndrome and effects on prognosis. *Hepatol. Baltim. Md* 62, 243–252. doi: 10.1002/hep.27849

Klein, L. M., Chang, J., Gu, W., Manekeller, S., Jansen, C., Lingohr, P., et al. (2020). The development and outcome of acute-on-chronic liver failure after surgical interventions. *Liver Transplant.* 26, 227–237. doi: 10.1002/lt.25675

Klein, S., Rick, J., Lehmann, J., Schierwagen, R., Schierwagen, I. G., Verbeke, L., et al. (2017). Janus-kinase-2 relates directly to portal hypertension and to complications in rodent and human cirrhosis. *Gut* 66, 145–155. doi: 10.1136/gutjnl-2015-309600

Leeming, D. J., Karsdal, M. A., Byrjalsen, I., Bendtsen, F., Trebicka, J., Nielsen, M. J., et al. (2013). Novel serological neo-epitope markers of extracellular matrix proteins for the detection of portal hypertension. *Aliment. Pharmacol. Ther.* 38, 1086–1096. doi: 10.1111/apt.12484

Leeming, D. J., Veidal, S. S., Karsdal, M. A., Nielsen, M. J., Trebicka, J., Busk, T., et al. (2015). Pro-C5, a marker of true type V collagen formation and fibrillation, correlates with portal hypertension in patients with alcoholic cirrhosis. *Scand. J. Gastroenterol.* 50, 584–592. doi: 10.3109/00365521.2014.996590

Lehmann, J., Praktiknjo, M., Nielsen, M. J., Schierwagen, R., Meyer, C., Thomas, D., et al. (2019). Collagen type IV remodelling gender-specifically predicts mortality in decompensated cirrhosis. *Liver Int.* 39, 885–893. doi: 10.1111/liv.14070

Moreau, R., Jalan, R., Gines, P., Pavesi, M., Angeli, P., Cordoba, J., et al. (2013). Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 144, 1426–1437. doi: 10.1053/j.gastro.2013.02.042

Nevzorova, Y. A., Boyer-Diaz, Z., Cubero, F. J., and Gracia-Sancho, J. (2020). Animal models for liver disease – a practical approach for translational research. *J. Hepatol.* 73, 423–440. doi: 10.1016/j.jhep.2020.04.011

Nielsen, M. J., Lehmann, J., Leeming, D. J., Schierwagen, R., Klein, S., Jansen, C., et al. (2015). Circulating elastin fragments are not affected by hepatic, renal and hemodynamic changes, but reflect survival in cirrhosis with TIPS. *Dig. Dis. Sci.* 60, 3456–3464. doi: 10.1007/s10620-015-3783-9

Praktiknjo, M., Book, M., Luetkens, J., Pohlmann, A., Meyer, C., Thomas, D., et al. (2018b). Fat-free muscle mass in magnetic resonance imaging predicts acute-on-chronic liver failure and survival in decompensated cirrhosis. *Hepatol. Baltim. Md* 67, 1014–1026. doi: 10.1002/hep.29602

Praktiknjo, M., Clees, C., Pigliacelli, A., Fischer, S., Jansen, C., Lehmann, J., et al. (2019). Sarcopenia is associated with development of acute-on-chronic liver failure in decompensated liver cirrhosis receiving transjugular intrahepatic portosystemic shunt. *Clin. Transl. Gastroenterol.* 10:e00025. doi: 10.14309/ctg.0000000000000025

Praktiknjo, M., Lehmann, J., Nielsen, M. J., Schierwagen, R., Uschner, F. E., Meyer, C., et al. (2018a). Acute decompensation boosts hepatic collagen type III deposition and deteriorates experimental and human cirrhosis. *Hepatol. Commun.* 2, 211–222. doi: 10.1002/hep4.1135

Praktiknjo, M., Monteiro, S., Grandt, J., Kimer, N., Madsen, J. L., Werge, M. P., et al. (2020). Cardiodynamic state is associated with systemic inflammation and fatal acute-on-chronic liver failure. *Liver Int*. 40, 1457–1466. doi: 10.1111/liv.14433

Reverter, E., Cirera, I., Albillos, A., Debernardi-Venon, W., Abraldes, J. G., Llop, E., et al. (2019). The prognostic role of hepatic venous pressure gradient in cirrhotic patients undergoing elective extrahepatic surgery. *J. Hepatol.* 71, 942–950. doi: 10.1016/j.jhep.2019.07.007

Schierwagen, R., Maybüchen, L., Zimmer, S., Hittatiya, K., Bäck, C., Klein, S., et al. (2015). Seven weeks of Western diet in apolipoprotein-E-deficient mice induce metabolic syndrome and non-alcoholic steatohepatitis with liver fibrosis. *Sci. Rep.* 5:12931. doi: 10.1038/srep12931

Trebicka, J., Amoros, A., Pitarch, C., Titos, E., Alcaraz-Quiles, J., Schierwagen, R., et al. (2019). Addressing profiles of systemic inflammation across the different clinical phenotypes of acutely decompensated cirrhosis. *Front. Immunol.* 10:476. doi: 10.3389/fimmu.2019.00476

Trebicka, J., Fernandez, J., Papp, M., Caraceni, P., Laleman, W., Gambino, C., et al. (2020a). PREDICT identifies precipitating events associated with the clinical course of acutely decompensated cirrhosis. *J. Hepatol.* 74, 1097–1108. doi: 10.1016/j.jhep.2020.11.019

Trebicka, J., Fernandez, J., Papp, M., Caraceni, P., Laleman, W., Gambino, C., et al. (2020b). The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. *J. Hepatol.* 73, 842–854. doi: 10.1016/j.jhep.2020.06.013

Trebicka, J., Racz, I., Siegmund, S. V., Cara, E., Granzow, M., Schierwagen, R., et al. (2011). Role of cannabinoid receptors in alcoholic hepatic injury: steatosis and fibrogenesis are increased in CB2 receptor-deficient mice and decreased in CB1 receptor knockouts. *Liver Int*. 31, 860–870. doi: 10.1111/j.1478-3231.2011.02496.x

Uschner, F. E., Ranabhat, G., Choi, S. S., Granzow, M., Klein, S., Schierwagen, R., et al. (2015). Statins activate the canonical hedgehog-signaling and aggravate non-cirrhotic portal hypertension, but inhibit the non-canonical hedgehog signaling and cirrhotic portal hypertension. *Sci. Rep.* 5:14573. doi: 10.1038/srep14573

Wetterkamp, M., van Beekum, C. J., Willis, M. A., Glowka, T. R., Manekeller, S., Fimmers, R., et al. (2020). Risk factors for postoperative morbidity and mortality after small bowel surgery in patients with cirrhotic liver disease-a retrospective analysis of 76 cases in a tertiary center. *Biology* 9:349. doi: 10.3390/biology9110349

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 04 October 2021
doi: 10.3389/fmed.2021.737984

Comparison of Transjugular Intrahepatic Portosystemic Shunt in the Treatment of Cirrhosis With or Without Portal Vein Thrombosis: A Retrospective Study

Hong-Liang Wang[1,2], *Wei-Jie Lu*[1], *Yue-Lin Zhang*[1,3], *Chun-Hui Nie*[1,3], *Tan-Yang Zhou*[1,2], *Guan-Hui Zhou*[1,2], *Tong-Yin Zhu*[1,2], *Bao-Quan Wang*[1,2], *Sheng-Qun Chen*[1,2], *Zi-Niu Yu*[1,2], *Li Jing*[1,2] *and Jun-Hui Sun*[1,2,3]*

[1] *Hepatobiliary and Pancreatic Interventional Treatment Center, Division of Hepatobiliary and Pancreatic Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China,* [2] *Zhejiang Provincial Research Center for Diagnosis and Treatment of Hepatobiliary Diseases, Hangzhou, China,* [3] *Zhejiang Clinical Research Center of Hepatobiliary and Pancreatic Diseases, Hangzhou, China*

Aim: The purpose of our study was to conduct a retrospective analysis to compare the effectiveness of transjugular intrahepatic portosystemic shunts (TIPS) in the treatment of patients with cirrhosis with or without portal vein thrombosis (PVT).

Methods: We included a total of 203 cirrhosis patients successfully treated with TIPS between January 2015 and January 2018, including 72 cirrhosis patients with PVT (35.5%) and 131 without PVT (64.5%). Our subjects were followed for at least 1 year after treatment with TIPS. Data were collected to estimate the mortality, shunt dysfunction, and complication rates after TIPS creation.

Results: During the mean follow-up time of 19.5 ± 12.8 months, 21 (10.3%) patients died, 15 (7.4%) developed shunt dysfunction, and 44 (21.6%) experienced overt hepatic encephalopathy (OHE). No significant differences in mortality ($P = 0.134$), shunt dysfunction ($P = 0.214$), or OHE ($P = 0.632$) were noted between the groups. Age, model for end-stage liver disease (MELD) score, and refractory ascites requiring TIPS were risk factors for mortality. A history of diabetes, percutaneous transhepatic variceal embolization (PTVE), 8-mm diameter stent, and platelet (PLT) increased the risk of shunt dysfunction. The prevalence of variceal bleeding and recurrent ascites was comparable between the two groups (16.7 vs. 16.7% $P = 0.998$ and 2.7 vs. 3.8% $P = 0.678$, respectively).

Conclusions: Transjugular intrahepatic portosystemic shunts are feasible in the management of cirrhosis with PVT. No significant differences in survival or shunt dysfunction were noted between the PVT and no-PVT groups. The risk of recurrent variceal bleeding, recurrent ascites, and OHE in the PVT group was generally similar to that in the no-PVT group. TIPS represents a potentially feasible treatment option in cirrhosis patients with PVT.

Keywords: transjugular intrahepatic portosystemic shunt, cirrhosis, portal vein thrombosis, survival, shunt dysfunction

OPEN ACCESS

Edited by:
Jonel Trebicka,
Goethe University Frankfurt, Germany

Reviewed by:
Michael Praktiknjo,
University Hospital Bonn, Germany
Maximilian Joseph Brol,
University Hospital Bonn, Germany

***Correspondence:**
Jun-Hui Sun
1307005@zju.edu.cn

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *08 July 2021*
Accepted: *06 September 2021*
Published: *04 October 2021*

Citation:
Wang H-L, Lu W-J, Zhang Y-L, Nie C-H, Zhou T-Y, Zhou G-H, Zhu T-Y, Wang B-Q, Chen S-Q, Yu Z-N, Jing L and Sun J-H (2021) Comparison of Transjugular Intrahepatic Portosystemic Shunt in the Treatment of Cirrhosis With or Without Portal Vein Thrombosis: A Retrospective Study. Front. Med. 8:737984. doi: 10.3389/fmed.2021.737984

INTRODUCTION

Portal vein thrombosis (PVT) is an important complication of cirrhosis but is not common in the general population. However, in cirrhosis patients, the occurrence rate is ∼10–25%, and this figure increases with the severity of cirrhosis (1–4). PVT can further aggravate portal vein hypertension and lead to repeated variceal bleeding or refractory ascites (5). PVT in cirrhosis may be associated with a reduction in the portal vein blood flow velocity, a high coagulation state, and vascular endothelial injury (6–8). Currently, anticoagulant therapy is recommended as the preferred treatment option for PVT, but anticoagulation is a challenging therapy in patients with liver cirrhosis given the well-recognized coagulation abnormalities (9). In addition, the occurrence of PVT is typically not evident, and most patients are complicated with portal hypertension (10, 11). At present, thrombosis exhibits different degrees of transformation into chronic thrombosis, and clinical treatment is very difficult. Transjugular intrahepatic portosystemic shunts (TIPS) can be used to establish a shunt between the hepatic vein and the portal vein to reduce portal vein pressure. TIPS improve portal vein blood flow and promote blood clot absorption and recanalization (12–15). Thus, the purpose of our study was to conduct a retrospective analysis to compare the effectiveness of TIPS in the treatment of patients with cirrhosis with or without PVT in our center.

MATERIALS AND METHODS

Patients

This retrospective study was reviewed and approved by the ethics committee of The First Affiliated Hospital, Zhejiang University School of Medicine. Given its retrospective nature and the lack of a need to collect samples from patients, a waiver of written informed consent was applied. This study included all patients with cirrhosis (any etiology) with or without PVT characterized as non-neoplastic (no tumor vein invasion) according to criteria validated in previous studies (16) between January 2015 and January 2018 in our center. The exclusion criteria for our study included previous TIPS placement, missing clinical and demographic information, hepatocellular carcinoma, previous liver transplantation (LT), technical failure of TIPS, <12 months of follow-up, any active tumor at the time of PVT diagnosis, and incomplete baseline data. However, our study did not exclude patients with cavernomatous transformation of the portal vein. Patients were categorized according to whether they had PVT or not before TIPS. Patients were followed until the occurrence of end points, including death, LT, or the end of the study, in January 2018.

TIPS Procedure

As previously described (17), TIPS procedures were performed by the same team of interventional radiologists who had >10 years of experience in TIPS procedures. With the exception of emergencies, all patients underwent routine computed tomography angiography (CTA) examination prior to the TIPS procedure to clearly visualize the anatomical relationship between the start (hepatic vein) and the end point (portal vein). After a successful puncture, bare stents (Boston Scientific, USA) plus a self-expandable polytetrafluoroethylene (ePTFE)-covered stent (GORE VIABAHN, USA), either 8 or 10 mm in diameter, were inserted primarily based on the data from the procedure that was performed. The portosystemic pressure gradient (PSG) was measured using the difference between the portal vein pressure and the right atrial pressure. If the PSG was not reduced below the target threshold (12 mmHg), balloon dilation was performed. TIPS revision using balloon dilatation or parallel TIPS was performed. Variceal embolization was based on post-TIPS portography using a metal coil (Cook, Bloomington, USA), glue (Guangzhou Baiyun, Guangdong, China), or a metal coil plus glue.

Data Collection and Follow-Up

Clinical, epidemiologic, laboratory, and radiologic data were extracted from the medical records of the patients, including demographics, etiology of cirrhosis, previous splenectomy, history of diabetes, history of overt HE, and laboratory testing results (aspartate aminotransferase, alanine aminotransferase, albumin, international normalized ratio, platelet, hemoglobin, white blood cell, creatinine, and total bilirubin). Child–Pugh score, Child–Pugh class, model for end-stage liver disease (MELD) score, and Eastern Cooperative Oncology Group (ECOG) score were also calculated for each patient. The following TIPS outcomes were assessed in the entire cohort: duration of follow-up, indications for TIPS, refractory ascites, variceal bleeding (gastric plus esophageal), 90-day mortality, mortality (liver failure, multiorgan failure, gastrointestinal bleeding, hepatorenal syndrome, sepsis, cerebral hemorrhage, or unknown), stent (8 or 10 mm), overt hepatic encephalopathy (OHE), recurrent variceal bleeding, recurrent ascites, antiplatelet treatments (aspirin or aspirin plus dipyridamole), percutaneous transhepatic variceal embolization (PTVE) (coil, glue, or glue plus coil), portosystemic gradient before TIPS, portosystemic gradient after TIPS, LT, and shunt dysfunction. The presence of PVT was determined according to computed tomography (CT) or magnetic resonance imaging and confirmed by portal angiography at the time of TIPS creation. Follow-up visits were performed when patients presented to the follow-up clinic and were scheduled 1, 2, 3, and 6 months after TIPS and every 6 months thereafter. Clinical, laboratory, and liver CTA evaluations were performed at each visit, and the occurrence of any liver-related complications since the last visit was collected by the clinical research coordinator. Patients underwent follow-up until death, LT, or the end date of the study on January 31, 2018.

Statistical Analysis

All continuous variables are presented as the mean ± SD, and categorical variables are expressed as counts and frequencies. Student's *t*-test or a chi-square test was used to compare the significant differences between groups where appropriate. Survival was calculated as the time from TIPS creation to the time of death, transplantation, or the last follow-up. Survival curves and the cumulative incidence of shunt dysfunction curves were

TABLE 1 | Baseline demographic and clinical characteristics of patients.

Demographics	Overall (*N* = 203)	PVT (*N* = 72)	No-PVT (*N* = 131)	*P*-value
Male	141 (69.5.0%)	47 (65.3%)	94 (71.7%)	0.424
Age (mean ±SD) (years)	55.4 ± 10.8	55.2 ± 10.5	55.6 ± 11.4	0.736
Etiology of cirrhosis				
Hepatitis B virus	111 (54.7%)	42 (58.3%)	69 (52.7%)	0.594
Hepatitis C virus	4 (1.9%)	1 (1.4%)	3 (2.1%)	0.421
Alcohol	34 (16.7%)	12 (16.7%)	22 (16.8%)	0.675
NASH/cryptogenic	26 (12.8%)	11 (15.3%)	15 (11.5%)	0.321
PBC/PSC	7 (3.4%)	1 (1.4%)	6 (4.6%)	0.257
Autoimmune	5 (2.5%)	3 (4.2%)	2 (1.5%)	0.132
Schistosome	10 (4.9%)	2 (2.8%)	8 (6.1%)	0.171
Previous splenectomy	27 (13.3%)	18 (25.0%)	9 (6.8%)	<0.001
History of diabetes	39 (19.3%)	11 (15.2%)	28 (21.4%)	0.262
History of overt HE	8 (3.9%)	2 (2.3%)	6 (4.6%)	0.324
Laboratory parameters (mean ± SD)				
AST (IU/l)	84 ± 212	86 ± 148	72 ± 256	0.232
ALT (IU/l)	65 ± 172	69 ± 124	62 ± 225	0.532
Albumin (g/dl)	34.3 ± 5.2	33.4 ± 4.4	34.6 ± 5.8	0.612
INR	1.3 ± 1.32	1.3 ± 1.22	1.3 ± 2.23	0.604
WBC	5.1 ± 5.1	5.8 ± 7.2	4.7 ± 3.2	0.231
PLT	76.1 ± 64.5	107.2 ± 82.4	65.4 ± 37.7	<0.001
HB	81.1 ± 24.7	76.4 ± 21.7	84.2 ± 256.2	0.021
Creatinine (mg/dl)	71.2 ± 31.2	67.4 ± 16.7	72.2 ± 35.5	0.325
Total bilirubin (mg/dl)	26.0 ± 19.45	24.6 ± 16.5	33.2 ± 22.4	0.432
Child-Pugh score	6.8 ± 1.4	6.8 ± 1.3	6.8 ± 1.4	0.632
Child-Pugh class				0.201
A	89 (43.6%)	29 (40.3%)	60 (45.8%)	
B	101 (49.5%)	40 (55.6%)	61 (46.6%)	
C	13 (6.4%)	3 (4.2%)	10 (7.6%)	
MELD	11.1 ± 3.20	10.9 ± 3.0	11.1 ± 3.3	0.324
ECOG	1.05 ± 0.27	1.04 ± 0.22	1.05 ± 0.23	0.703
Stage of PVT (chronic)	/	54 (75.0%)	/	
Degree of PVT	/		/	
Mural	/	12 (16.7%)	/	
Partial	/	54 (75.0%)	/	
Complete	/	4 (5.5%)	/	
Extent of PVT	/		/	
MPV alone	/	12 (16.7%)	/	
MPV + SMV	/	19 (26.4%)	/	
MPV + SV/splenectomy	/	32 (44.4%)	/	
MPV + SMV + SV/splenectomy	/	7 (9.7%)	/	

NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; HE, hepatic encephalopathy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; WBC, White Blood Cell; PLT, Platelet; HB, Hemoglobin; MELD, Model for End-stage Liver Disease; PVT, portal vein thrombosis. ECOG, Eastern Cooperative Oncology Group; MPV, main portal vein; SMV, superior mesenteric vein; SV, splenic vein.

estimated using the Kaplan–Meier method, and the differences were compared using the log-rank test. Risk factors associated with survival were explored using the Cox hazard multivariate regression model. To rule out the effect that splenectomy might have in our findings, we also performed the analysis excluding patients who had undergone splenectomy. We regard a two-tailed $p < 0.05$ as statistically significant. All the statistical analyses were conducted with R 3.5.0 (18).

RESULTS

Baseline Characteristics

A total of 208 patients underwent TIP creation, but five patients experienced technical failure of TIPS, including two in the non-PVT group and three in the PVT group. Finally, a total of 203 patients who underwent TIP creation, including 72 patients with PVT, were included in our analysis. **Table 1** presents the baseline

TABLE 2 | Outcomes of Transjugular intrahepatic portosystemic shunt in the Entire Cohort.

Characteristics	Overall (*N* = 203)	PVT (*N* = 72)	No-PVT (*N* = 131)	*P*-value
Duration of follow-up (month)	19.5 ± 12.8	20.5 ± 10.2	18.8 ± 13.2	0.234
Indications for TIPS				
Refractory ascites	23 (11.3%)	3 (4.2%)	20 (15.4%)	0.021
Variceal bleeding (Gastric + Esophageal)	184 (91.5%)	70 (97.2%)	114 (87.0%)	0.018
90-day mortality	8 (5.8%)	2 (2.6%)	6 (7.5%)	0.206
Mortality	21 (14.3%)	5 (10.3%)	16 (16.4%)	0.134
Liver failure	8 (3.9%)	2 (2.8%)	6 (4.5%)	
Multiorgan failure	6 (2.9%)	1 (1.4%)	5 (3.8%)	
Gastrointestinal bleeding	2 (0.9%)	1 (1.4%)	1 (0.7%)	
Hepatorenal syndrome	1 (0.5%)	0 (0.0%)	1 (0.7%)	
Sepsis	1 (0.5%)	0 (0.0%)	1 (0.7%)	
Cerebral hemorrhage	1 (0.5%)	0 (0.0%)	1 (0.7%)	
Unknown	2 (0.9%)	1 (1.4%)	1 (0.7%)	
Diameter of stent				0.622
8 mm	189 (92.6%)	68 (94.4%)	121 (92.3%)	
10 mm	14 (7.4%)	4 (5.6%)	10 (7.7%)	
Overt hepatic encephalopathy	44 (21.6%)	14 (19.4%)	30 (22.9%)	0.632
Recurrent variceal bleeding	34 (16.7%)	12 (16.7%)	22 (16.7%)	0.998
Recurrent ascites	7 (3.4%)	2 (2.7%)	5 (3.8%)	0.678
Antiplatelet treatments	114 (55.9%)	50 (69.4%)	64 (45%)	0.032
Aspirin	80 (39.2%)	36 (50.0%)	44 (30.1%)	0.021
Aspirin + Dipyridamole	34 (16.7%)	13 (18.1%)	21 (16.0%)	0.632
PTVE	152 (74.5%)	47 (65.3%)	105 (80.1%)	0.256
Coil	72 (35.3%)	25 (17.3%)	47 (36.3%)	0.782
Glue	22 (10.8%)	7 (9.7%)	15 (10.3%)	0.421
Glue + Coil	48 (23.5)	15 (20.8%)	33 (25.3%)	0.421
Portosystemic gradient before TIPS (mmHg)	25.2 ± 5.6	27.4 ± 6.2	24.1 ± 4.5	0.234
Portosystemic gradient after TIPS	10.5 ± 5.8	12.1 ± 6.2	9.5 ± 3.2	0.432
Shunt dysfunction	15 (7.4%)	7 (9.7%)	8 (6.1%)	0.214

Indications for TIPS, 90-Day Mortality, Mortality, Diameter of stent, Overt hepatic encephalopathy, Recurrent variceal bleeding, Recurrent ascites, Antiplatelet treatments, PTVE, PSG before TIPS (mmHg), PSG after TIPS (mmHg), Liver transplantation, Shunt dysfunction compared between patients with and without PVT. OHE, Overt hepatic encephalopathy; PSG, Portosystemic gradient; PTVE, percutaneous transhepatic variceal embolization.

characteristics of the patients and comparisons between the two groups. Age ($P = 0.736$) and sex ($P = 0.424$) were similar between the two groups. Hepatitis B, non-alcoholic steatohepatitis (NASH)/cryptogenesis, and liver disease caused by alcohol were identified as the most common causes of cirrhosis. The prevalence of hepatitis B infection, NASH/cryptogenesis, and liver disease due to alcohol consumption were generally comparable between the PVT and no-PVT groups [(58.3 vs. 52.7%, $P = 0.594$), (15.1 vs. 11.5%, $P = 0.321$), and (16.7 vs. 16.8%, $P = 0.672$), respectively].

Comparing the two groups of patients, the PVT group had a greater proportion of patients with a history of splenectomy (25.0 vs. 6.8%, $P < 0.001$). History of diabetes and OHE did not significantly differ between PVT and no-PVT groups [(15.2 vs. 21.4%, $P = 0.262$) and (2.3 vs. 4.6%, $P = 0.324$)]. The following laboratory parameters were collected before TIPS: AST, ALT, albumin, INR, WBC, PLT, HB, creatinine, and total bilirubin. The PVT group had higher platelet counts (107.2 ± 82.4 vs. 65.4 ± 37.7, $P < 0.001$), but no obvious differences in other parameters were noted between the two groups. Child–Pugh, MELD, and ECOG scores were similar between the PVT and no-PVT groups [(6.8 ± 1.3 vs. 6.8 ± 1.4 $P = 0.632$), (10.9 ± 3.0 vs. 11.1 ± 3.3, $P = 0.324$), and (1.05 ± 0.27 vs. 1.05 ± 0.23, $P = 0.703$), respectively].

Morbidity Following TIPS

During the mean follow-up time of 19.5 ± 12.8 months, 21 (10.3%) patients died. The cause of death included liver failure in eight patients (3.9%), multiorgan failure in six (2.9%), gastrointestinal bleeding in two (0.9%), hepatorenal syndrome in one (0.5%), sepsis in one (0.5%), cerebral hemorrhage in one (0.5%), and other conditions in two (0.9%). The cumulative incidence of death at 90 days and in the overall follow-up period was not significantly different between the PVT and no-PVT groups [(2.6 vs. 7.5%, $P = 0.206$) and (10.3 vs. 16.4%, $P = 0.134$), respectively] (**Table 2**, **Figure 1**). No significant difference was observed in the proportion of patients experiencing transplantation

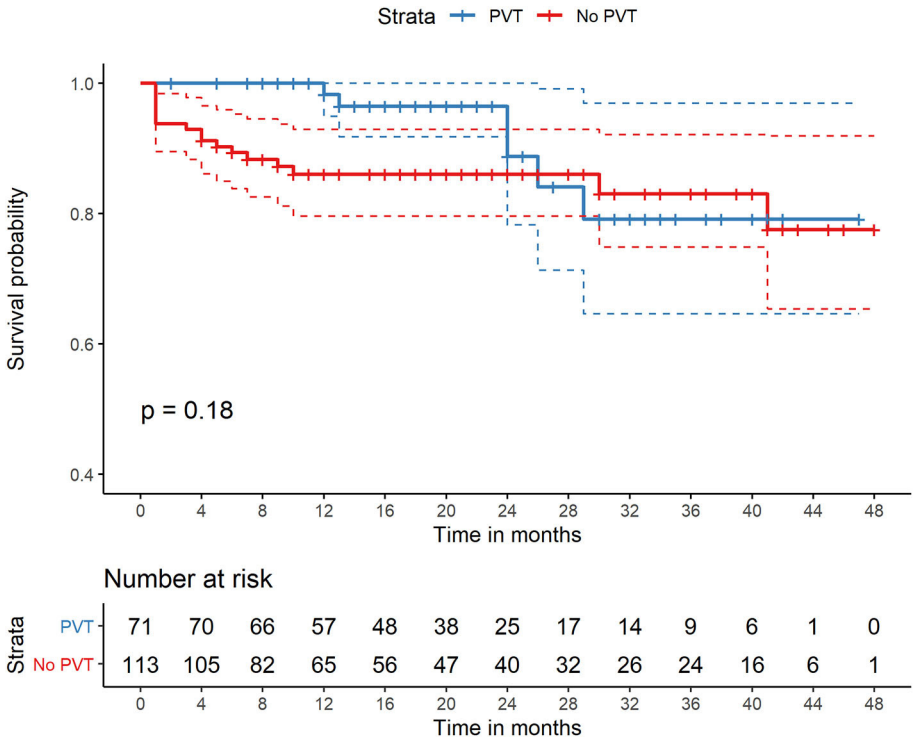


FIGURE 1 | Comparison of survival of patients with PVT and without PVT who were all treated with TIPS. PVT, portal vein thrombosis; TIPS, transjugular intrahepatic portosystemic shunt.

between the PVT and no-PVT groups (9.0 vs. 5.5%, $P = 0.319$).

Univariate analysis showed that age (per year increase), refractory ascites requiring TIPS, variceal bleeding (gastric plus esophageal) as an indication for TIPS, creatinine, MELD score, and ECOG score was associated with mortality risk. The Cox hazard multivariate regression model showed that only older age (hazard ratio (HR), 1.05; 95% CI, 1.04–1.09), higher MELD score (HR, 1.23; 95% CI, 1.08–1.41), and refractory ascites requiring TIPS (HR, 0.46; 95% CI, 0.32–0.65) were statistically significant predictors of mortality (**Table 3**). In addition, the usage of glue in PTVE (HR, 0.31 95% CI, 0.08–1.18) may improve overall survival compared with coil alone in PTVE (HR, 0.66 95% CI, 0.14–3.16).

Post-operative Complications and Shunt Dysfunction

Figure 2 presents an example of a patient with PVT in the main portal vein, and the superior mesenteric vein was treated with TIPS. All 203 patients successfully accepted TIPS between January 2015 and January 2018. Variceal bleeding (gastric plus esophageal) was the main indication for TIPS, accounting for 97.2 and 87.0% of patients with and without PVT, respectively ($P = 0.018$). The PSG was 25.3 ± 5.6 mmHg before TIPS and 10.5 ± 5.8 mmHg after TIPS. The PSG values in patients with and without PVT were similar before ($P = 0.234$) and after TIPS establishment ($P = 0.432$). The diameter of the stent required to achieve the desired reduction in the PSG was not significantly different between the PVT and no-PVT groups ($P = 0.622$). The main complications of TIPS, including recurrent variceal bleeding, recurrent ascites, and overt HE, were similar between the two groups (16.7 vs. 16.7%, $P = 0.998$; 2.7 vs. 3.8%, $P = 0.678$; and 19.4 vs. 22.9%, $P = 0.632$, respectively) (**Table 2**).

During the follow-up period, 15 (7.4%) patients reported one or more episodes of shunt dysfunction. No significant difference in the cumulative incidence of shunt dysfunction was noted between the two groups during the follow-up period (9.7 vs. 6.7%, $P = 0.214$) (**Table 2**, **Figure 3**). Univariate and multivariate analyses showed that 8-mm stent diameter (HR, 1.24; 95% CI, 1.09–1.41) were associated with increased shunt dysfunction risk during follow-up (**Table 4**).

DISCUSSION

In the past, PVT was considered a relative contraindication to TIPS; however, many previous studies have shown that similar outcomes were reported in patients with non-oncologic PVT and those without PVT after the creation of TIPS (13, 19, 20). A recent systematic review and meta-analysis suggested that TIPSs in patients with PVT yielded satisfactory outcomes. For example, the 1-year portal vein recanalization rate was 77.7%, the TIPS patency rate was 84.2%, and the overall 1-year survival

TABLE 3 | Factors associated with risk of mortality after Transjugular intrahepatic portosystemic shunt.

Variable	Univariate analysis				Multivariate analysis			
	Hazard ratio† (95% CI)			*P*-value	Hazard ratio† (95% CI)			*P*-value
Gender (male vs. female)	1.44	0.63	3.31	0.387	0.89	0.54	1.47	0.832
Age (per year increase)	1.05	1.01	1.09	0.010	1.04	1.00	1.09	0.032
Antiplatelet treatments (yes vs. no)	0.42	0.18	0.99	0.048	0.48	0.20	1.15	0.100
History of ascites (yes vs. no)	0.90	0.40	2.03	0.800				
History of diabetes (yes vs. no)	0.49	0.12	2.11	0.340				
Previous splenectomy (yes vs. no)	0.62	0.27	1.29	0.231				
Refractory ascites (yes vs. no)	0.48	0.28	0.81	<0.05	0.46	0.32	0.65	<0.05
Varices bleeding (Gastric + Esophageal) (yes vs. no)	0.71	0.31	1.63	0.414				
PTVE (vs. no PTVE)								
Coil	0.83	0.32	2.17	0.708				
Glue	0.25	0.03	2.00	0.192				
Glue + Coil	0.86	0.31	2.43	0.784				
Diameter of stent (8 vs. 10 mm)	0.62	0.23	1.70	0.356				
Total bilirubin	1.00	0.97	1.02	0.817				
INR	1.71	0.32	9.06	0.530				
Creatinine	1.01	1.01	1.02	<0.001	1.01	1.00	1.02	0.067
PLT	1.00	0.99	1.01	0.647				
WBC	0.99	0.92	1.07	0.851				
HB	1.00	0.99	1.02	0.811				
Child	1.21	0.94	1.55	0.136				
MELD	1.15	1.05	1.27	0.003	1.23	1.08	1.41	<0.05
ECOG	3.03	1.23	7.44	0.016	2.11	0.75	5.93	0.158

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; WBC, White Blood Cell; PLT, Platelet; HB, Hemoglobin; MELD, Model for End-stage Liver Disease; PVT, portal vein thrombosis. ECOG, Eastern Cooperative Oncology Group. †CI, Confidence interval.

was 87.4% (21). The results from our retrospective study also suggest that TIPS represents an alternative for the treatment of refractory ascites and variceal bleeding (gastric plus esophageal) in patients with cirrhosis with PVT. These findings based on the results of our study revealed no statistically significant difference in recurrent variceal bleeding, recurrent ascites, OHE, or shunt dysfunction between the PVT and no-PVT groups.

The mortality, complication, and shunt dysfunction rates reported in our study align with those reported in previous studies (22, 23). Our results indicate that age, MELD score, and refractory ascites requiring TIPS were risk factors for mortality in multivariate analysis. PVT is not a risk factor for mortality after TIPS. Comparisons of high-risk factors for mortality have revealed differences in some epidemiological data (age, MELD score, and refractor ascites). Mortality after TIPS increases, and this finding is closely related to increasing MELD scores, which is consistent with that reported in previous literature (23, 24). PVT increased the risk of variceal rebleeding in patients with cirrhosis (25), especially in cases of portal cavernoma and acute PVT with an increase in portal hypertension, which may cause life-threatening acute refractory variceal bleeding in those with refractory ascites (26). Thus, these patients might be at a high risk and receive benefits from TIPS insertion *via* resolution of the thrombosed portal vein and simultaneous reductions in the PSG. Furthermore, our results demonstrated that refractory ascites before TIPS may be associated with poor mortality. We believe there may be one possible explanation for this finding. The refractory ascites requiring TIPS, criteria for patient selection, can improve survival, as previously reported in the literature (3). For example, most patients were <65 years old, classified with an early stage (Child B) of disease, and did not experience prior encephalopathy, which might partially explain these excellent results (27). However, most of the patients with refractory ascites requiring TIPS in our entire cohort were classified as Child C or were elderly patients. In addition, the number of patients in this study was small, and so these patients may have had worse mortality. Our results showed that 15 (7.4%) of the entire cohort experienced shunt dysfunction, and this prevalence is lower than results from previous studies (23).

Multivariate analysis showed that smaller stent diameters were associated with increased shunt dysfunction risk in the follow-up, which is consistent with previous studies (25). The choice of stent diameter (8 vs. 10 mm) remains controversial in the literature, and there is no consensus. A previous clinical trial suggested that the use of 8-mm diameter stents for TIPS construction leads to unsatisfactory control of portal hypertension with recurrence or persistence of complications in the majority of patients (28).

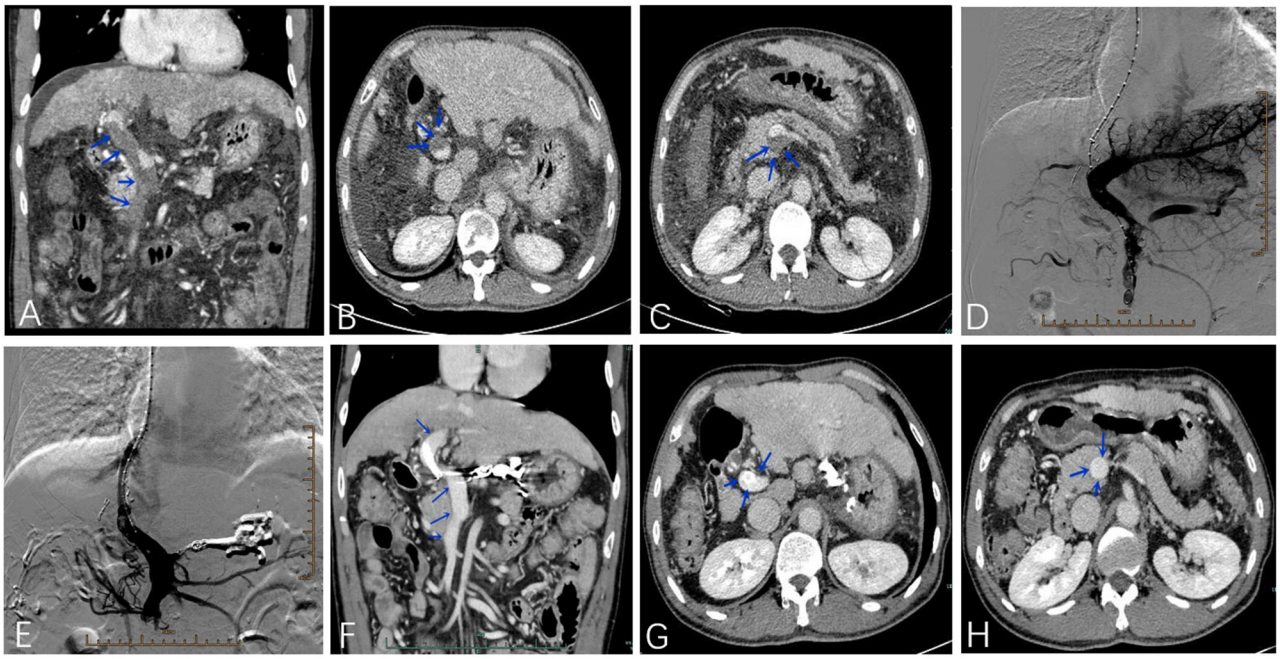


FIGURE 2 | A 55-year-old male patient was treated with TIPS due to esophagogastric varices bleeding with PVT in the main portal vein and superior mesenteric vein **(A–C)**. The portal venogram before **(D)** and after **(E)** stent placement. Four months after the operation, the thrombosis disappeared in the main portal vein and superior mesenteric vein **(F–H)**.

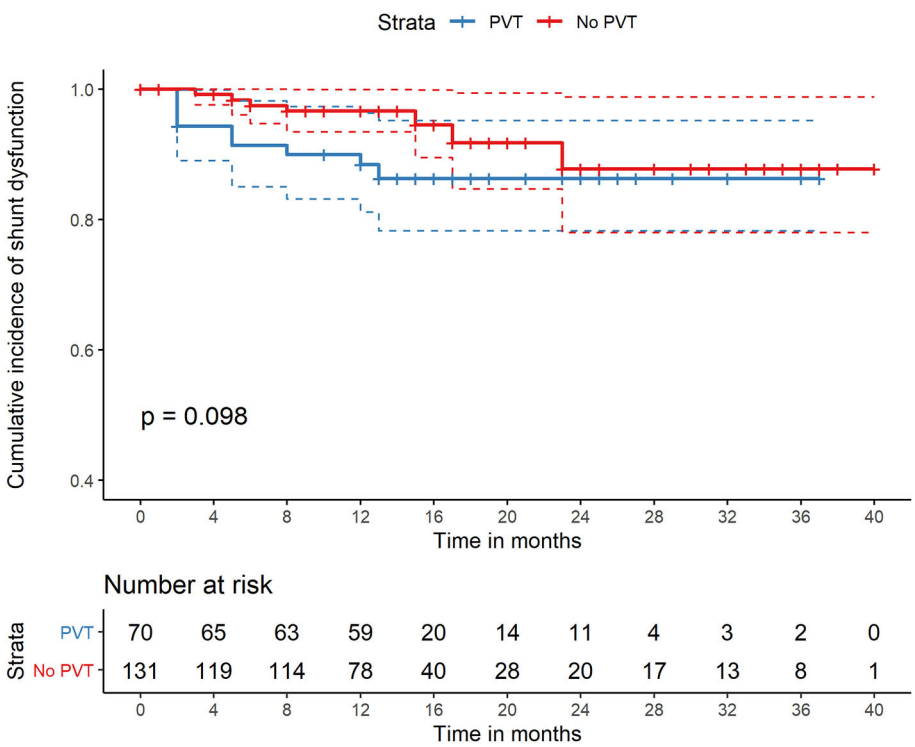


FIGURE 3 | Comparison of cumulative incidence of shunt dysfunction of patients with PVT and without PVT who were all treated with TIPS. PVT, portal vein thrombosis; TIPS, transjugular intrahepatic portosystemic shunt.

TABLE 4 | Factors associated with risk of shunt dysfunction after Transjugular intrahepatic portosystemic shunt.

Variable	Univariate analysis				Multivariate analysis			
	Hazard ratio† (95% CI)			*P*-value	Hazard ratio† (95% CI)			*P*-value
Gender (male vs. female)	0.82	0.26	2.55	0.733	0.81	0.29	2.36	0.478
Age (per year increase)	1.02	0.98	1.07	0.321	1.08	0.88	1.33	0.234
Antiplatelet treatments (yes vs. no)	1.19	0.44	3.20	0.735				
History of Ascites (yes vs. no)	0.71	0.26	1.91	0.495				
History of diabetes (yes vs. no)	0.40	0.05	3.01	0.372				
Previous splenectomy (yes vs. no)	1.19	0.44	3.20	0.735				
Refractory Ascites (yes vs. no)	1.22	0.28	5.38	0.791				
Varices bleeding (Gastric + Esophageal) (yes vs. no)	0.41	0.15	1.08	0.072	0.69	0.30	1.59	0.381
PTVE								
Coil	0.66	0.14	3.16	0.607				
Glue	0.31	0.08	1.18	0.087				
Glue+Coil	0.48	0.13	1.80	0.275				
Diameter of stent (8 vs. 10 mm)	1.32	1.08	1.61	0.0012	1.24	1.09	1.41	0.023
Total bilirubin	0.96	0.91	1.02	0.151				
INR	0.47	0.04	5.78	0.554				
Creatinine	1.01	0.99	1.02	0.490				
PLT	1.01	1.00	1.01	0.074	1.00	0.99	1.01	0.587
WBC	0.99	0.88	1.11	0.851				
HB	1.01	0.99	1.03	0.491				
Child	0.97	0.68	1.38	0.854				
MELD	0.93	0.76	1.13	0.445				
ECOG	0.79	0.58	1.08	0.352				

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; WBC, White Blood Cell; PLT, Platelet; HB, Hemoglobin; MELD, Model for End-stage Liver Disease; PVT, portal vein thrombosis. ECOG, Eastern Cooperative Oncology Group. †CI, Confidence interval.

However, a recent clinical trial suggested that TIPS with 8-mm diameter covered stents showed similar shunt function to TIPS with 10-mm diameter stents (29). In addition, recent studies have revealed that a smaller 8-mm diameter (V. S 10-mm diameter) TIPS stent graft appears to improve patient outcomes, such as survival (30, 31). The inconsistent findings may be due to the heterogeneity of patients and the small sample size in different studies. Therefore, further clinical trials on this topic based on restricted inclusion criteria and larger sample sizes are warranted.

Our study reveals that the usage of glue in PTVE may improve overall survival and prevent shunt dysfunction, which may be preferable over coil embolization alone. This finding was consistent with the results of a recent study (32). One explanation for this finding may be that, due to the physical properties of the glue embolization material, it may propagate more readily and thoroughly into the network of PTVE, thus, leading to a cast-like formation accumulating in the periphery of PTVE. However, in practice it is more challenging to use fluid embolism materials, particularly glue, and these are more prone to off-target embolism. Coils are more precise and easier to apply, especially if removable coils are used. In addition, the cost for glue and coils (pushable and detachable) may vary in different countries. Therefore, the decision for either embolization method needs to take these regional conditions into account.

Overt hepatic encephalopathy post-TIPS occurred in 21.6% of patients in our study, and this finding is consistent with the literature (20, 33). Our results also demonstrated that PVT is not related to the incidence of OHE after TIPS. Furthermore, no patient who required a small shunt diameter developed refractory hepatic encephalopathy, and this finding may be because we excluded patients with spontaneous or recent HE. However, a randomized controlled trial (RCT) (34) suggested that either lactitol or rifaximin was not able to prevent post-TIPS encephalopathy. We still routinely prescribed lactulose and/or lactic acid powder to all patients post-TIPS to reduce the time of feces in the gut. To date, post-TIPS OHE remains a problem associated with the use of ePTFE-covered stents (19). In the future, multidisciplinary cooperative analyses will be required to identify a better method to prevent and treat post-TIPS OHE. Our study also revealed that the rates of recurrent variceal bleeding and recurrent ascites after TIPS creation were similar to those reported in previous literature (35, 36). No difference was noted between the PVT group and the no-PVT group. Patients with TIPS and PVT in principle still received antiplatelet treatment in our study useless they were evaluated as having a high risk of rebleeding.

In total, 15 (7.4%) patients (seven (9.7%) in the PVT group and eight (6.1%) in the no-PVT group) cohort exhibited shunt

dysfunction, and the cumulative incidence of shunt dysfunction among the entire cohort in the overall follow-up period was not significantly different between the PVT and no-PVT groups. This percentage is less than that reported for covered stents in patients with cirrhosis with or without PVT in the literature (37). In total, fifteen patients with shunt dysfunction were successfully treated, including eight patients who underwent balloon angioplasty treatment with stent placement, five patients who underwent balloon angioplasty treatment without stent placement, and two patients who were treated with parallel TIPS. As expected, an increased rate of portal vein recanalization was observed in our entire cohort, which may be largely due to the increased flow velocity established by TIPS as it promotes mechanical lysis of residual non-occlusive thrombi (the so-called "washout effect") (19, 20, 38, 39).

There are several limitations of our study. First, our study was a retrospective analysis, which was conducted in a single center with a limited sample size. The majority of included patients had hepatitis B-related cirrhosis. Therefore, the generalization of our findings to other settings, especially Western countries, is difficult. International, multicenter, and large-sample studies may be needed in the future to better understand the effectiveness of TIPs. Second, the epidemiological features noted in the large proportion of patients in our study included HBV-related cirrhosis, which may limit the generalizability of the findings to patients with cirrhosis for other reasons. Third, an ePTFE-covered stent combined with a bare stent was implanted during the TIPS procedure rather than a Viatorr-covered stent that was not available in China during the study period. Fourth, patients with acute bleeding during TIPS creation require urgent treatment. However, acute bleeding is an indication for emergency TIPS, which may lead to differences in procedural urgency.

In conclusion, our study shows that TIPS is feasible in the management of cirrhosis with PVT. No significant differences in survival and shunt dysfunction were noted between the PVT and no-PVT groups. Age, MELD score, and refractory ascites requiring TIPS were risk factors for mortality. A history of diabetes, PTVE, 8-mm diameter stent, and PLT increase the risk of shunt dysfunction. The occurrence of recurrent variceal bleeding, recurrent ascites, and OHE was similar between the two groups. Therefore, TIPS could be considered an alternative treatment option in cirrhosis patients with PVT.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This retrospective study has been reviewed and approved by the Ethics Committee in The First Affiliated Hospital, Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

H-LW and J-HS designed the study. W-JL, Y-LZ, C-HN, T-YZho, and G-HZ collected the data. H-LW, T-YZhu, B-QW, S-QC, Z-NY, and LJ analyzed the data. H-LW, W-JL, and J-HS wrote the paper with input from all authors.

FUNDING

The present work was funded by the Zhejiang Provincial Natural Science Foundation of China (Grant No. LZ18H180001), the National Natural Science Foundation of China (Grant No. 81971713), the National S&T Major Project of China (No. 2018ZX10301201), Grant from the Health Commission of Zhejiang Province (JBZX-202004), the Research Unit of Collaborative Diagnosis and Treatment For Hepatobiliary and Pancreatic Cancer, the Chinese Academy of Medical Sciences (2019RU019), the Key Research Development Program of Zhejiang province (Grant No. 2018C03018), the Key Science and Technology Program of Zhejiang province (No. WKJ-ZJ-1923), and the National Key R&D Program of China (No. 2017YFC0114102).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.737984/full#supplementary-material

REFERENCES

1. Harding DJ, Perera MTPR, Chen F, Olliff S, Tripathi D. Portal vein thrombosis in cirrhosis: controversies and latest developments. *World J Gastroenterol*. (2015) 21:6769–84. doi: 10.3748/wjg.v21.i22.6769
2. Rodríguez-Castro KI, Porte RJ, Nadal E, Germani G, Burra P, Senzolo M. Management of nonneoplastic portal vein thrombosis in the setting of liver transplantation: a systematic review. *Transplantation*. (2012) 94:1145–53. doi: 10.1097/TP.0b013e31826e8e53
3. Bureau C, Thabut D, Oberti F, Dharancy S, Carbonell N, Bouvier A, et al. Transjugular intrahepatic portosystemic shunts with covered stents increase transplant-free survival of patients with cirrhosis and recurrent ascites. *Gastroenterology*. (2017) 152:157–63. doi: 10.1053/j.gastro.2016.09.016
4. Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou P-E, et al. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology*. (2015) 61:660–7. doi: 10.1002/hep.27546
5. Njei B, McCarty TR, Laine L. Early transjugular intrahepatic portosystemic shunt in US patients hospitalized with acute esophageal variceal

bleeding. *J Gastroenterol Hepatol*. (2017) 32:852–8. doi: 10.1111/jgh.13593
6. Sobhonslidsuk A, Reddy KR. Portal vein thrombosis: a concise review. *Am J Gastroenterol*. (2002) 97:535–41. doi: 10.1111/j.1572-0241.2002.05527.x
7. Amitrano L, Guardascione MA, Brancaccio V, Margaglione M, Manguso F, Iannaccone L, et al. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. *J Hepatol*. (2004) 40:736–41. doi: 10.1016/j.jhep.2004.01.001
8. Zocco MA, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F, et al. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein thrombosis development. *J Hepatol*. (2009) 51:682–9. doi: 10.1016/j.jhep.2009.03.013
9. Ghazaleh S, Beran A, Aburayyan K, Nehme C, Patel D, Khader Y, et al. Efficacy and safety of anticoagulation in non-malignant portal vein thrombosis in patients with liver cirrhosis: a systematic review and meta-analysis. *Ann Gastroenterol*. (2021) 34:104–10. doi: 10.20524/aog.2020.0544
10. Qi X, De Stefano V, Li H, Dai J, Guo X, Fan D. Anticoagulation for the treatment of portal vein thrombosis in liver cirrhosis: a systematic review and meta-analysis of observational studies. *Eur J Intern Med*. (2015) 26:23–9. doi: 10.1016/j.ejim.2014.12.002
11. Cai M, Zhu K, Huang W, Meng X, He K, Zhou B, et al. Portal vein thrombosis after partial splenic embolization in liver cirrhosis: efficacy of anticoagulation and long-term follow-up. *J Vasc Interv Radiol*. (2013) 24:1808–16. doi: 10.1016/j.jvir.2013.08.018
12. Senzolo M, Tibbals J, Cholongitas E, Triantos CK, Burroughs AK, Patch D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with and without cavernous transformation. *Aliment Pharmacol Ther*. (2006) 23:767–75. doi: 10.1111/j.1365-2036.2006.02820.x
13. Luca A, Miraglia R, Caruso S, Milazzo M, Sapere C, Maruzzelli L, et al. Short- and long-term effects of the transjugular intrahepatic portosystemic shunt on portal vein thrombosis in patients with cirrhosis. *Gut*. (2011) 60:846–52. doi: 10.1136/gut.2010.228023
14. Rössle M. TIPS: 25 years later. *J Hepatol*. (2013) 59:1081–93. doi: 10.1016/j.jhep.2013.06.014
15. Han G, Qi X, Guo W, Niu J, Bai M, Fan D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis in cirrhosis. *Gut*. (2012) 61:326–7. doi: 10.1136/gutjnl-2011-300577
16. Piscaglia F, Gianstefani A, Ravaioli M, Golfieri R, Cappelli A, Giampalma E, et al. Criteria for diagnosing benign portal vein thrombosis in the assessment of patients with cirrhosis and hepatocellular carcinoma for liver transplantation. *Liver Transpl*. (2010) 16:658–67. doi: 10.1002/lt.22044
17. Luo X, Zhao M, Wang X, Jiang M, Yu J, Li X, et al. Long-term patency and clinical outcome of the transjugular intrahepatic portosystemic shunt using the expanded polytetrafluoroethylene stent-graft. *PLoS ONE*. (2019) 14:e0212658. doi: 10.1371/journal.pone.0212658
18. R Core Team. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. (2018).
19. Han G, Qi X, He C, Yin Z, Wang J, Xia J, et al. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with symptomatic portal hypertension in liver cirrhosis. *J Hepatol*. (2011) 54:78–88. doi: 10.1016/j.jhep.2010.06.029
20. Tan HK, James PD, Sniderman KW, Wong F. Long-term clinical outcome of patients with cirrhosis and refractory ascites treated with transjugular intrahepatic portosystemic shunt insertion. *J Gastroenterol Hepatol*. (2015) 30:389–95. doi: 10.1111/jgh.12725
21. Zhang JB, Chen J, Zhou J, Wang XM, Chen S, Chu JG, et al. Systematic review and meta-analysis of trans-jugular intrahepatic portosystemic shunt for cirrhotic patients with portal vein thrombosis. *World J Clin Cases*. (2021) 9:5179–90. doi: 10.12998/wjcc.v9.i19.5179
22. Chalasani N, Clark WS, Martin LG, Kamean J, Khan MA, Patel NH, et al. Determinants of mortality in patients with advanced cirrhosis after transjugular intrahepatic portosystemic shunting. *Gastroenterology*. (2000) 118:138–44. doi: 10.1016/S0016-5085(00)70422-7
23. Lv Y, He C, Wang Z, Guo W, Wang J, Bai W, et al. Association of nonmalignant portal vein thrombosis and outcomes after transjugular intrahepatic portosystemic shunt in patients with cirrhosis. *Radiology*. (2017) 285:999–1010. doi: 10.1148/radiol.2017162266
24. Merola J, Fortune BE, Deng Y, Ciarleglio M, Amirbekian S, Chaudhary N, et al. Transjugular intrahepatic portosystemic shunt creation for cirrhotic portal hypertension is well tolerated among patients with portal vein thrombosis. *Eur J Gastroenterol Hepatol*. (2018) 30:668–75. doi: 10.1097/MEG.0000000000001097
25. Riggio O, Ridola L, Lucidi C, Angeloni S. Emerging issues in the use of transjugular intrahepatic portosystemic shunt (TIPS) for management of portal hypertension: time to update the guidelines? *Dig Liver Dis*. (2010) 42:462–7. doi: 10.1016/j.dld.2009.11.007
26. Perarnau J-M, Baju A, D'Alteroche L, Viguier J, Ayoub J. Feasibility and long-term evolution of TIPS in cirrhotic patients with portal thrombosis. *Eur J Gastroenterol Hepatol*. (2010) 22:1093–8. doi: 10.1097/MEG.0b013e328338d995
27. Salerno F, Cammà C, Enea M, Rössle M, Wong F. Transjugular intrahepatic portosystemic shunt for refractory ascites: a meta-analysis of individual patient data. *Gastroenterology*. (2007) 133:825–34. doi: 10.1053/j.gastro.2007.06.020
28. Riggio O, Ridola L, Angeloni S, Cerini F, Pasquale C, Attili AF, et al. Clinical efficacy of transjugular intrahepatic portosystemic shunt created with covered stents with different diameters: results of a randomized controlled trial. *J Hepatol*. (2010) 53:267–72. doi: 10.1016/j.jhep.2010.02.033
29. Wang Q, Lv Y, Bai M, Wang Z, Liu H, He C, et al. Eight millimetre covered TIPS does not compromise shunt function but reduces hepatic encephalopathy in preventing variceal rebleeding. *J Hepatol*. (2017) 67:508–16. doi: 10.1016/j.jhep.2017.05.006
30. Praktiknjo M, Abu-Omar J, Chang J, Thomas D, Jansen C, Kupczyk P, et al. Controlled underdilation using novel VIATORR(R) controlled expansion stents improves survival after transjugular intrahepatic portosystemic shunt implantation. *JHEP Rep*. (2021) 3:100264. doi: 10.1016/j.jhepr.2021.100264
31. Trebicka J, Bastgen D, Byrtus J, Praktiknjo M, Terstiegen S, Meyer C, et al. Smaller-diameter covered transjugular intrahepatic portosystemic shunt stents are associated with increased survival. *Clin Gastroenterol Hepatol*. (2019) 17:2793–9.e1. doi: 10.1016/j.cgh.2019.03.042
32. Wolter K, Praktiknjo M, Boie J, Decker G, Nadal J, Jansen C, et al. Glue embolization of gastroesophageal varices during Transjugular Intrahepatic Portosystemic Shunt (TIPS) improves survival compared to coil-only embolization-a single-center retrospective study. *Cardiovasc Intervent Radiol*. (2021) 44:1240–50. doi: 10.1007/s00270-021-02852-y
33. Holster IL, Tjwa ETTL, Moelker A, Wils A, Hansen BE, Vermeijden JR, et al. Covered transjugular intrahepatic portosystemic shunt versus endoscopic therapy + β-blocker for prevention of variceal rebleeding. *Hepatology*. (2016) 63:581–9. doi: 10.1002/hep.28318
34. Riggio O, Masini A, Efrati C, Nicolao F, Angeloni S, Salvatori FM, et al. Pharmacological prophylaxis of hepatic encephalopathy after transjugular intrahepatic portosystemic shunt: a randomized controlled study. *J Hepatol*. (2005) 42:674–9. doi: 10.1016/j.jhep.2004.12.028
35. Qi X, He C, Guo W, Yin Z, Wang J, Wang Z, et al. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with variceal bleeding in liver cirrhosis: outcomes and predictors in a prospective cohort study. *Liver Int*. (2016) 36:667–76. doi: 10.1111/liv.12929
36. Luca A, D'Amico G, La Galla R, Midiri M, Morabito A, Pagliaro L. TIPS for prevention of recurrent bleeding in patients with cirrhosis: meta-analysis of randomized clinical trials. *Radiology*. (1999) 212:411–21. doi: 10.1148/radiology.212.2.r99au46411
37. Wang Z, Jiang M-S, Zhang H-L, Weng N-N, Luo X-F, Li X, et al. Is post-TIPS anticoagulation therapy necessary in patients with cirrhosis and portal vein thrombosis? A randomized controlled trial. *Radiology*. (2016) 279:943–51. doi: 10.1148/radiol.2015150369
38. Thornburg B, Desai K, Hickey R, Kulik L, Ganger D, Baker T, et al. Portal vein recanalization and transjugular intrahepatic portosystemic shunt creation for chronic portal vein thrombosis: technical considerations. *Tech Vasc Interv Radiol*. (2016) 19:52–60. doi: 10.1053/j.tvir.2016.01.006
39. Salem R, Vouche M, Baker T, Herrero JI, Caicedo JC, Fryer J, et al. Pretransplant portal vein recanalization-transjugular intrahepatic portosystemic shunt in patients with complete obliterative

portal vein thrombosis. *Transplantation*. (2015) 99:2347–55. doi: 10.1097/TP.0000000000000729

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Physiology

REVIEW
published: 29 November 2021
doi: 10.3389/fphys.2021.795298

Translational Control in Liver Disease

*Alexandra Balvey and Mercedes Fernandez**

Laboratory of Translational Control in Liver Disease and Cancer, IDIBAPS Biomedical Research Institute, University of Barcelona, Barcelona, Spain

Chronic liver disease is one of the biggest threats to public health worldwide. Worryingly, the incidence of liver disease is dramatically rising due to the aging of the population and the global epidemics of obesity. Both are major risk factors for chronic liver disease and adverse prognostic factors, causing an increase in mortality rate. It is of great concern that 80–95% of obese people have non-alcoholic fatty liver disease, the major precursor for liver failure and a global health challenge. Currently, the only curative treatment for advanced chronic liver disease is liver transplantation, which is, however, hampered by high treatment costs and the scarcity of donor organs. New strategies are therefore urgently needed to prevent and reverse chronic liver disease. And for that it is essential to understand better the molecular mechanisms underlying human disease. This review focuses on the abnormalities in the regulation of translation by RNA-binding proteins during chronic liver disease and their pathological impact on portal hypertension, fibrosis, steatosis, neovascularization, and cancer development.

Keywords: translation, RNA-binding proteins, CPEB proteins, liver disease, portal hypertension

OPEN ACCESS

Edited by:
Jonel Trebicka,
Goethe University, Germany

Reviewed by:
Michael Praktiknjo,
University Hospital Bonn, Germany
Tilman Sauerbruch,
University of Bonn, Germany

***Correspondence:**
Mercedes Fernandez
mercefernandez@ub.edu

Specialty section:
This article was submitted to Gastrointestinal Sciences, a section of the journal Frontiers in Physiology

Received: *14 October 2021*
Accepted: *08 November 2021*
Published: *29 November 2021*

Citation:
Balvey A and Fernandez M (2021) Translational Control in Liver Disease. Front. Physiol. 12:795298. doi: 10.3389/fphys.2021.795298

Abbreviations: A-site, acceptor site; Akt, RAC-alpha serine/threonine protein kinase, also known as PKB (protein kinase B); ARE, AU-rich element; Calpain2, CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CCL2, monocyte chemoattractant protein 1; CDC2, gene encoding for Cyclin Dependent Kinase 1 (Cdk1), also called p34; CPE, cytoplasmic polyadenylation element; CPEB, cytoplasmic polyadenylation element binding protein; CPSF, cleavage polyadenylation specific factor (complex). It binds the AAUAAA/AUUAAA sequence and recruits PAP.; CTD, RNA-binding C-terminal domain; DAMPs, damage-associated molecular patterns; Dcp1, decapping enzyme 1; Dcp2, decapping enzyme 2; E-site, exit site; eEF1A-B, eukaryotic elongation factor 1A-B; eEF2α, eukaryotic elongation factor 2α; p-eEF2α, phosphorylated eukaryotic elongation factor 2α; EC, endothelial cells; ECM, extracellular matrix; eIF, eukaryotic initiation factor; eIF4E, eukaryotic initiation factor 4E; eIF4G, eukaryotic initiation factor 4G; eIF5-A, eukaryotic initiation factor 5-A; ER, endoplasmic reticulum; eRF1, eukaryotic release factor 1; eRF3, eukaryotic release factor 3; GLD-2, germ line development protein 2; GTP, guanosine triphosphate; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HepG2, immortal hepatocellular carcinoma cell line; HFD, high fat diet; HIF1α, hypoxia-inducible factor α; HSC, hepatic stellate cells; Huh7, human hepatoma derived cell line; IκBα, NF-κB Inhibitor α, also known as major histocompatibility complex enhancer binding protein MAD3; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; iKO, inducible knock-out; IRES, internal ribosome entry site; JAK/STAT, janus kinases/signal transducer and activator of translation pathway; KC, Kupffer Cells (macrophages); KO, knock-out; LSEC, liver sinusoidal endothelial cells; m^7Gppp, 5′cap; MAFLD, metabolic-associated fatty liver disease; MAPK/ERK, mitogen activated protein kinases/extracellular signal regulated kinases pathway; miRNA, micro RNA; MMP9, matrix metalloproteinase 9; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor κB p65 subunit; NTD, N-terminal regulatory domain; p21, protein 21; p34, protein 34; p-Akt, phosphorylated Akt; P-site, Peptidyl site; PABP, Poly(A) binding protein (family of proteins).; PAP, Poly(A) polymerase; PARN, Poly(A) ribonuclease; PAS, polyadenylation site; PDGF, platelet-derived growth factor; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PHT, portal hypertension; PI3K-Akt, phosphatidylinositol-3-kinase-Akt pathway; PlGF, placental growth factor; PTEN, phosphatidylinositol 3,4,5-triphosphate 3-phosphatase and dual-specificity protein phosphatase; RBPs, RNA binding proteins; ROS, reactive oxygen species; RRM, RNA recognition motif; SMC, smooth muscle cells; STAT3, signal transducer and activator of transcription 3.; TAM, tumor associated macrophages; TAK1, TGF-β activated kinase 1; TGF-β, transforming growth factor β; TNFα, tumoral necrosis factor α; TLR4, toll-like receptor 4; Twist1, twist-related protein 1; uORF, upstream open reading frame; UPR, unfolded protein response; UTR, untranslated region; VEGF, vascular endothelial growth factor; VSPC, vascular stem/progenitor cells; WT, wild type; Xrn1p, 5′ to 3′ exoribonuclease 1 protein.

INTRODUCTION

Chronic liver diseases, along with hepatocellular carcinoma (HCC), have increasingly become a global significant health burden, affecting as much as 5.5 million people worldwide (Younossi et al., 2016; Pimpin et al., 2018). This set of diseases are characterized by decreased hepatic function as a result of chronic inflammation and repeated insults to the liver, often leading to an irreversible and fatal outcome. It can no longer be ignored that the dramatically increasing global epidemic of obesity greatly helps at fueling metabolic conditions, which will often manifest through the liver in the form of non-alcoholic or metabolic associated fatty liver disease (NA/MAFLD), predisposing to a spectrum of diseases including non-alcoholic steatohepatitis (NASH), fibrosis (Mejias et al., 2020a), cirrhosis and HCC (Younes and Bugianesi, 2018; Zhang, 2018). Under such circumstances, the liver and its cellular population are forced to undergo metabolic reprogramming to compensate the new condition. Underlying inflammation and other metabolic changes, regulation of mRNA translation through the control of poly(A) elongation has been observed as a key factor (Curinha et al., 2014; Chen et al., 2016), where the main actors have been found to be the cytoplasmic polyadenylation element binding (CPEB) family of proteins, mostly implicated in cell proliferation, tumorigenesis, invasiveness, angiogenesis and fibrogenesis. This review aims to stand out the role of key CPEB proteins in the regulation of mRNA translation under metabolic stress in the liver, contributing to gather and bring further the limited knowledge we have on the underlying molecular mechanisms, in order to find alternative approaches to treat these diseases.

POSTTRANSCRIPTIONAL CONTROL OF GENE EXPRESSION

Gene expression regulation is an intricate, interconnected and multi-layered process involving three main players (DNA, RNA and proteins), in which every step is tightly monitored and controlled to ensure optimal cellular adaptation to the environmental and physiological demands, while remaining robust to transient perturbations (Moore, 2005). Whereas the nucleotide sequence of a gene determines the sequence of its mRNA product, and whereas an mRNA's sequence determines the amino acid sequence of the resulting polypeptide, there is no trivial relationship between the concentration of a transcript and the concentration(s) of the protein(s) derived from it (Liu et al., 2016). Although most of the research from the last decades has focused on the first step of the pathway (the regulation of transcription), novel systematic studies quantifying transcripts and proteins at genomic scales exposed the importance of multiple processes beyond transcript concentration, contributing to establishing the expression level of a protein (McManus et al., 2015). Translational efficiency thus becomes the single best predictor of protein expression (Schwanhäusser et al., 2011), underlining the importance of the last step in the gene expression cascade.

mRNA translation is a cyclical process that has been described and investigated for many years now, identifying three steps from which initiation has been the most studied, followed by elongation and termination. Translation initiation is the most complex step and rate-limiting, involving a myriad of proteins (with new ones being linked to it as research progresses), typically divided in a standard cap-dependent initiation and an alternative cap-independent initiation. Both mechanisms have the purpose of recruiting the mRNA and assembling the ribosomal subunits so that translation can begin.

Cap-dependent translation is the most general mechanism of translation initiation (Ruggero and Shimamura, 2014). A set of proteins called eukaryotic initiation factors (eIFs) are required for the recruitment of the 40S subunit on the 5′UTR, right where the m^7Gppp group is. Once assembled, the 40S subunit scans the mRNA until reaching the start codon (AUG), which is then pinpointed and paired with the anticodon tRNA at the peptidyl site (or P-site), culminating with the recruitment of the 60S ribosomal subunit and thus forming the whole ribosomal complex, ready to proceed to the elongation phase. However, the alternative mechanism of translation initiation, called Internal Ribosome Entry Site (IRES) mediated translation, will allow the translation of mRNAs in a cap-independent manner, sparing the need of 5′UTR recognition as well as the mRNA scanning process, by directly recruiting the 40S subunit nearby the codon where the translation must be initiated.

Translational elongation is a mechanism conserved in all kingdoms of life, assisted by a minimal set of factors (Dever et al., 2018). During this step, the ribosome reads the mRNA sequence and consequently adds the corresponding amino acids matching each codon, mediated by elongation factors eEF1A-B, eEF2, and eIF5A, which keep the cognate aminoacyl-tRNAs flowing through the A- (acceptor), P- (peptidyl) and E- (exit) sites, as the lecture proceeds.

The last step in translation is known as termination, and it is triggered when the ribosome reaches the stop codon. Two release factors (eRF1 and eRF3), with the help of GTP, constitute a ternary complex that execute the release of the nascent peptide (Hellen, 2018). The complex follows disassembly, allowing its constituents to integrate further rounds of translation in a posterior recycling phase.

Once the mRNA transcript has completed its function, it is required to undergo physiological exonuclease-mediated degradation by diverse decay pathways (Halbeisen et al., 2008), each one being carefully controlled to recognize its target mRNAs. Oddly, the major cytoplasmic mRNA degradation pathway in eukaryotes begins with shortening of the poly(A) tail by a variety of deadenylases, before the removal of the 5′cap structure by decapping enzymes Dcp1 and Dcp2 (Parker and Song, 2004). After that, the decapped intermediates are consequently digested either by Xrn1p exonuclease ($5' \rightarrow 3'$) or by the exosome complex ($3' \rightarrow 5'$).

The poly(A) tail is a dynamic structure constituted by a sequence of 200–250 adenine residues (its length varying among species), found at the 3′UTR end of all nuclear transcribed eukaryotic mRNAs. One of its main essential functions is blocking mRNA degradation by ubiquitous exonucleases, and

thus providing an additional regulatory opportunity to extend (or shorten) the transcript's life while remaining in the cytoplasm by just allowing the addition or deletion of adenine residues from the 3′end, respectively. Moreover, a long poly(A) tail will allow stabilization of the mRNA during translation by circularization, assisted by initiation factors eIF4E and eIF4G, and PABP, all of which are target of a number of factors that will stimulate or inhibit the translation of specific mRNAs (Fernández-Miranda and Méndez, 2012).

RNA-BINDING PROTEINS

The eIF4E, eIF4G, and PABP belong to the great family of RNA-binding proteins (RBPs), together with a myriad of other proteins. RBPs are central components in RNA metabolism, since they regulate all aspects in the life of mRNAs; from their synthesis, processing and maturation, to their export, stability, transport and translation, in addition to generating connections and regulatory networks between these processes, so that perturbations in the pathway can be quickly neutralized or, at least modulated. This crucial role is also played by the CPEB (cytoplasmic-polyadenylation element binding protein) family, which belongs to the RBP category as well. The first clues on the existence of this family came around the 1990s when Richter and colleagues were observing the involvement of $p34^{cdc2}$ kinase in cyclin-mediated polyadenylation (Paris et al., 1991; Hake and Richter, 1994). They found that $p34^{cdc2}$ kinase was phosphorylating another kinase, probably Aurora-A kinase, which in turn phosphorylated a CPE-binding protein, happening to be CPEB1, years of research after. Fast forward, up-to-date genome-wide studies found that around 20% of vertebrate genome transcripts contain CPE elements, thus turning into potential CPEB targets (Pavlopoulos et al., 2011), although the specific roles and regulation of each CPEB are still poorly understood in adult tissues.

Cytoplasmic polyadenylation element binding are of special interest for their active role in cytoplasmic polyadenylation modulation, which they execute through binding specific structural elements on targeted mRNAs and by interacting with other proteins (**Figures 1A,B**). Intriguingly, CPEBs have the ability to both activate and repress translation by turning on polyadenylation and deadenylation, respectively; they do so by sticking to specific sequences located on the 3′UTR end of the transcripts, the most common one being the CPE (cytoplasmic polyadenylation element), an AU-rich domain (Belloc and Méndez, 2008). Whether they bind with higher or lower affinity to these sequences, and whether they end up activating or repressing translation, will greatly depend on the number and type of sequences, distance between them and the proteins and complexes surrounding that region. Furthermore, each of the four mammalian paralogs comprised within the CPEB family (herein CPEB1-4) have also been attributed different degrees of affinity with the above mentioned sequences (Fernández-Miranda and Méndez, 2012). This is due to the fact that they all share common domains, such as the NTD (N-terminal regulatory domain) and CTD (RNA-binding C-terminal domain) ones. While CTD remains largely conserved across the family, NTD is highly variable regarding its RRM (RNA-recognition motifs), and strongly susceptible to several post-transcriptional modifications (Mendez et al., 2000; Theis et al., 2003; Drisaldi et al., 2015), which leads to contemplate the fact that the different CPEBs will trigger different signals over a same mRNA target, thus engaging distinct expression patterns. To add up another level of complexity, it has been described by Igea and Mendez (Igea and Méndez, 2010; Hu et al., 2014) that some CPEBs (particularly CPEB1 and CPEB4) are able to self-regulate their expression using the CPE elements sheltered in their very own mRNA. This set of facts, adding up to the empirical behavioral observation of the different CPEBs in different tissues and disease stages, suggests that this family of proteins is capable to accurately adjust spatial and temporal signals in such a complex and precise manner; there is no doubt that there's a long way ahead until we can start elucidating the specific mechanisms and circumstances under which CPEBs operate.

Early studies on the CPEB family members' sequences show close similarities between CPEB2-4 compared to CPEB1, which have been clustered into a separated subfamily (Fernández-Miranda and Méndez, 2012; Ivshina et al., 2014). Intriguingly, CPEB orthologs have been found to be better conserved across different species than between paralogs (Wang and Cooper, 2010), for what we can assume their identities had a strong role to be selectively maintained through evolution, although the number of CPEBs within their family can vary between species. The first investigations on CPEBs used *Xenopus laevis* oocytes as a model to study cytoplasmic polyadenylation dependent translation. Later on, CPEB investigations began to use other models and move across other fields, unraveling different identities and roles for each CPEB member, all of them regarding the regulation of the poly(A) tail. CPEB1 has been the most studied member of this puzzling family, partly because it was the first to be discovered. Studies from Mendez lab have shown that CPEB1 can act both as an activator and a repressor of mRNA translation, depending on its phosphorylation status (Fernández-Miranda and Méndez, 2012); while the activator mechanism has been quite well characterized, the repression mechanism (when CPEB1 is unphosphorylated) remains debatable. CPEB1 will change its affinity for F (cleavage polyadenylation specific factor) upon phosphorylation, following PARN (poly(A) ribonuclease) eviction from the complex, and enabling Gld2 (a poly(A) polymerase) to enter the scene and begin polyadenylation during the meiotic (Kim and Richter, 2007) and mitotic stages (Novoa et al., 2010). CPEB1 has also been found to be implicated in cellular senescence (Burns and Richter, 2008), tumor development (Nagaoka et al., 2016), inflammation (Ivshina et al., 2015), synaptic plasticity (Udagawa et al., 2012), and liver homeostasis (Alexandrov et al., 2012), beyond meiosis. Conversely, CPEB2 was suggested some years ago to act as a translational repressor upon the elongation phase, via interaction with eEF2 (Chen and Huang, 2012), and very little information has been obtained on possible activation mechanisms on specific target mRNAs (Hägele et al., 2009). We know CPEB2 is expressed in the liver, as well as in the brain and in testis, that it interacts with b-catenin and CaMKII (which are both targets of CPEB1) (Turimella et al., 2015), and that it could

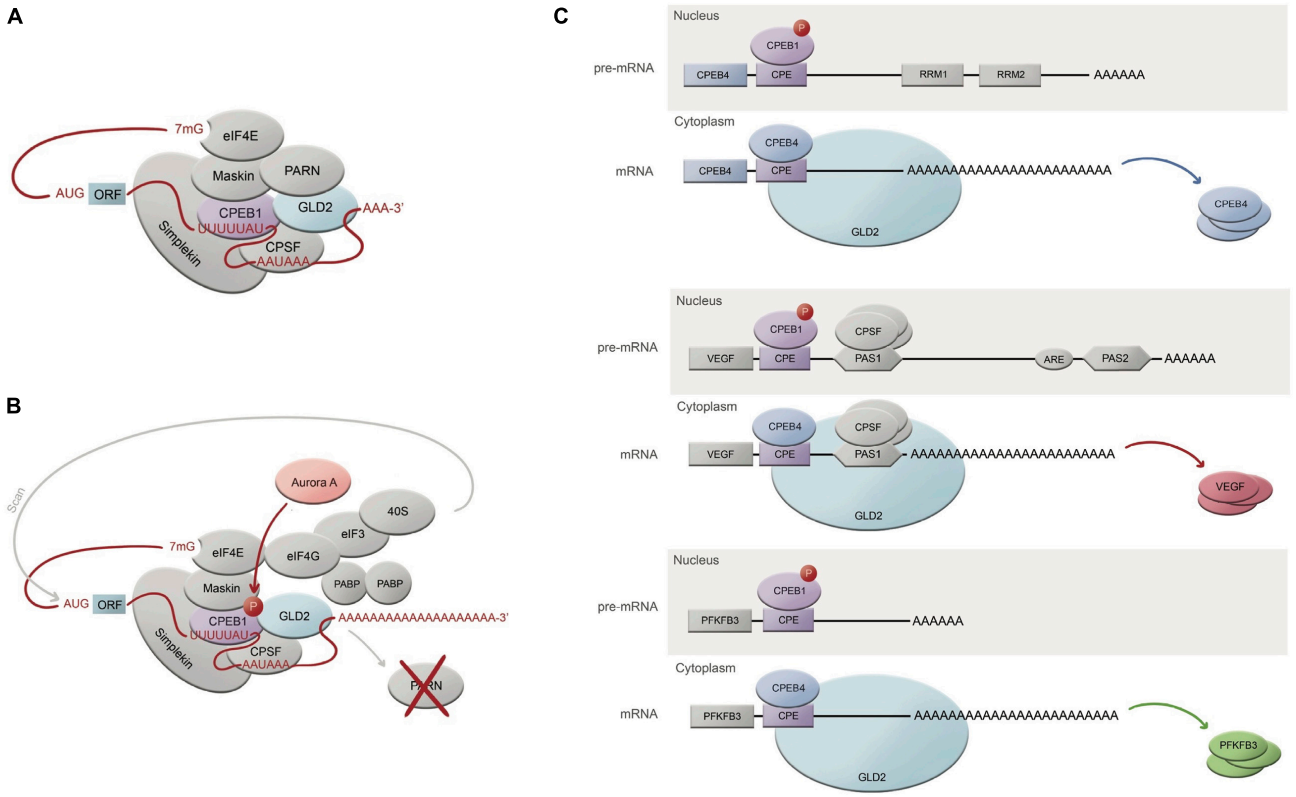


FIGURE 1 | CPEB-regulated mechanism of translational activation through polyadenylation. **(A)** Translationally repressed mRNA where CPEB1 is bound to the CPE domain (U-rich UUUUUAU sequence), while at the same time, CPSF is associated to a nuclear pre-mRNA site AAUAAA. CPEB1 is at the same time interacting with GLD2 (adenylating enzyme), which is inhibited by its binding to PARN (deadenylating enzyme). PARN shortens the poly(A) tail in this translational repression state. CPEB1 is also bound to Maskin, which interacts with eIF4E, which in turn also cooperates in translational repression by taking the place of eIF4G. Simplekin acts as a platform for the protein complex tethering the repressed mRNA. **(B)** Upon Aurora A phosphorylation, CPEB1 is thus phosphorylated and activated, which will cause PARN ejection, liberating GLD2 to finally be able to resume polyadenylation. The resulting long poly(A) tail will facilitate the 40S ribosomal subunit to find it, guided by eIF4G-eIF3 complex, finally allowing translation. **(C)** Roles and mechanism of CPEB1 and CPEB4 in 3 different mRNAs related with the pathophysiology of chronic liver disease; active CPEB1 will bind to the CPE sequence in pre-mRNAs within the nucleus, allowing CPSF to cleave the hexanucleotide domain, originating the mature mRNA. Once the mature mRNA has exited the nucleus, CPEB4 will bind the CPE sequence to enhance polyadenylation and thus prioritizing the translation of poly(A)-rich mRNAs.

be involved in HIF1a activity regulation, at least in neuroblastoma cells (Hägele et al., 2009). To date, the knowledge on CPEB2 and its role in cancer and liver is still modest but not less deserving of further research. CPEB3, in turn, has been mainly studied in the frame of synapse activity, where studies indicate this member of the family can both enhance activation and degradation of target mRNAs upon either monoubiquitinylation (Pavlopoulos et al., 2011), either cleavage by Calpain2 or either by forming prion-like aggregates (Drisaldi et al., 2015; Fioriti et al., 2015; Stephan et al., 2015). CPEB3 has been related to tumorigenesis and it seems it would also play a role in HCC progression (Zhang et al., 2020), but again, very little has been researched on that matter regarding CPEB3.

Ultimately, CPEB4 was also attributed repression and activation roles regarding cytoplasmic polyadenylation in specific contexts, such as terminal erythroid differentiation (Hu et al., 2014), circadian rhythms (also involving CPEB2) (Kojima et al., 2012; Maillo et al., 2017), oocyte maturation, somatic cell cycle and tumor progression and malignancy (Novoa et al., 2010; Ortiz-Zapater et al., 2012), cell survival (Kan et al., 2010; Chang and Huang, 2014), and pathological angiogenesis in the liver context (Calderone et al., 2016), strongly indicating a pro-tumoral role in cancer progression (Han et al., 2015; Hu et al., 2015; Zhong et al., 2015; Boustani et al., 2016), despite some reports pointing toward opposite directions (Peng et al., 2014).

CYTOPLASMIC POLYADENYLATION ELEMENT BINDING PROTEINS IN LIVER DISEASE

The liver is a complex organ, just as the family of proteins we're writing about. It is also the most affected organ by the aging population and the modern lifestyle in industrialized societies, often fueling chronic liver diseases, which are currently still an underestimated and growing global public health problem. The epidemiology of liver disease is diverse; alcohol abuse, HCV/HBC viral infections and non-alcoholic fatty liver disease (NAFLD) are

the most common causes, and studies cannot find agreement in which of them is the most prevalent one. One must note, before keeping on with this review, that the trend in the clinical field for the past years has strived to redefine NAFLD so that this affection can be rather diagnosed by inclusion criteria instead of exclusion criteria; this is how "metabolism-associated fatty liver disease" (MAFLD) is born, a term recently coined by an international panel, with growing popularity in literature (Eslam et al., 2020; Yamamura et al., 2020). This set of chronic liver diseases affects no less than 5.5 million people worldwide (Pimpin et al., 2018), with NA/MAFLD leading the rank, estimated to affect approximately 25% of the world population (Younossi et al., 2019). This affection, while remaining untreated, will continue to progress to non-alcoholic steatohepatitis (NASH), which very often will derive in serious liver injury stage by stage until reaching cirrhosis or even HCC. Associated metabolic risk factors will range from hypertension and dyslipidaemia, obesity and diabetes (Neuschwander-Tetri, 2010); cardiovascular disease is the most common cause of mortality in individuals affected by NA/MAFLD, followed by HCC (Adams and Angulo, 2005). However, when the patient reaches the stage of cirrhosis, liver disease will prevail as the number-one risk for mortality.

In all of these phases of the chronic liver disease, we find a cytoplasmic polyadenylation binding protein (CPEB) involved, acting in one way or another (**Figures 1C**, **2**). This review aims to put together the investigations from the last decades on this family of proteins to shed some light on the purpose of their role in this particular organ, and to show how significant research is on this field, given the current inevitable background of these diseases, the magnitude of people affected by them, and the socioeconomic and health burden they imply. But before tackling the multiple roles the CPEB proteins play in chronic liver diseases, it might be useful to first have a brief insight into the cell composition, structure and basic physiological functions of this organ, to have a better global understanding.

The liver is the most extensive organ in our body after the skin, weighting around 2–3% of the whole bodyweight. Its function is essential for the body's homeostasis, participating in the most important biological mechanisms amongst which stand detoxification of waste compounds, erythrocyte recycling, synthesis and secretion of bile, plasma protein synthesis and energetic metabolism homeostasis, among others (Fernandez, 2015). Because of its detoxification function, the liver is constantly being exposed to toxins and stress-inducing molecules, which greatly favor the staging of chronic liver disease, especially under an excess calorie intake and nutritionally imbalanced diet (mostly in the form of carbohydrates and fat) accompanied by a sedentary lifestyle. The most abundant cell population in the liver are hepatocytes (around 80%), their distribution shaping polygonal conformations organized in radial layers; the space between these layers is known as the liver sinusoid, where the nutrient exchange takes place. The rest of cells (20%) are grouped into the so-called non-parenchymal type: LSEC (liver sinusoidal endothelial cells), HSC (hepatic stellate cells), KC (Kupffer cells). LSECs are highly specialized cells which are found enclosing the liver sinusoid. They are characterized by being highly permeable and forming fenestrations in order to facilitate the process of nutrient delivery to hepatocytes. In addition, they are in charge of maintaining a low portal pressure regardless of global pressure fluctuations, and also maintain HSC quiescent to repress fibrogenesis. HSCs are pericyte-like cells found within the space of Disse (subendothelial area contained between LSEC and hepatocytes, where the exchange of molecules is given); their main function is stocking up on vitamin A, but they also play a big role in immune response over stress, besides mediating injury response and tissue regeneration. Finally, KCs are resident macrophages whose function is to process aged erythrocytes and other circulating waste, besides coping with immunological imbalance in the liver, when in need. Structurally, the liver tissue is mostly composed of multiple parcels named portal triads, which comprise branches from the hepatic artery and portal vein, and a bile duct; understanding this architecture is essential to follow the stages of disease development.

Cytoplasmic Polyadenylation Element Binding Proteins and Cellular Stress

After a life of poor diet habits and physical inactivity, the liver starts to be forced to overcompensate for the cellular damage and dysfunction derived from oxidative stress over a net accumulation of energy in the form of triglycerides. Hepatocyte injury and death are at the center of the progression of NA/MAFLD to NASH, since they amplify inflammatory and fibrotic signaling in the pericellular milieu (Ibrahim et al., 2018). It is still unclear how the vicious cycle of progressive destruction and regeneration starts and the role that CPEBs have in it, but a study from Maillo et al. (2017) could be useful to bring some light over how CPEBs are involved in the pathogenesis of NA/MAFLD. Their work (Maillo et al., 2017) focuses on CPEB4, whose mRNA levels are intriguingly regulated in a circadian manner in the liver. This is also the case of CPEB2 (Kojima et al., 2012), although this study leaves it unrelated to hepatosteatosis. The absence of CPEB4 in high fat diet (HFD)-fed mice, though, resulted in exacerbated steatosis, sometimes accompanied by fibrosis, originated by lipid accumulation in the liver and impaired lipid metabolism.

The liver is complexly governed by a cell-autonomous circadian clock, which regulates the unfolded protein response (UPR) in hepatocytes. The maintenance of endoplasmic reticulum (ER) integrity by the UPR is crucial for glucose and lipid metabolism homeostasis; thus, alterations in UPR pathway are known to lead to hepatosteatosis and possibly type-2 diabetes. Although CPEB4 protein levels are not circadian themselves, its mRNA levels oscillate, generating a circadian mediator of UPR in order to anticipate periods of elevated ER overexertion (Maillo et al., 2017). CPEB4 and UPR are closely related because in hepatocytes under metabolic stress, such as the one induced by HFD, the ER triggers the UPR to maintain tissue homeostasis; eIF2a is then phosphorylated and global protein synthesis attenuation follows, in order to help the cell to adapt to the ER stress. However, p-eIF2a will selectively increase the translation of upstream open reading frame (uORF)-rich transcripts, amongst which stands CPEB4 mRNA (which actually contains eight uORFs). In a second wave of translational activation,

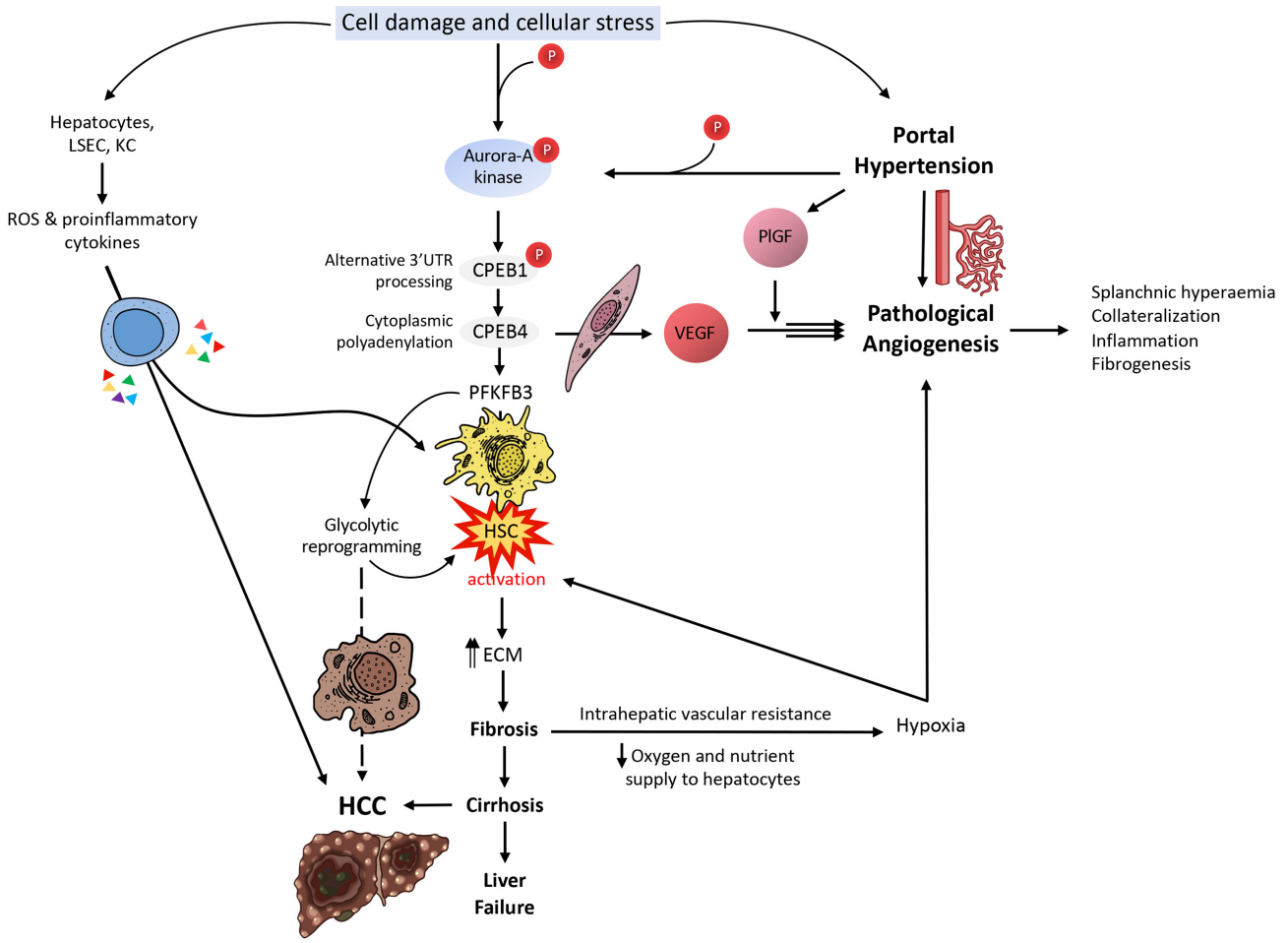


FIGURE 2 | CPEB-mediated translational control in liver disease. Upon cell damage and cellular stress in the liver, Aurora-A kinase is phosphorylated; Aurora-A will in turn phosphorylate CPEB1, which will process mRNAs in their alternative 3′UTR during a first wave of translational activation. One of these mRNAs is CPEB4 mRNA; in a second wave of translational activation, CPEB4 will enhance the polyadenylation of several mRNAs, such as VEGF and PFKFB3, besides its own mRNA. VEGF will cause pathological angiogenesis and its effect will be greatly boosted by PlGF, leading to splanchnic hyperemia, collateralization of blood vessels, inflammation and fibrogenesis. These will cause an increase in portal hypertension, which will increase PlGF levels and increase Aurora-A kinase phosphorylation, both feeding back the VEGF loop. PFKFB3, on the other side, will cause the activation of HSCs, which will start synthesizing ECM elements that will contribute to liver fibrosis and later cirrhosis, if unresolved. Liver fibrosis will cause intrahepatic vascular resistance and thus a reduction in oxygen and nutrient supply to hepatocytes, causing them to enter a hypoxic state; upon this situation, hepatocytes will produce angiogenic factors, attempting to resolve the situation, but actually making it worse. On the other hand, cirrhosis is well-known for being the most common prelude for HCC. The glycolytic switch caused by the overexpression of PFKFB3 in this specific context might likely facilitate the survival of transformed cells in a hypoxic millieu. Hepatocytes, LSECs and KCs will also respond to cell damage and cellular stress by producing ROS and liberating proinflammatory cytokines, contributing to the activation of HSCs and the progression of HCC.

CPEB4 will therefore bind CPE-containing mRNAs, encoding for multiple chaperones and other proteins involved in ER homeostasis and stress resolution.

Under CPEB4 depletion in mice, it is argued that impaired mitochondrial fatty acid oxidation and respiration will cause lipid accumulation and toxicity, besides also favoring the induction of apoptotic UPR branch, failing to adaptively attenuating HFD-induced ER stress. In addition, HFD-fed and CPEB4 KO mice showed hyperglycemia, and although CPEB4 KO was not directly related to glucose metabolism, it is indirectly associated to it through impaired lipid homeostasis, as the accumulation of lipids has an inhibitory effect on hepatic insulin signaling (Maillo et al., 2017), leaving liver gluconeogenesis unaffected. The authors of the study point out that their results indicate a cell-autonomous defect in hepatocytes, rather than a metabolic impairment in adipose tissue regarding the pathological response to HFD. One can fathom that CPEB4 depletion will induce hepatosteatosis as a result of unfolded protein accumulation, according to this study.

Other interactions described in literature that may potentially play a role in chronic liver disease initiation are, for example, CPEB1 on PTEN and STAT3 mRNAs; though CPEB1 interacts in a direct manner with these CPE-containing mRNAs by repressing them, in the absence of CPEB1 these factors become upregulated, interfering in glucose metabolism and causing insulin resistance in the liver in response to stressful ongoing events, such as high fat diet (Alexandrov et al., 2012). In addition,

this interaction has been associated to elevated IL-6 serum levels in the same study upon CPEB1 knock-out mice; this finding is known to be correlated to insulin resistance and even cancer (Fève and Bastard, 2009). This complex interplay between pro-inflammatory and insulin-related factors will, if not cause, trigger and fuel stressful metabolic conditions in hepatocytes that might be hard to overcome (Kucukoglu et al., 2021).

Cytoplasmic Polyadenylation Element Binding Proteins and Portal Hypertension

Portal hypertension (PHT) is usually one of the most significant and devastating complications of chronic liver disease, often silently manifesting at early stages of the disease. The profound hemodynamic disturbances are a consequence of vascular architecture distortion, and they are not limited to intrahepatic circulation as one would think; it is a fact that they will eventually involve the splanchnic and systemic vascular beds, characterized by a pathological increase of blood flow and consequent portosystemic collateral vessel formation in the form of pathological angiogenesis (Fernandez et al., 2016). It is in this scenario of PHT and pathological angiogenesis where we find a CPEB enrolled again.

Upon hemodynamic shear and mechanical stress trigger, increased blood flow and vascular growth factors, there is a rapid phosphorylation of Aurora-A kinase, a serine/threonine kinase that is able to activate CPEB1. Activated CPEB1 will then initiate alternative nuclear processing within 3′UTR of vascular endothelial growth factor (VEGF) and CPEB4 mRNAs; the latter activating cytoplasmic polyadenylation of VEGF mRNA, which will rapidly rise its translational rate (**Figures 1C**, **2**). VEGF will activate endothelial cells to turn on mobility and filopodia protrusion in order to form tip cells and to initiate new sprouts (Fernandez et al., 2016). These cells are supported by stalk cells, which are also activated endothelial cells whose function is to establish the vessel's lumen. Endothelial cells will then begin to secrete platelet-derived growth factor (PDGF) to attract pericytes and smooth muscle cells to stabilize the nascent vessel. This elaborated mechanism contributes to increase splanchnic neovascularization and splanchnic blood flow during PHT and chronic liver disease. It also participates in the formation of portosystemic collateral vessels, which try to alleviate the increased portal pressure by redirecting the enhanced portal venous inflow through the new collaterals. This angiogenic mechanism involving CPEB1 and CPEB4 overexpression is purely pathologic and plays a big role in quite every stage of the chronic liver disease; in cirrhotic patients, for instance, angiogenesis will contribute to the establishment and maintenance of abnormal hepatic architecture, perpetuating PHT and promoting fibrogenesis and inflammation. In this sense, CPEB silencing ameliorates major hallmarks of PHT, such as portosystemic collateral vessel formation, mesenteric arterial hyperdynamic circulation, and several surrogate markers of disease severity, such as increased von Willebrand factor plasma levels and splenic enlargement and hyperactivation, according to animal models (Calderone et al., 2016; **Figure 3**).

Cytoplasmic Polyadenylation Element Binding Proteins and Neovascularization

For many years, research has focused on the overproduction of VEGF as the target for new therapies, but the problem with these drugs is that they target both physiological and pathological angiogenesis, and their use becomes restricted because of significant side effects, such as collapsing normal vasculature, leakage and bleeding (Calderone et al., 2016). In this sense, the research on the posttranscriptional mechanisms mediated by CPEBs opens a new door to target pathological angiogenesis solely.

Pathological angiogenesis is a process where new vessels sprout and branch from preexisting blood vessels (Ramirez-Pedraza and Fernández, 2019), and becomes clinically relevant in a context of PHT and cirrhosis, where CPEB1 and CPEB4 are upregulated in a similar way than the previously mentioned. Even though VEGF is directly guilty for launching neovascularization processes, it does not discern between a physiological or a pathological setting; but CPEBs do. Analysis on VEGF's mRNA uncovered multiple regulating elements with diverse functions; the already known CPE domains at the 3′UTR allow CPEB1 and CPEB4 binding, and so do the PAS (putative polyadenylation site) domains. In addition, important feed-back loops where CPEB4 binds its own CPE boosts its translation and will surely cause an additional increase on VEGF overexpression. Otherwise, other AU-rich elements (AREs) will act as negative regulators of VEGF mRNA when its synthesis is not needed (Calderone et al., 2016).

When VEGF is overexpressed by hepatocytes, it is released into the extracellular matrix with the aim of recruiting endothelial cells and promoting their proliferation and differentiation to start creating new vessels, whereas when VEGF is produced by endothelial cells, it will alternatively strengthen their angiogenic phenotype. Knock-down of either CPEB1 or CPEB4 in endothelioma cells causes a reduction in VEGF protein expression, leaving its mRNA levels untouched (Calderone et al., 2016). This is translated in a halt of tubular-like structure formation *in vitro*. In knock-out mice, physiological angiogenesis will remain unharmed and global vasculature unaffected by CPEB depletion. In addition, induction of CPEB1 knock-out compared to induced CPEB4 knock-out reveals different preferences for different PAS domains, leading to a longer/shorter 3′UTR of CPEB4 and VEGF mRNAs. In the absence of the nuclear CPEB1 activated form, PAS2 will be the default polyadenylation site for CPEB4 and VEGF mRNAs; this domain will originate the longest 3′UTR transcript variant, containing more AREs and microRNA-binding sites, which will repress CPEB4 cytoplasmic translation, in this case. Removing these motifs from the CPEB4 transcript will cause CPEB4 mRNA stabilization and activation.

Vasculogenesis is in turn another major hallmark of chronic PHT that contributes to disease aggravation, modestly differing from the pathogenic angiogenesis above described (which is initiated by mature endothelial cells that activate upon PHT and start to proliferate). Vasculogenesis is originated by vascular stem/progenitor cells (VSPC), harbored in healthy mesenteric vessels before PHT steps in. Upon an unresolved increase in blood pressure, these cells will start to proliferate and differentiate

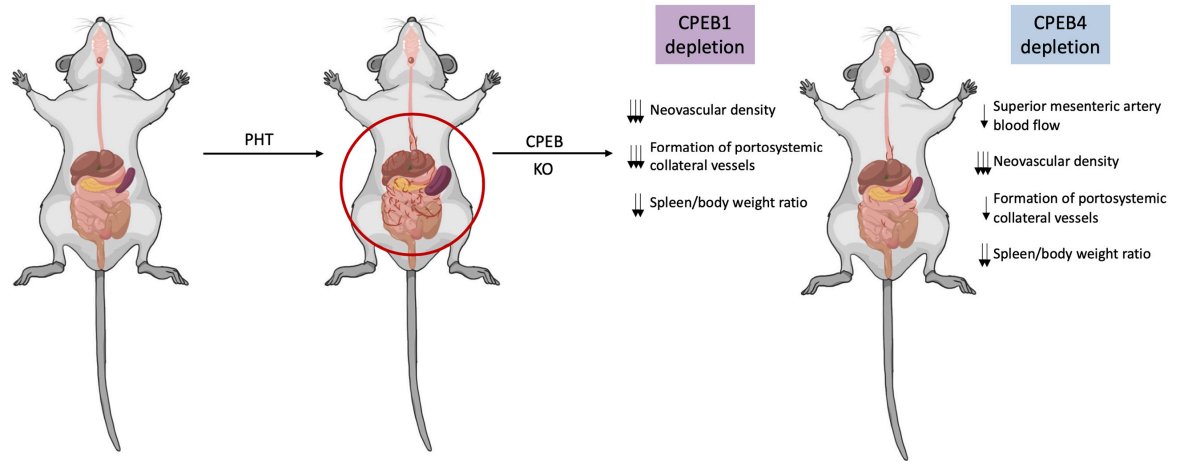


FIGURE 3 | CPEB silencing limits pathologic angiogenesis in portal hypertension and protects against disease progression. In a context of portal hypertension (PHT), pathological neovascularization increases significantly contributing to increase splanchnic blood flow and portosystemic collateral vessels. Upon CPEB1 or CPEB4 depletion in animal models of PHT, splanchnic neovascular density is markedly and significantly reduced, while preexisting vasculature density remains untouched. The formation of portosystemic collateral vessels is also diminished after CPEB1 or CPEB4 depletion in a context of PHT. CPEB1 or CPEB4 ablation also prevents the PHT-induced increase in the spleen/body weight ratio, suggesting attenuation of portal hypertension.

into different vasculogenic types (such as mature endothelial cells – ECs – or – smooth muscle cells – SMCs – lineages) in order to trigger the formation of abnormal vessels (Garcia-Pras et al., 2017). CPEB4 is a considerable factor regarding the proliferation and proper differentiation of VSPC; Garcia-Pras and colleagues argue that the underlying molecular mechanism is probably mediated by two sequential and non-redundant translational waves again implicating CPEB1 and CPEB4, resulting in the upregulation of hundreds of mRNAs encoding factors that are differentially expressed during cell cycle. *In vivo* evidence with knock-out mice is comparable to the prior mentioned study (Calderone et al., 2016) and essentially agrees on the same terms. Garcia-Pras and colleagues observed that mesenteric CPEB4 levels correlate spatiotemporally with VSPC expansion and neovascularization under PHT circumstances, and *in vitro* studies determined that CPEB4 is critical for cell division, SPC function and pathological angiogenesis in cancer and PHT. In addition, the differentiation potential of VSPCs is subordinated to multiple factors including VEGF and PDGF and their corresponding receptors, which are in turn also upregulated in a PHT environment (Garcia-Pras et al., 2017).

Cytoplasmic Polyadenylation Element Binding Proteins and Fibrosis

Unresolved and sustained insults in the liver with settled PHT will often entail scarring processes and consequent dysregulation of extracellular matrix (ECM), tipping the scale in favor of excessive matrix deposition. In addition, the nascent hypoxic areas due to distorted liver structure will also enhance angiogenesis and neovascularization attempting to resolve the ongoing condition, and far from making it better, it will strongly worsen the already established feedback loop of PHT-angiogenesis-hypoxia-fibrosis, rapidly and irreversibly deteriorating the patient's health.

Fibrosis is described by Mejias et al. (2020a) as the abnormal and excessive deposition of collagen-rich ECM, resulting in compromised tissue and organ structure, and it is the sole histologic feature of NASH that currently anticipates clinical outcomes. In this advanced stage, angiogenesis is now stimulated by hypoxia, which is in turn caused by overcompensated tissue repairing processes in response to continuous insults to the liver. Wound healing responses will also trigger inflammation processes that will attract macrophages and activate HSC upon secretion of proinflammatory cytokines.

At a molecular level, the phosphorylation of Aurora-A kinase in the liver territory within a PHT context keeps activating translational waves implicating CPEB1 first and CPEB4 later. We saw how intimately related were VEGF and CPEB4 in pathological angiogenesis and neovascularization; the same happens in fibrogenesis, where CPEB4 binds the CPE sequences in PFKFB3′s mRNA, upregulating its translation and driving HSC activation and the expression of fibrogenic markers (Mejias et al., 2020b). In addition to this molecular mechanism, other cell populations (hepatocytes, LSECs, and KCs) will generate ROS and proinflammatory cytokines under cellular stress, also contributing to HSC transdifferentiation. Activated HSC shift into a myofibroblastic phenotype, characterized by being highly proliferative and producing and secreting ECM; VEGF-A and MMP9 are, among others, two factors that will mediate fibrosis-associated angiogenesis within the early stages. This highly proliferative HSC phenotype demands lots of energy to maintain its function, and it is fueled by PFKFB3-mediated glycolytic reprogramming as an additional energetic and synthetic supply. This metabolic shift frequently anticipates an advantage in many malignant transformed cells; following this trend, it is not crazy to assume that this mechanism will be later utilized by transformed HCC cells, in a scenario where the cirrhotic liver succeeds at evading global liver failure for a while.

Mejias and colleagues analyzed CPEB4 and PFKFB3 trends in human and mouse primary HSC cell lines and livers from cirrhotic patients and rat and mice experimental models, which showed correlating overexpressed levels upon HSC activation. Following this observation, they tested different approaches to understand the transdifferentiation mechanism and the main characters playing in it. From this study (Mejias et al., 2020b), they confirmed that (a) PFKFB3 antagonists inhibit dependent glycolysis and CPEB4 overexpression is attenuated in HSC, for what PFKFB3 drives glycolytic switch in HSC, (b) CPEB4 knock-out prevents PFKFB3 overexpression (and thus HSC activation) for what PFKFB3 is a direct target of CPEB4, and (c) CPEB4 silencing in HSC during liver injury fails at increasing PFKFB3 levels for what translational regulation by CPEB4 outranks transcriptional control (**Figures 1C**, **2**).

Of course, and given this intricate context, the amount of activated signaling pathways and markers becomes huge and complex, involving multiple cell types, simultaneously cross-talking in different directions and chaotically influencing each other, which makes it difficult to establish a clear sequential script on the progression of liver disease. But at the same time, it represents an advantage in this specific context; targeting a single moiety, CPEB4 in this case, would synchronously act on all the processes involved in the progression of chronic liver disease.

Cytoplasmic Polyadenylation Element Binding Proteins and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) will be the last stage in chronic liver disease, commonly appearing in cirrhotic patients before fatal liver failure. In addition, a significant share of NASH-related HCC thrives in livers unaffected (or minimally affected) by fibrosis (Kucukoglu et al., 2021). Together, it is globally estimated that HCC is the first primary liver cancer and the third most usual cause of cancer-related mortality (Chalasani et al., 2018). We have seen a correlation between chronic inflammation caused by a poor diet, but mechanisms linking NA/MAFLD and NASH to HCC are still poorly understood. At a molecular level, this is most likely initiated by unresolved ROS and ER stress, which can fuel tumor cell proliferation through UPR pathway (Jialal et al., 2014; Engin, 2017) as previously explained. In a fat-rich environment, increased adipokines and proinflammatory cytokines will often perpetuate chronic inflammation via TNFa, IL-6 and activation of NF-kB, which contribute to inhibit apoptosis in the liver and promote proliferation, invasion and metastasis (Lin et al., 2010; Kucukoglu et al., 2021).

As translational mediators, CPEBs are involved in many processes related to the cell cycle progression, such as cellular division, differentiation and senescence, among others. The only CPEB so far expendable to the mitotic cell division is CPEB3 (Giangarrà et al., 2015). In a global cancer context, aberrant CPEB expression has been linked to cell proliferation, invasion, malignant transformation and angiogenesis through translational reprogramming in numerous types of cancer (D'Ambrogio et al., 2013), indicating that mRNA processing is important for tumor growth; however, not all CPEBs play the same role in tumorigenesis. According to literature, CPEB1 and CPEB3 act as tumor suppressors, while CPEB2 rather displays oncogenic features (Chen et al., 2016); in contrast, CPEB4 remains to be classified into one of these two categories. Thus, ectopic or imbalanced levels of CPEB subtypes may modulate the behavior of cancer cells and tilt the cell fate toward tumor development instead of senescence or controlled proliferation (Fernández-Miranda and Méndez, 2012; Ortiz-Zapater et al., 2012; Giangarrà et al., 2015).

Despite the diverse roles CPEBs play in different cancer types, very limited knowledge has been gathered regarding HCC. While CPEB4 is physiologically expressed in the brain, heart, kidney and lung (Ortiz-Zapater et al., 2012), it has been the most related CPEB to HCC, although its role comes as quite paradoxical; whilst it seems to play a tumor-suppressor role in HCC, it's been described as an oncogenic promoter in other kinds of cancer (Tsai et al., 2016). Observations of CPEB4 levels in different HCC stages suggest CPEB4 could play a phase-dependent role in HCC; but whether CPEB4 can be considered as a diagnostic marker or therapeutic target in HCC needs to be further researched. *In vitro* studies on liver cancer cells such as HepG2 revealed that CPEB4 knock-out promotes colony formation, and CPEB4 knock-down accelerates growth in xenograft mice (Tsai et al., 2016). Tsai and colleagues also collected and analyzed data from 49 human HCC samples, where they found CPEB4 to be mostly overexpressed in early stages of HCC and greatly decreased in late stages, suggesting CPEB4's role at later stages of HCC intends to facilitate HCC progression, manifesting a complicated biphasic role in tumorigenesis. Other molecular mechanisms implicating CPEB2 and mostly CPEB1 have been described for example by Nairismagi and colleagues (Nairismägi et al., 2012), regarding the role of Twist1 in epithelial-to-mesenchymal transition (Yang et al., 2004). This transcription factor seems to be repressed by CPEB1 and CPEB2 in physiological conditions, and upon lack of these CPEBs, it is then up-regulated, promoting E-cadherin loss and inducing cell migration. Although this mechanism has not yet been studied in a HCC context, it is worthy to bear in mind that Twist1 is activated under hypoxic circumstances (Sun et al., 2009), when HIF1a is also active and not repressed by CPEB1 and CPEB2 (Hägele et al., 2009; Chen and Huang, 2012), a situation that could be perfectly given in a tumoral and fibrotic environment lacking nutrient and oxygen influx. Another manner CPEBs influence HCC progression is through miRNAs, a booming field in the past decade. Some studies have been able to find multiple miRNA binding sites in CPEB mRNAs (Morgan et al., 2010) suggesting a regulating role of CPEB functions (Richter, 2007). A quite recent study by Zou and colleagues (Zou et al., 2016) describes a mechanism of cell proliferation, migration and invasion enhancement in HCC, concerning CPEB3 and miRNA-107. According to this study, miRNA-107, which is implicated in various cancers (Song et al., 2014; Zhou et al., 2014; Tao et al., 2015), was overexpressed in Huh7 and HepG2 cell lines, hindering CPEB3 mRNA and protein levels through binding to its 3′UTR. This CPEB3 down-regulation was accompanied by an increase in p-Akt and EGFR levels and a decrease in p21 levels, assigning a tumor-suppressor role for CPEB3 in a context of HCC.

Cytoplasmic Polyadenylation Element Binding Proteins and the Immune System

Non-alcoholic or metabolic associated fatty liver disease is very often attributed an immune pro-inflammatory component as one of the underlying mechanisms involved in disease progression, but to which extent is it? And more importantly, are CPEBs again involved in immune dysregulation in chronic liver disease? There are solid evidences of altered immune cell behavior in different stages of chronic liver disease, and a couple of studies have found a link to CPEBs for now.

In 2015, Richter's lab was focusing on CPEB1's role in inflammation (Ivshina et al., 2015), which was later confirmed and extended by Cui et al. (2020). In this study, they uncovered a mechanism by which depletion of CPEB1 causes a substantial increase in macrophage IL-6 synthesis, a well characterized pro-inflammatory cytokine with an active role both in chronic inflammation in a context of obesity and HCC progression (Alexandrov et al., 2012; Taniguchi and Karin, 2014; Ramirez-Pedraza and Fernández, 2019). In normal conditions, CPEB1 is bound to the CPE elements in TAK-1 mRNA, repressing the translation of the transcript. Upon CPEB1 depletion, TAK-1 is thus translated, causing the phosphorylation of IkBa, which will then dissociate from cytoplasmic NF-kB, allowing its internalization in the nucleus. NF-kB is a transcription factor that will activate the transcription of IL-6 in macrophages and polarized monocytes. The *in vivo* part of this study included HFD-fed mice; they observed that not only KO mice exhibited more IL-6 compared to WT, but also that HFD-fed KO mice presented insulin resistance, which has been many times related to chronic inflammation in literature, and also related to CPEB1 and CPEB2 depletion (Alexandrov et al., 2012). In the liver, KCs are the main producers of IL-6, and hepatocytes display high amounts of IL-6R, the IL-6 receptor; this causes them to be more susceptible to the up-regulation of signaling pathways such as JAK/STAT, MAPK/ERK and PI3K-Akt, all mediated and fueled by IL-6 (Taniguchi and Karin, 2014).

Conversely, our lab has been working for the past years in studying the role of CPEB4 in high fat diet induced obesity (Pell et al., 2021), and the results have elucidated another mechanism by which macrophages are altered by the adipose tissue, not previously described in literature. Indeed, CPEB4 drives a posttranscriptional reprogramming in adipocytes of white adipose tissue under obesity conditions. This rewiring stimulates the production and release of proinflammatory factors from obese adipocytes, which in turn promote a proinflammatory switch in macrophages and increase their migratory capacity. RIP-seq analysis from this study also revealed that CPEB4 is necessary for CCL2 and TLR4 production, which are implicated in the activation and recruitment of proinflammatory macrophages (Ramirez-Pedraza and Fernández, 2019) and also in liver fibrosis (Loomba et al., 2021). Moreover, the depletion of CPEB4 significantly attenuates CCL2 and TLR4 levels in adipose tissue, besides releasing higher levels of IL-10, an anti-inflammatory cytokine.

Activated pro-inflammatory macrophages are also related to pathological angiogenesis; it's been described by Ramirez-Pedraza and Fernandez (Ramirez-Pedraza and Fernández, 2019) that they actively support the formation of new vasculature through the secretion of pro-angiogenic factors (such as TGF-b, VEGF, PlGF, PDGF and matrix-remodeling proteases) and physical interaction with sprouting areas in the liver. While VEGF upregulation is directly linked to new vessel formation as described in prior sections, PlGF, for instance, which is also upregulated in this context of chronic inflammation (Li et al., 2017), will amplify VEGF activity by modifying the binding affinity of VEGF's receptors (Carmeliet et al., 2001; Fischer et al., 2007; Van Steenkiste et al., 2009). The nascent vascular network therefore allows macrophages to disseminate more easily throughout the tissue and interact with different cell types, guided by factors released by hepatocytes and often helping at HSC activation, promoting ECM accumulation and thus exacerbating fibrogenesis.

Other immune cells have been associated to chronic inflammation and many studies have described the cross-talk between the adipose tissue and the immune system (Gomes et al., 2016; Nati et al., 2016; Loomba et al., 2021); although macrophages have been at the spotlight of CPEB mediation of inflammatory processes regarding chronic liver disease and obesity until now, the door is open for other cell types, such as neutrophils and T-cells, to share an equally significant role in this scenario.

SUMMARY AND CONCLUSION

Recent findings from our group and other researchers unveil previously unrecognized posttranscriptional regulatory circuits orchestrated by the RNA-binding proteins CPEBs, which are required for portal hypertension, neovascularization, steatosis and fibrogenesis in liver disease. This improved understanding may create new opportunities to develop better treatments to combat chronic liver disease. In this regard, a potential therapeutic approach would be to block the binding of the CPEB proteins to their target mRNAs. For that, in collaboration with other groups, we are currently developing small compounds with CPEB4 inhibitory activity. These compounds specifically block the RNA binding site for CPEB4, preventing the binding of CPEB4 to their target mRNAs. It will be important to deliver these small compound CPEB4 inhibitors to the specific cell type of interest. For that, we are also collaborating with experts to develop drug-carrier therapeutic strategies to specifically deliver small compounds to any cell of interest. It is hoped that these CPEB4 inhibitors will make it to the clinic in the near future.

AUTHOR CONTRIBUTIONS

AB wrote the manuscript and designed figures. MF wrote and corrected sections of the manuscript and got funding. Both authors contributed to the article and approved submitted version.

FUNDING

This work was supported by grants from the FEDER/Spanish Ministry of Science, Innovation and Universities (SAF2017-87988-R, PID2020-118937RB-I00 MCIN/AEI/10.13039/501100011033, and PRE2018-083718), the Spanish Association Against Cancer (GCB15152955MEND), Worldwide Cancer Research Foundation (20_0284), World Cancer Research Fund International (IIG_FULL_2020_021), BBVA Foundation (28/2019), La Caixa Foundation (HR18-00302), and La Marató TV3 Foundation (2019-0259) to MF. IDIBAPS is supported by the CERCA Programme (Catalan Government).

REFERENCES

Adams, L. A., and Angulo, P. (2005). Recent concepts in non-alcoholic fatty liver disease. *Diabet. Med.* 22, 1129–1133.

Alexandrov, I. M., Ivshina, M., Jung, D. Y., Friedline, R., Ko, H. J., Xu, M., et al. (2012). Cytoplasmic polyadenylation element binding protein deficiency stimulates PTEN and Stat3 mRNA translation and induces hepatic insulin resistance. *PLoS Genet.* 8:e1002457. doi: 10.1371/journal.pgen.1002457

Belloc, E., and Méndez, R. (2008). A deadenylation negative feedback mechanism governs meiotic metaphase arrest. *Nature* 452, 1017–1021. doi: 10.1038/nature06809

Boustani, M. R., Mehrabi, F., Yahaghi, E., Khoshnood, R. J., Shahmohammadi, M., Darian, E. K., et al. (2016). Somatic CPEB4 and CPEB1 genes mutations spectrum on the prognostic predictive accuracy in patients with high-grade glioma and their clinical significance. *J. Neurol. Sci.* 363, 80–83. doi: 10.1016/j.jns.2016.02.032

Burns, D. M., and Richter, J. D. (2008). CPEB regulation of human cellular senescence, energy metabolism, and p53 mRNA translation. *Genes Dev.* 22, 3449–3460. doi: 10.1101/gad.1697808

Calderone, V., Gallego, J., Fernandez-Miranda, G., Garcia-Pras, E., Maillo, C., Berzigotti, A., et al. (2016). Sequential functions of CPEB1 and CPEB4 regulate pathologic expression of vascular endothelial growth factor and angiogenesis in chronic liver disease. *Gastroenterology* 150, 982.e30–997.e30. doi: 10.1053/j.gastro.2015.11.038

Carmeliet, P., Moons, L., Luttun, A., Vincenti, V., Compernolle, V., De Mol, M., et al. (2001). Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat. Med.* 7, 575–583. doi: 10.1038/87904

Chalasani, N., Younossi, Z., Lavine, J. E., Charlton, M., Cusi, K., Rinella, M., et al. (2018). The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the study of liver diseases. *Hepatology* 67, 328–357. doi: 10.1002/hep.29367

Chang, Y.-W., and Huang, Y.-S. (2014). Arsenite-activated JNK signaling enhances CPEB4-vinexin interaction to facilitate stress granule assembly and cell survival. *PLoS One* 9:e107961. doi: 10.1371/journal.pone.0107961

Chen, P.-J., and Huang, Y.-S. (2012). CPEB2-eEF2 interaction impedes HIF-1α RNA translation: translation control at elongation. *EMBO J.* 31, 959–971. doi: 10.1038/emboj.2011.448

Chen, Y., Tsai, Y.-H., and Tseng, S.-H. (2016). Regulation of the expression of cytoplasmic polyadenylation element binding proteins for the treatment of cancer. *Anticancer Res.* 36, 5673–5680. doi: 10.21873/anticanres.11150

Cui, H. S., Joo, S. Y., Cho, Y. S., Kim, J.-B., and Seo, C. H. (2020). CPEB1 or CPEB4 knockdown suppresses the TAK1 and Smad signalings in THP-1 macrophage-like cells and dermal fibroblasts. *Arch. Biochem. Biophys.* 683:108322.

Curinha, A., Oliveira Braz, S., Pereira-Castro, I., Cruz, A., and Moreira, A. (2014). Implications of polyadenylation in health and disease. *Nucleus* 5, 508–519. doi: 10.4161/nucl.36360

D'Ambrogio, A., Nagaoka, K., and Richter, J. D. (2013). Translational control of cell growth and malignancy by the CPEBs. *Nat. Rev. Cancer* 13, 283–290. doi: 10.1038/nrc3485

Dever, T. E., Dinman, J. D., and Green, R. (2018). Translation elongation and recoding in eukaryotes. *Cold Spring Harb. Perspect. Biol.* 10:a032649. doi: 10.1101/cshperspect.a032649

Drisaldi, B., Colnaghi, L., Fioriti, L., Rao, N., Myers, C., Snyder, A. M., et al. (2015). SUMOylation is an inhibitory constraint that regulates the prion-like aggregation and activity of CPEB3. *Cell Rep.* 11, 1694–1702. doi: 10.1016/j.celrep.2015.04.061

Engin, A. B. (2017). "Adipocyte-macrophage cross-talk in obesity," in *Obesity and Lipotoxicity [Internet]*, eds A. B. Engin and A. Engin (Cham: Springer International Publishing).

Eslam, M., Newsome, P. N., Sarin, S. K., Anstee, Q. M., Targher, G., Romero-Gomez, M., et al. (2020). A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J. Hepatol.* 73, 202–209.

Fernandez, M. (2015). Molecular pathophysiology of portal hypertension: FERNANDEZ. *Hepatology* 61, 1406–1415. doi: 10.1002/hep.27343

Fernandez, M., Mejias, M., Garcia-Pras, E., Gallego, J., and Pell, N. (2016). Pathogenesis of portal hypertension: extrahepatic mechanisms. *Curr. Hepatol. Rep.* 15, 199–207.

Fernández-Miranda, G., and Méndez, R. (2012). The CPEB-family of proteins, translational control in senescence and cancer. *Ageing Res. Rev.* 11, 460–472.

Fève, B., and Bastard, J.-P. (2009). The role of interleukins in insulin resistance and type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* 5, 305–311.

Fioriti, L., Myers, C., Huang, Y.-Y., Li, X., Stephan, J. S., Trifilieff, P., et al. (2015). The persistence of hippocampal-based memory requires protein synthesis mediated by the prion-like protein CPEB3. *Neuron* 86, 1433–1448. doi: 10.1016/j.neuron.2015.05.021

Fischer, C., Jonckx, B., Mazzone, M., Zacchigna, S., Loges, S., Pattarini, L., et al. (2007). Anti-PlGF inhibits growth of VEGF(R)-inhibitor-resistant tumors without affecting healthy vessels. *Cell* 131, 463–475. doi: 10.1016/j.cell.2007.08.038

Garcia-Pras, E., Gallego, J., Coch, L., Mejias, M., Fernandez-Miranda, G., Pardal, R., et al. (2017). Role and therapeutic potential of vascular stem/progenitor cells in pathological neovascularisation during chronic portal hypertension. *Gut* 66, 1306–1320. doi: 10.1136/gutjnl-2015-311157

Giangarrà, V., Igea, A., Castellazzi, C. L., Bava, F.-A., and Mendez, R. (2015). Global analysis of CPEBs reveals sequential and non-redundant functions in mitotic cell cycle. *PLoS One* 10:e0138794. doi: 10.1371/journal.pone.0138794

Gomes, A. L., Teijeiro, A., Burén, S., Tummala, K. S., Yilmaz, M., Waisman, A., et al. (2016). Metabolic inflammation-associated IL-17A causes non-alcoholic steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 30, 161–175. doi: 10.1016/j.ccell.2016.05.020

Hägele, S., Kühn, U., Böning, M., and Katschinski, D. M. (2009). Cytoplasmic polyadenylation-element-binding protein (CPEB)1 and 2 bind to the HIF-1a mRNA 3'-UTR and modulate HIF-1a protein expression. *Biochem. J.* 417, 235–246. doi: 10.1042/BJ20081353

Hake, L. E., and Richter, J. D. (1994). CPEB is a specificity factor that mediates cytoplasmic polyadenylation during *Xenopirs oocyte* maturation. *Cell* 79, 617–627. doi: 10.1016/0092-8674(94)90547-9

Halbeisen, R. E., Galgano, A., Scherrer, T., and Gerber, A. P. (2008). Post-transcriptional gene regulation: from genome-wide studies to principles. *Cell Mol. Life Sci.* 65, 798–813.

Han, T., Sun, H., Wen, X., Liu, Z., Li, S., Wang, J.-G., et al. (2015). Expression of CPEB4 in invasive ductal breast carcinoma and its prognostic significance. *Onco Targets Ther.* 2015:3499. doi: 10.2147/OTT.S87587

Hellen, C. U. T. (2018). Translation termination and ribosome recycling in eukaryotes. *Cold Spring Harb. Perspect. Biol.* 10:a032656. doi: 10.1101/cshperspect.a032656

Hu, W., Yang, Y., Xi, S., Sai, K., Su, D., Zhang, X., et al. (2015). Expression of CPEB4 in human glioma and its correlations with prognosis. *Medicine* 94:e979. doi: 10.1097/md.0000000000000979

Hu, W., Yuan, B., and Lodish, H. F. (2014). Cpeb4-mediated translational regulatory circuitry controls terminal erythroid differentiation. *Dev. Cell* 30, 660–672. doi: 10.1016/j.devcel.2014.07.008

Ibrahim, S. H., Hirsova, P., and Gores, G. J. (2018). Non-alcoholic steatohepatitis pathogenesis: sublethal hepatocyte injury as a driver of liver inflammation. *Gut* 67, 963–972. doi: 10.1136/gutjnl-2017-315691

Igea, A., and Méndez, R. (2010). Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4. *EMBO J.* 29, 2182–2193. doi: 10.1038/emboj.2010.111

Ivshina, M., Alexandrov, I. M., Vertii, A., Doxsey, S., and Richter, J. D. (2015). CPEB regulation of TAK1 synthesis mediates cytokine production and the inflammatory immune response. *Mol. Cell Biol.* 35, 610–618. doi: 10.1128/MCB.00800-14

Ivshina, M., Lasko, P., and Richter, J. D. (2014). Cytoplasmic polyadenylation element binding proteins in development, health, and disease. *Annu. Rev. Cell Dev. Biol.* 30, 393–415. doi: 10.1146/annurev-cellbio-101011-155831

Jialal, I., Kaur, H., and Devaraj, S. (2014). Toll-like receptor status in obesity and metabolic syndrome: a translational perspective. *J. Clin. Endocrinol. Metab.* 99, 39–48. doi: 10.1210/jc.2013-3092

Kan, M.-C., Oruganty-Das, A., Cooper-Morgan, A., Jin, G., Swanger, S. A., Bassell, G. J., et al. (2010). CPEB4 is a cell survival protein retained in the nucleus upon ischemia or endoplasmic reticulum calcium depletion. *Mol. Cell Biol.* 30, 5658–5671. doi: 10.1128/mcb.00716-10

Kim, J. H., and Richter, J. D. (2007). RINGO/cdk1 and CPEB mediate poly(A) tail stabilization and translational regulation by ePAB. *Genes Dev.* 21, 2571–2579. doi: 10.1101/gad.1593007

Kojima, S., Sher-Chen, E. L., and Green, C. B. (2012). Circadian control of mRNA polyadenylation dynamics regulates rhythmic protein expression. *Genes Dev.* 26, 2724–2736.

Kucukoglu, O., Sowa, J.-P., Mazzolini, G. D., Syn, W.-K., and Canbay, A. (2021). Hepatokines and adipokines in NASH-related hepatocellular carcinoma. *J. Hepatol.* 74, 442–457. doi: 10.1016/j.jhep.2020.10.030

Li, X., Jin, Q., Yao, Q., Zhou, Y., Zou, Y., Li, Z., et al. (2017). Placental growth factor contributes to liver inflammation, angiogenesis, fibrosis in mice by promoting hepatic macrophage recruitment and activation. *Front. Immunol.* 8:801. doi: 10.3389/fimmu.2017.00801

Lin, Y., Bai, L., Chen, W., and Xu, S. (2010). The NF-κB activation pathways, emerging molecular targets for cancer prevention and therapy. *Expert Opin. Ther. Targets* 14, 45–55. doi: 10.1517/14728220903431069

Liu, Y., Beyer, A., and Aebersold, R. (2016). On the dependency of cellular protein levels on mRNA abundance. *Cell* 165, 535–550.

Loomba, R., Friedman, S. L., and Shulman, G. I. (2021). Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* 184, 2537–2564. doi: 10.1016/j.cell.2021.04.015

Maillo, C., Martín, J., Sebastián, D., Hernández-Alvarez, M., García-Rocha, M., Reina, O., et al. (2017). Circadian- and UPR-dependent control of CPEB4 mediates a translational response to counteract hepatic steatosis under ER stress. *Nat. Cell Biol.* 19, 94–105. doi: 10.1038/ncb3461

McManus, J., Cheng, Z., and Vogel, C. (2015). Next-generation analysis of gene expression regulation – comparing the roles of synthesis and degradation. *Mol. BioSyst.* 11, 2680–2689. doi: 10.1039/c5mb00310e

Mejias, M., Balvey, A., and Fernandez, M. (2020a). Crosstalk between angiogenesis and fibrogenesis in liver disease. *Curr. Tissue Microenviron. Rep.* 1, 121–129. doi: 10.1007/s43152-020-00013-w

Mejias, M., Gallego, J., Naranjo-Suarez, S., Ramirez, M., Pell, N., Manzano, A., et al. (2020b). CPEB4 increases expression of PFKFB3 to induce glycolysis and activate mouse and human hepatic stellate cells, promoting liver fibrosis. *Gastroenterology* 159, 273–288. doi: 10.1053/j.gastro.2020.03.008

Mendez, R., Murthy, K. G. K., Ryan, K., Manley, J. L., and Richter, J. D. (2000). Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. *Mol. Cell* 6, 1253–1259.

Moore, M. J. (2005). From birth to death: the complex lives of eukaryotic mRNAs. *Science* 309, 1514–1518.

Morgan, M., Iaconcig, A., and Muro, A. F. (2010). CPEB2, CPEB3 and CPEB4 are coordinately regulated by miRNAs recognizing conserved binding sites in paralog positions of their 3′-UTRs. *Nucleic Acids Res.* 38, 7698–7710. doi: 10.1093/nar/gkq635

Nagaoka, K., Fujii, K., Zhang, H., Usuda, K., Watanabe, G., Ivshina, M., et al. (2016). CPEB1 mediates epithelial-to-mesenchyme transition and breast cancer metastasis. *Oncogene* 35, 2893–2901. doi: 10.1038/onc.2015.350

Nairismägi, M.-L., Vislovukh, A., Meng, Q., Kratassiouk, G., Beldiman, C., Petretich, M., et al. (2012). Translational control of TWIST1 expression in MCF-10A cell lines recapitulating breast cancer progression. *Oncogene* 31, 4960–4966. doi: 10.1038/onc.2011.650

Nati, M., Haddad, D., Birkenfeld, A. L., Koch, C. A., Chavakis, T., and Chatzigeorgiou, A. (2016). The role of immune cells in metabolism-related liver inflammation and development of non-alcoholic steatohepatitis (NASH). *Rev. Endocr. Metab. Disord.* 17, 29–39. doi: 10.1007/s11154-016-9339-2

Neuschwander-Tetri, B. A. (2010). Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology* 52, 774–788. doi: 10.1002/hep.23719

Novoa, I., Gallego, J., Ferreira, P. G., and Mendez, R. (2010). Mitotic cell-cycle progression is regulated by CPEB1 and CPEB4-dependent translational control. *Nat. Cell Biol.* 12, 447–456. doi: 10.1038/ncb2046

Ortiz-Zapater, E., Pineda, D., Martínez-Bosch, N., Fernández-Miranda, G., Iglesias, M., Alameda, F., et al. (2012). Key contribution of CPEB4-mediated translational control to cancer progression. *Nat. Med.* 18, 83–90. doi: 10.1038/nm.2540

Paris, J., Swenson, K., Piwnica-Worms, H., and Richter, J. D. (1991). Maturation-specific polyadenylation: in vitro activation by p34cdc2 and phosphorylation of a 58-kD CPE-binding protein. *Genes Dev.* 5, 1697–1708. doi: 10.1101/gad.5.9.1697

Parker, R., and Song, H. (2004). The enzymes and control of eukaryotic mRNA turnover. *Nat. Struct. Mol. Biol.* 11, 121–127. doi: 10.1038/nsmb724

Pavlopoulos, E., Trifilieff, P., Chevaleyre, V., Fioriti, L., Zairis, S., Pagano, A., et al. (2011). Neuralized1 activates CPEB3: a function for nonproteolytic ubiquitin in synaptic plasticity and memory storage. *Cell* 147, 1369–1383. doi: 10.1016/j.cell.2011.09.056

Pell, N., Garcia-Pras, E., Gallego, J., Naranjo-Suarez, S., Balvey, A., Suñer, C., et al. (2021). Targeting the cytoplasmic polyadenylation element binding protein CPEB4 protects against diet-induced obesity and microbiome dysbiosis. *Mol. Metab.* doi: 10.1016/j.molmet.2021.101388 [Epub ahead of print].

Peng, F., Xiao, X., Jiang, Y., Luo, K., Tian, Y., Peng, M., et al. (2014). HBx down-regulated Gld2 plays a critical role in HBV-related dysregulation of miR-122. *PLoS One* 9:e92998. doi: 10.1371/journal.pone.0092998

Pimpin, L., Cortez-Pinto, H., Negro, F., Corbould, E., Lazarus, J. V., Webber, L., et al. (2018). Burden of liver disease in Europe: epidemiology and analysis of risk factors to identify prevention policies. *J. Hepatol.* 69, 718–735.

Ramirez-Pedraza, M., and Fernández, M. (2019). Interplay between macrophages and angiogenesis: a double-edged sword in liver disease. *Front. Immunol.* 10:2882. doi: 10.3389/fimmu.2019.02882

Richter, J. D. (2007). CPEB: a life in translation. *Trends Biochem. Sci.* 32, 279–285. doi: 10.1016/j.tibs.2007.04.004

Ruggero, D., and Shimamura, A. (2014). Marrow failure: a window into ribosome biology. *Blood* 124, 2784–2792. doi: 10.1182/blood-2014-04-526301

Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., et al. (2011). Global quantification of mammalian gene expression control. *Nature* 473, 337–342.

Song, Y., Ma, X., Ma, G., Lin, B., Liu, C., Deng, Q., et al. (2014). MicroRNA-107 promotes proliferation of gastric cancer cells by targeting cyclin dependent kinase 8. *Diagn. Pathol.* 9:164. doi: 10.1186/s13000-014-0164-1

Stephan, J. S., Fioriti, L., Lamba, N., Colnaghi, L., Karl, K., Derkatch, I. L., et al. (2015). The CPEB3 protein is a functional prion that interacts with the actin cytoskeleton. *Cell Rep.* 11, 1772–1785. doi: 10.1016/j.celrep.2015.04.060

Sun, S., Ning, X., Zhang, Y., Lu, Y., Nie, Y., Han, S., et al. (2009). Hypoxia-inducible factor-1α induces Twist expression in tubular epithelial cells subjected to hypoxia, leading to epithelial-to-mesenchymal transition. *Kidney Int.* 75, 1278–1287. doi: 10.1038/ki.2009.62

Taniguchi, K., and Karin, M. (2014). IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin. Immunol.* 26, 54–74. doi: 10.1016/j.smim.2014.01.001

Tao, J., Ji, J., Li, X., Ding, N., Wu, H., Liu, Y., et al. (2015). Distinct anti-oncogenic effect of various microRNAs in different mouse models of liver cancer. *Oncotarget* 6, 6977–6988. doi: 10.18632/oncotarget.3166

Theis, M., Si, K., and Kandel, E. R. (2003). Two previously undescribed members of the mouse CPEB family of genes and their inducible expression in the principal cell layers of the hippocampus. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9602–9607. doi: 10.1073/pnas.1133424100

Tsai, L.-Y., Chang, Y.-W., Lee, M.-C., Chang, Y.-C., Hwang, P.-I., Huang, Y.-S., et al. (2016). Biphasic and stage-associated expression of CPEB4 in hepatocellular carcinoma. *PLoS One* 11:e0155025. doi: 10.1371/journal.pone.0155025

Turimella, S. L., Bedner, P., Skubal, M., Vangoor, V. R., Kaczmarczyk, L., Karl, K., et al. (2015). Characterization of cytoplasmic polyadenylation element binding 2 protein expression and its RNA binding activity: expression and RNA Binding of CPEB2. *Hippocampus* 25, 630–642. doi: 10.1002/hipo.22399

Udagawa, T., Swanger, S. A., Takeuchi, K., Kim, J. H., Nalavadi, V., Shin, J., et al. (2012). Bidirectional control of mRNA translation and synaptic plasticity by the cytoplasmic polyadenylation complex. *Mol. Cell.* 47, 253–266. doi: 10.1016/j.molcel.2012.05.016

Van Steenkiste, C., Geerts, A., Vanheule, E., Van Vlierberghe, H., De Vos, F., Olievier, K., et al. (2009). Role of placental growth factor in mesenteric neoangiogenesis in a mouse model of portal hypertension. *Gastroenterology* 137, 2112.e6–2124.e6. doi: 10.1053/j.gastro.2009.08.068

Wang, X.-P., and Cooper, N. G. F. (2010). Comparative in silico analyses of Cpeb1–4 with functional predictions. *Bioinform. Biol. Insights* 4, 61–83. doi: 10.4137/bbi.s5087

Yamamura, S., Eslam, M., Kawaguchi, T., Tsutsumi, T., Nakano, D., Yoshinaga, S., et al. (2020). MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. *Liver Int.* 40, 3018–3030. doi: 10.1111/liv.14675

Yang, J., Mani, S. A., Donaher, J. L., Ramaswamy, S., Itzykson, R. A., Come, C., et al. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117, 927–939. doi: 10.1016/j.cell.2004.06.006

Younes, R., and Bugianesi, E. (2018). Should we undertake surveillance for HCC in patients with NAFLD? *J. Hepatol.* 68, 326–334. doi: 10.1016/j.jhep.2017.10.006

Younossi, Z. M., Golabi, P., de Avila, L., Paik, J. M., Srishord, M., Fukui, N., et al. (2019). The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. *J. Hepatol.* 71, 793–801. doi: 10.1016/j.jhep.2019.06.021

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., and Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64, 73–84. doi: 10.1002/hep.28431

Zhang, H., Zou, C., Qiu, Z., E, F., Li, Q., Chen, M., et al. (2020). CPEB3-mediated MTDH mRNA translational suppression restrains hepatocellular carcinoma progression. *Cell Death Dis.* 11:792. doi: 10.1038/s41419-020-02984-y

Zhang, X. (2018). "NAFLD related-HCC: the relationship with metabolic disorders," in *Obesity, Fatty Liver and Liver Cancer [Internet]*, ed. J. Yu (Singapore: Springer), 55–62. doi: 10.1007/978-981-10-8684-7_5

Zhong, X., Xiao, Y., Chen, C., Wei, X., Hu, C., Ling, X., et al. (2015). MicroRNA-203-mediated posttranscriptional deregulation of CPEB4 contributes to colorectal cancer progression. *Biochem. Biophys. Res. Commun.* 466, 206–213. doi: 10.1016/j.bbrc.2015.09.008

Zhou, C., Li, G., Zhou, J., Han, N., Liu, Z., and Yin, J. (2014). miR-107 activates ATR/Chk1 pathway and suppress cervical cancer invasion by targeting MCL1. *PLoS One* 9:e111860. doi: 10.1371/journal.pone.0111860

Zou, C.-D., Zhao, W.-M., Wang, X.-N., Li, Q., Huang, H., Cheng, W.-P., et al. (2016). MicroRNA-107: a novel promoter of tumor progression that targets the CPEB3/EGFR axis in human hepatocellular carcinoma. *Oncotarget* 7, 266–278. doi: 10.18632/oncotarget.5689

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 23 December 2021
doi: 10.3389/fmed.2021.761517

COX-2/sEH Dual Inhibitor PTUPB Alleviates CCl_4-Induced Liver Fibrosis and Portal Hypertension

Zhifeng Zhao[1†], Chihao Zhang[1†], Jiayun Lin[1], Lei Zheng[1], Hongjie Li[1], Xiaoliang Qi[1], Haizhong Huo[1], Xiaolou Lou[1], Bruce D. Hammock[2], Sung Hee Hwang[2], Yongyang Bao[3] and Meng Luo[1*]*

[1] Department of General Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2] Department of Entomology, Nematology and UC Davis Comprehensive Cancer Center, Davis, CA, United States, [3] Department of Pathology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

OPEN ACCESS

Edited by:
Jonel Trebicka,
Goethe University Frankfurt, Germany

Reviewed by:
Michael Praktiknjo,
University Hospital Bonn, Germany
Maximilian Joseph Brol,
University Hospital Bonn, Germany

***Correspondence:**
Yongyang Bao
yybaodr28@sina.com
Meng Luo
luosh9hospital@sina.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 20 August 2021
Accepted: 02 December 2021
Published: 23 December 2021

Citation:
Zhao Z, Zhang C, Lin J, Zheng L, Li H, Qi X, Huo H, Lou X, Hammock BD, Hwang SH, Bao Y and Luo M (2021) COX-2/sEH Dual Inhibitor PTUPB Alleviates CCl_4-Induced Liver Fibrosis and Portal Hypertension. Front. Med. 8:761517. doi: 10.3389/fmed.2021.761517

Background: 4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl) -benzenesulfonamide (PTUPB), a dual cyclooxygenase-2 (COX-2)/soluble epoxide hydrolase (sEH) inhibitor, was found to alleviate renal, pulmonary fibrosis and liver injury. However, few is known about the effect of PTUPB on liver cirrhosis. In this study, we aimed to explore the role of PTUPB in liver cirrhosis and portal hypertension (PHT).

Method: Rat liver cirrhosis model was established *via* subcutaneous injection of carbon tetrachloride (CCl_4) for 16 weeks. The experimental group received oral administration of PTUPB (10 mg/kg) for 4 weeks. We subsequently analyzed portal pressure (PP), liver fibrosis, inflammation, angiogenesis, and intra- or extrahepatic vascular remodeling. Additionally, network pharmacology was used to investigate the possible mechanisms of PTUPB in live fibrosis.

Results: CCl_4 exposure induced liver fibrosis, inflammation, angiogenesis, vascular remodeling and PHT, and PTUPB alleviated these changes. PTUPB decreased PP from 17.50 ± 4.65 to 6.37 ± 1.40 mmHg, reduced collagen deposition and profibrotic factor. PTUPB alleviated the inflammation and bile duct proliferation, as indicated by decrease in serum interleukin-6 (IL-6), liver cytokeratin 19 (CK-19), transaminase, and macrophage infiltration. PTUPB also restored vessel wall thickness of superior mesenteric arteries (SMA) and inhibited intra- or extrahepatic angiogenesis and vascular remodeling *via* vascular endothelial growth factor (VEGF), von Willebrand factor (vWF), etc. Moreover, PTUPB induced sinusoidal vasodilation by upregulating endothelial nitric oxide synthase (eNOS) and GTP-cyclohydrolase 1 (GCH1). In enrichment analysis, PTUPB engaged in multiple biological functions related to cirrhosis, including blood pressure, tissue remodeling, immunological inflammation, macrophage activation, and fibroblast proliferation. Additionally, PTUPB suppressed hepatic expression of sEH, COX-2, and transforming growth factor-β (TGF-β).

Conclusion: 4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl)- benzenesulfonamide ameliorated liver fibrosis and PHT by inhibiting fibrotic deposition, inflammation, angiogenesis, sinusoidal, and SMA remodeling. The molecular mechanism may be mediated *via* the downregulation of the sEH/COX-2/TGF-β.

Keywords: PTUPB, liver fibrosis, portal hypertension, inflammation, angiogenesis

BACKGROUND

As a prevalent and challenging illness, liver cirrhosis is characterized by abnormal buildup of hepatic extracellular matrix (ECM) as a result of inflammation or damage (1). The deposition of fibrotic tissue increases intrahepatic circulatory resistance and extrahepatic circulatory pressure which lead to portal hypertension (PHT) (2). PHT can result in esophageal and gastric varices, and also severe bleeding in the upper gastrointestinal system; thus, early management is critical. However, there is currently no specific treatment for liver cirrhosis, especially PHT.

As one of the most abundant lipid mediators, arachidonic acid (ARA) and its metabolites play a critical role in the vasoactivity, inflammation, fibrosis, etc. The ARA can be metabolized and transformed *via* three pathways: cyclooxygenase (COX), cytochrome P450 (CYP450), and lipoxygenase (LOX). Among them, COX and CYP450 pathways are most strongly associated with liver cirrhosis (2). COX is divided into two categories: COX-1 and COX-2. As a biomarker of inflammation, immune system, and cell proliferation, COX-2 was deeply involved in the progression and deterioration of liver cirrhosis (3). The CYP450 pathway performed a variety of activities, including antiinflammatory, antihypertension, and antifibrosis, mostly *via* the epoxyeicosatrienoic acids (EETs) (4–6). However, EETs are often catalyzed by soluble epoxide hydrolase (sEH) into the less biologically active metabolite in stress situations such as hypertension (7, 8). As a result, the biological activities of ARA pathways depend primarily on COX-2 and sEH.

In recent years, a COX-2/sEH dual inhibitor, PTUPB, has been developed. PTUPB was later discovered to have impacts on pulmonary fibrosis (9), renal fibrosis (10), and liver injury (11). Those studies above suggested that PTUPB shows an alleviating effect in fibrosis. But the effect of PTUPB in liver cirrhosis and PHT has not been researched yet. In this article, the effect of PTUPB on liver cirrhosis and PHT was explored.

This study is made up of the following sections. To begin, we investigated PTUPB's remission impact on hepatic fibrosis. Following that, we investigated the impact of PTUPB on liver inflammation and function. Then, we investigated the angiogenesis pathways in the liver and the influence of PTUPB on mesenteric vascular remodeling. Finally, we investigated the mechanism and pathways.

Abbreviations: ALT, alanine transaminase; ANGPT1, angiopoietin 1; ARA, arachidonic acid; AST, aspartate aminotransferase; CCl4, carbon tetrachloride; COL1A1, collagen type I alpha 1 chain; COX-2, cyclooxygenase-2; CYP450, cytochrome P450; CK-19, cytokeratin 19; DBIL, direct bilirubin; eNOS, endothelial nitric oxide synthase; ELISA, enzyme-linked immunosorbent assay; EPHX2, epoxide hydrolase 2; EETs, epoxyeicosatrienoic acids; ECM, extracellular matrix; GCH1, GTP-cyclohydrolase 1; HR, heart rate; IL-6, interleukin-6; LOX, lipoxygenase; MMP-9, matrix metalloproteinase-9; MMP-2, matrix metalloproteinase-2; MAP, mean arterial pressure; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; PHT, portal hypertension; PP, portal pressure; PPI, protein–protein interaction; she, soluble epoxide hydrolase; SD, Sprague Dawley; SMA, superior mesenteric arteries; TBIL, total bilirubin; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor; GGT, γ-glutamyl transferase.

METHOD

Animals and Reagents

All animal-related protocols were approved by the Ethical Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). Sprague Dawley (SD) rats (male, 6–8 weeks old) weighing 200–250 g were purchased from the Experimental Animal Center of School of Medicine, Shanghai Jiao Tong University (Shanghai, China). The rats were maintained in our specific pathogen-free facility under controlled conditions (22°C, 40–60% humidity, and 12-h light/dark cycle), and free access to tap water and standard rat food was given to the rats.

1-(4-trifluoromethoxyphenyl)-3-(1-propionylpiperidin-4-yl)-urea, TPPU, and 4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide, PTUPB, were synthesized according to the previous procedures (12). TPPU was generously provided by the laboratory of Dr. Bruce Hammock (UC Davis, USA) and stored at 20°C. TPPU and PTUPB were dissolved in PEG-400 to give a 10g/L clear solution. This solution was then added to warm drinking water with rapid stirring to give the 100 mg/L solution of TPPU/PTUPB in drinking water. Based on the estimation of daily water consumption, a concentration of 100 mg/L inhibitor in drinking water will result in a dose of approximately 10 mg/kg/day. The other rats received vehicle (PEG 400 diluted in water) as control. PTUPB and TPPU were administered for 4 weeks from the 12th week in the CCl_4 model group.

A total of 20 rats were used in this study. Rats were divided into four subgroups as follows: control group (OO-VEH) received pure olive oil injection with vehicle administration ($n = 5$); PHT group received carbon tetrachloride (CCl_4) (50% in olive oil, v/v, 1 ml/kg) by subcutaneous injection (s.c.) two times a week for 16 weeks with vehicle administration (CCl_4-VEH) ($n = 5$); PHT group with TPPU administration (CCl_4-TPPU) ($n = 5$); and PHT group with PTUPB administration (CCl_4-PTUPB). The detailed grouping strategy was shown in **Figure 1**.

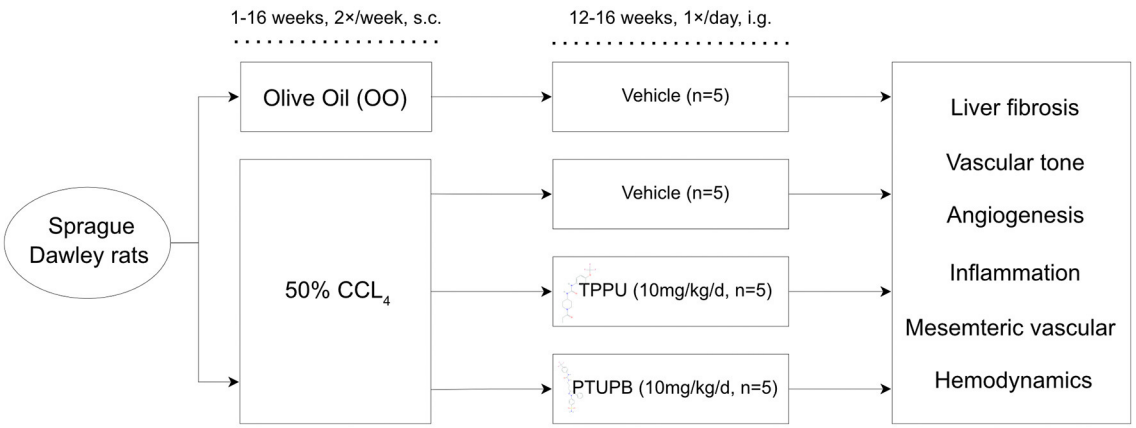


FIGURE 1 | Schematic diagram of animal models.

Hemodynamic Measurements

After finishing modeling, rats were anesthetized with 40 mg/kg zolazepam and tiletamine (Zoletil 50, France) and 8 μg/kg dexmedetomidine hydrochloride (Dexdomitor®, Pfizer Inc. USA) through intramuscular injections. A PE-50 catheter (Smiths Medical, UK) was inserted into the right femoral artery to determine the heart rate (HR) and mean arterial pressure (MAP). The catheter was then placed into the portal vein to determine the portal pressure (PP). A transducer linked the catheter to the monitor, and the values were acquired using a multichannel physiological signal collection system (ALC-MPA multichannel bioinformatics analysis system, Shanghai Alcott Biotechnology Co., Ltd., China). Following sacrifice of the rats, blood, liver, and superior mesenteric arteries (SMA) were taken for enzymatic analysis, histological, and molecular analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum levels of interleukin (IL)-6 and hyaluronan were determined by IL-6 rat ELISA kit (Thermo Fisher, USA) and hyaluronan rat ELISA Kit (R&D Systems, USA), respectively, according to the manufacturer's instructions.

Histological and Immunohistochemical (IHC) Examination

The liver sections from the right lobe and mesenteric tissues were fixed in 10% formalin buffer (pH 7.4) and embedded in a paraffin block. Hematoxylin–eosin (H&E), Masson, and Sirius Red staining were used on the liver sections, followed by random evaluation under a light microscope by an expert pathologist.

For IHC staining, liver sections were incubated with antimatrix metalloproteinase-2 (MMP-2) antibody (1:150, Servicebio, China), antimatrix metalloproteinase-9 (MMP-9) antibody (1:300, Servicebio, China), antivascular endothelial growth factor (VEGF) A antibody (1:250, Servicebio, China), antivon Willebrand factor (vWF) antibody (1:1,000, Servicebio, China), anticytokeratin 19 (CK-19) antibody (1:1,000, Servicebio, China), anti-sEH antibody (1:50, Absin, China), anti-CD31 antibody (1:300, Servicebio, China), and anti-CD68 antibody (1:150, Servicebio, China), overnight at 4°C with phosphate-buffered saline (PBS) as negative control. Subsequently, the sections were incubated with appropriate HRP-conjugated goat antirabbit secondary antibody for 60 min, followed by restaining with hematoxylin. The collagen deposition volume and stained area were calculated with IHC Profiler plugin in ImageJ (version 1.53, USA). The results were expressed as the proportions of the stained areas. The total area and average values were taken from five rats in each group.

Hepatic Functions

Hepatic functions, including the total bilirubin (TBIL), direct bilirubin (DBIL), aspartate aminotransferase (AST), alanine transaminase (ALT), and γ-glutamyl transferase (GGT), were examined using kits from Changchun Huili Biotech (China) according to the manufacturer's instruction.

Western Blotting Analysis

Samples from liver tissue were taken and kept at −80°C. To extract protein from the liver, the sample was crushed in liquid nitrogen and homogenized according to the manufacturer's instructions using RIPA buffer (Beyotime, China). The liver extracts were then centrifuged for 15 min at 10,000 g for 15 min at 4°C. The supernatant was immediately collected, and the BCA protein analysis kit was used to determine the total protein content (Beyotime, China). Equal amounts of proteins were electrophoresed on sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes. Primary antibodies against α-smooth muscle actin (α-SMA) (1:1,000, Servicebio, China), COX-2 (1: 750, Servicebio, China), and sEH (1:1,000, Absin, China) were used to incubate the blots (1:1,000, Servicebio, China). The membranes were then treated with corresponding secondary antibodies. Immunoreactive bands were visualized using an electrochemiluminescence instrument (Vilber Lourmat, France) and quantified using digital image software (Kodak, USA).

Real-Time Polymerase Chain Reaction (qRT-PCR)

The mRNA expressions of TGF-β, epoxide hydrolase 2 (EPHX2), COX2, α-SMA, collagen type I alpha 1 chain (COL1A1), MMP2, MMP9, VEGF, VWF, angiopoietin 1 (ANGPT1), endothelial nitric oxide synthase (eNOS), GTP cyclohydrolase 1 (GCH1), and adhesion G protein-coupled receptor E1 (Adgre1, F4-80) were determined. qRT-PCR was conducted on a Bio-Rad iCycleriQ real-time PCR detection system (Bio-Rad laboratories, Germany) using IQ SYBR Green supermix Kit (Bio-Rad). The reaction was carried out in triplicate. The data were analyzed using the iCycleriQ software system (Bio-Rad, Germany).

Bioinformatic Analysis

Potential target genes were obtained from Pharm Mapper (http://lilabecust.cn/pharmmapper/) and SwissTargetPrediction [https://swisstargetprediction.ch/; probability value ≥0.10 was selected (13)]. Afterward, we entered the keyword "liver fibrosis" into the GeneCards (https://www.genecards.org/) (14) to obtain target genes related to liver fibrosis. The differentially expressed genes related to PTUPB and liver fibrosis were intersected and depicted in Venn diagram by *ggVennDiagram* package.

Protein–protein interaction (PPI) network of common target genes was generated from STRING database [https://string-db.org/ (15)]. The PPI network was visualized using Cytoscape (version 3.6.1) (16). To determine the hub genes, we computed the centrality of each mRNA node using "MCC" method in CytoHubba, a Cytoscape plugin (17). The top ten genes were deemed hub mRNAs based on their degree of centrality.

The *clusterProfiler* package was used to perform gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on common target genes (18). The GO terms describe gene functions in three aspects: biological processes, molecular functions, and cellular components. The KEGG analysis predicts the involvement of the common target genes in various biological pathways. The modified $p < 0.05$ was used as a cutoff value (19, 20).

Statistical Analysis

All the statistical analyses were performed using R (version 4.1.0.). Continuous variables were expressed as means ± standard deviation (SD). N represents the number of rats. Statistical significance was calculated by Student's *t*-test or Mann–Whitney *U* test. Two-sided $p < 0.05$ was considered statistically significant.

RESULT

PTUPB Reduces Portal Hypertension

The hemodynamic and general characteristics were measured including weight, liver weight, HR, MAP, and PP. After the modeling of PHT, CCl4-VEH group presented with a higher PP (17.50 ± 4.65 vs. 5.40 ± 1.13 mmHg), lower weight (465.75 ± 11.15 vs. 562.25 ± 65.38 g), and lower liver weights (21.63 ± 0.93 vs. 26.93 ± 1.77 g) compared with OO-VEH significantly ($p <$ 0.05), although MAP and HR remained comparable. Following the treatment of PTUPB, the body and liver weights of PHT rats rebounded and PP decreased significantly from 17.50 ± 4.65 to 6.37 ± 1.40 mmHg ($p < 0.001$), whereas map and HR remained unchanged. Similar to PTUPB, TPPU treatment also increased weights (609.50 ± 25.12 vs. 465.75 ± 11.15 g) and liver weights (29.33 ± 2.76 vs. 21.63 ± 0.93 g) compared with CCl_4-VEH group and decreased PP (6.86 ± 1.44 vs. 17.50 ± 4.65 mmHg) significantly ($p < 0.05$). The results are shown in **Table 1**.

Antifibrotic Effects of PTUPB

After 16 weeks of CCl4 treatment, rats in the CCl4-VEH group exhibited marked liver fibrosis. Liver in PHT rats showed increased vacuolar degeneration and lysis in H&E staining, more collagen deposition in Masson (24% vs. 6%) and Sirius Red (23% vs. 15%) staining (**Figures 2A,E,F**). The hepatic expression of α-SMA, COL1A1, and serum hyaluronan also increased significantly (**Figures 2B–D**). PTUPB treatment resulted in significant reduced collagen deposition in Masson (9% vs. 24%) and Sirius Red (13% vs. 23%) staining which could also been confirmed by macroscopy and H&E (**Figures 2A,E,F**). In addition, the hepatic expression of α-SMA, COL1A1, and serum hyaluronan also decreased significantly after PTUPB treatment (**Figure 2B–D,G**). The treatment of TPPU was relatively less effective. Only the Masson staining of CCl4-TPPU group (13%) was significantly less than CCl4-VEH. No significant difference was observed in other indicators between CCl_4-TPPU with CCl_4-VEH group including the Sirius Red staining, serum hyaluronan, and hepatic expression of α-SMA and COL1A1 (**Figures 2B–F**).

PTUPB Ameliorate Inflammation and Liver Functions

Liver fibrosis is often accompanied by an increase hepatic and systemic inflammation. In CCl4-VEH group, hepatic expression of F4/80 mRNA and CD68 staining area increased significantly, indicating the upregulated hepatic mononuclear or macrophage infiltration (**Figures 3C,D**). The serum IL-6 also elevated, suggesting the systemic inflammation in PHT models (**Figure 3E**). The PTUPB treatment groups showed significant lower levels of F4/80 mRNA and CD68 staining in liver and also serum IL-6 (**Figures 3A–E**). Similar but less-effective results were observed in CCl_4-TPPU group.

In addition, we found that the liver structure was destroyed and its functions were decreased severely in PHT models. CK-19, a classical marker of bile duct cell proliferation, was upregulated in CCl4-VEH group compared with OO-VEH group (5% vs. 2%). Liver function markers, such as ALT, AST and DBIL, were also elevated significantly in CCl4-VEH rats (**Figures 3A,B,F–H**). In TPPU and PTUPB treatment groups, CK-19, ALT, and AST were observed to decrease significantly (**Figures 3B,F,G**) whereas TBIL, DBIL, and GGT did not change significantly (**Figures 3H–J**).

PTUPB Inhibits Pathological Angiogenesis and Sinusoidal Remodeling

Pathological angiogenesis and sinusoidal remodeling are remarkable pathological symptoms of cirrhosis which contribute to vascular resistance and PHT. Several mediators of angiogenesis and remodeling were significantly increased in PHT rats including MMP2, VEGF, vWF, and Angpt1 (**Figures 4B–E,J**),

TABLE 1 | Hemodynamic and general characteristics.

	OO-VEH($n = 5$)	CCl_4 -VEH($n = 5$)	CCl_4-TPPU($n = 5$)	CCl_4-PTUPB($n = 5$)	OO *vs.* CCl_4	CCl_4-VEH *vs.* TPPU	CCl_4-VEH *vs.* PTUPB
Weight/g	562.25 ± 65.38	465.75 ± 11.15	609.50 ± 25.12	555.50 ± 15.59	0.013*	0.001*	0.02*
Liver/g	26.93 ± 1.77	21.63 ± 0.93	29.33 ± 2.76	29.93 ± 2.71	0.022*	0.002*	<0.001*
MAP/mmHg	82.75 ± 8.42	67.67 ± 11.59	60.75 ± 3.50	70.00 ± 4.69	0.082	0.615	0.974
PP/mmHg	5.40 ± 1.13	17.50 ± 4.65	6.86 ± 1.44	6.37 ± 1.40	<0.001*	<0.001*	<0.001*
HR/bpm	370.50 ± 32.69	375.75 ± 21.62	352.50 ± 60.08	388.5 ± 19.96	0.997	0.814	0.961

**Statistical significance ($p < 0.05$) by Student's t-test.*

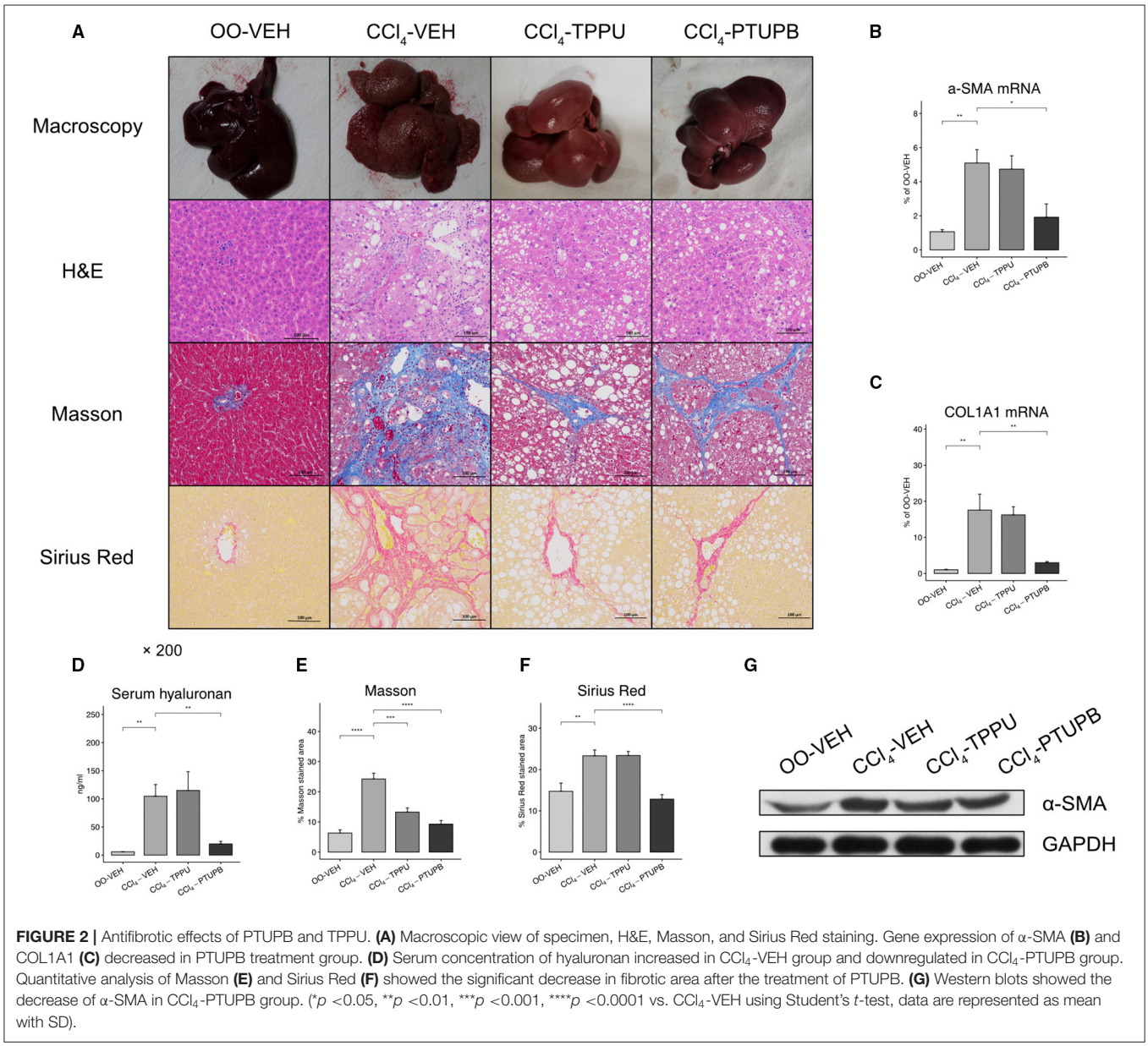


FIGURE 2 | Antifibrotic effects of PTUPB and TPPU. **(A)** Macroscopic view of specimen, H&E, Masson, and Sirius Red staining. Gene expression of α-SMA **(B)** and COL1A1 **(C)** decreased in PTUPB treatment group. **(D)** Serum concentration of hyaluronan increased in CCl_4-VEH group and downregulated in CCl_4-PTUPB group. Quantitative analysis of Masson **(E)** and Sirius Red **(F)** showed the significant decrease in fibrotic area after the treatment of PTUPB. **(G)** Western blots showed the decrease of α-SMA in CCl_4-PTUPB group. (*p <0.05, **p <0.01, ***p <0.001, ****p <0.0001 vs. CCl_4-VEH using Student's t-test, data are represented as mean with SD).

which were confirmed by immunohistochemical staining (**Figure 4A**). After PTUPB treatment, MMP2, VEGF, vWF, Angpt1, and CD31 were significantly reduced, whereas MMP9 remained unchanged (**Figures 4B–E,J,K**). However, in CCl_4-TPPU group, only vWF, Angpt1, and CD31 were reduced significantly. In addition to the sinusoid remodeling, sinusoidal dysfunction also contributes to vascular resistance. Our results indicated that the expression of GCH1 decreased significantly in PHT group. After PTUPB treatment, the hepatic GCH1 and eNOS mRNA increased remarkably (**Figures 4L,M**).

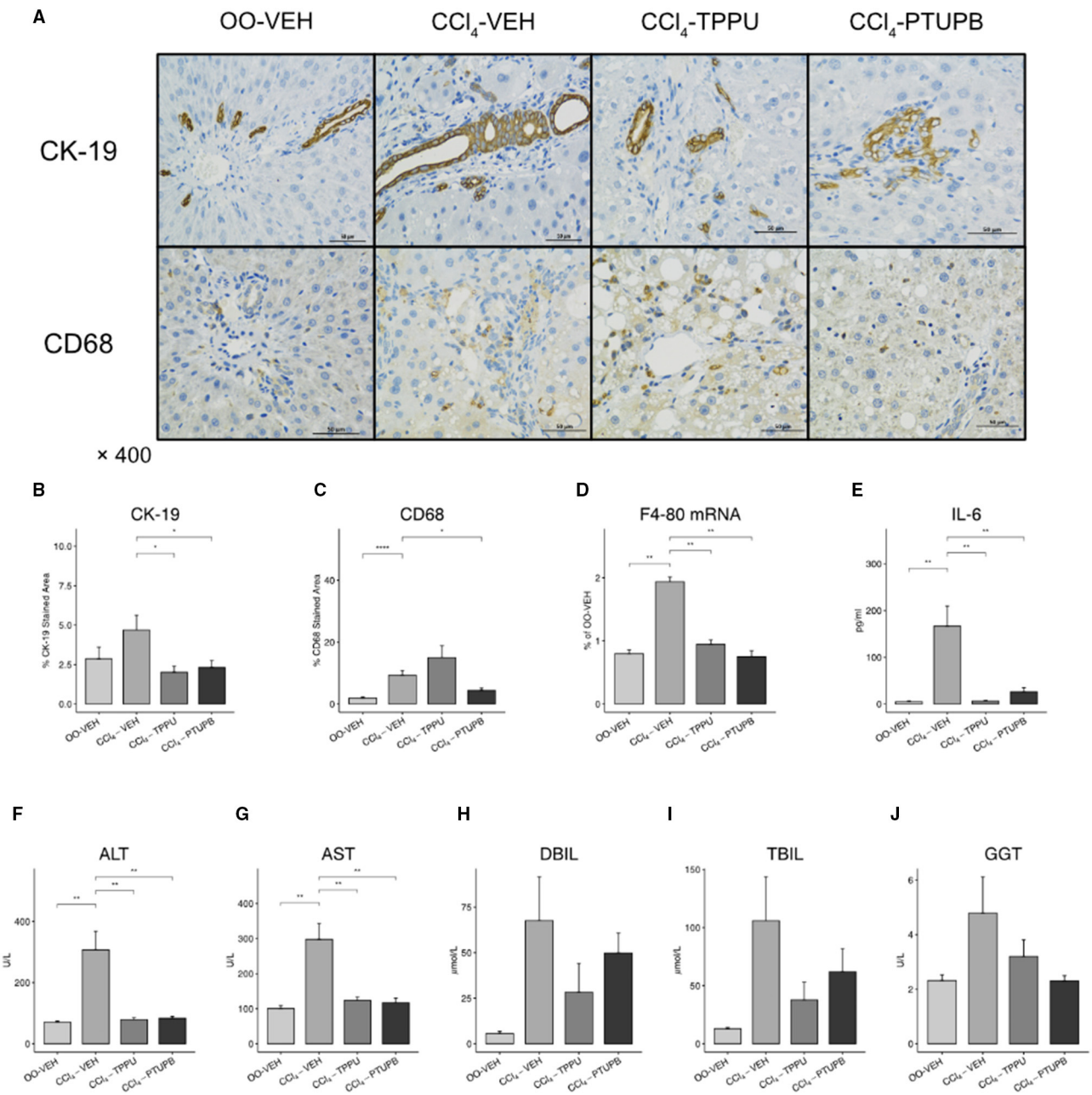


FIGURE 3 | PTUPB inhibits hepatic inflammation and bile duct cell proliferation. **(A)** Immunohistochemistry (IHC) on liver tissue with anti-CK-19 and anti-CD68 antibody. Quantification of IHC staining analyzed by IHC Profiler indicated significant decrease of CK-19 **(B)** and CD68 **(C)** in liver tissue. Furthermore, levels of hepatic expression of F4-80 **(D)**, serum IL-6 **(E)**, ALT **(F)**, AST**(G)**, DBIL **(H)**, TBIL **(I)**, and GGT **(J)** were measured. F4-80, IL-6, ALT, and AST were decreased significantly after PTUPB treatment. (*p <0.05, **p <0.01, ****p <0.0001 vs. CCl_4-VEH using Student's t-test, data are represented as mean with SD).

PTUPB Improves Vascular Remodeling in Mesenteric Artery

In CCl_4-induced PHT rats, the lumen wall of SMA became thinner while its vascular pattern became disrupted (**Figures 5A,B**). However, the lumen diameter stayed unchanged in CCl_4-VEH group (**Figure 5C**) despite the wall thickness or lumen diameter ratio still reduced remarkably (**Figure 5D**). Following the treatment of PTUPB and TPPU, SMA restored to normal state with thicker wall thickness and higher wall thickness or lumen diameter ratio (**Figures 5A,B,D**).

In addition, the expression of remodeling factors including MMP2 and VEGF in SMA also increased significantly in PHT

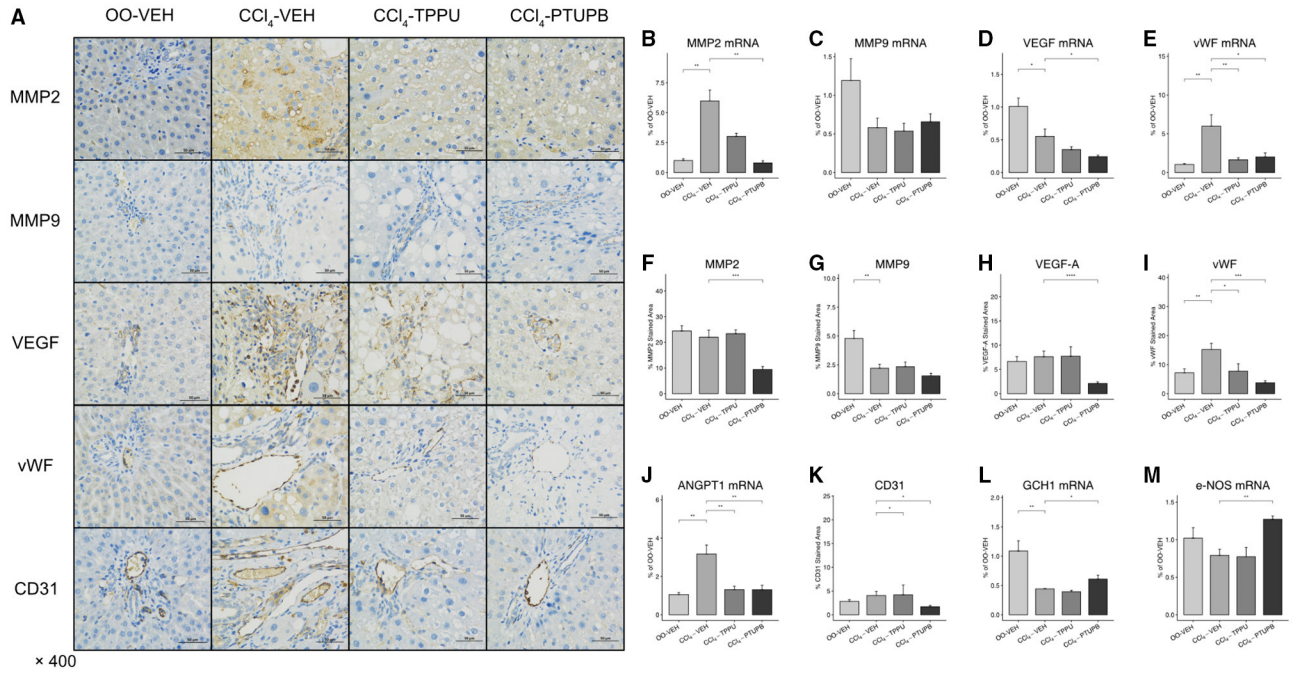


FIGURE 4 | PTUPB inhibits angiogenesis, sinusoidal remodeling, and vascular tone. **(A)** IHC staining for MMP-2, MMP-9, VEGF, vWF, and CD31 in liver tissue. After the PTUPB treatment, several gene expressions decreased including MMP2 **(B)**, VEGF **(D)**, vWF **(E)**, Angpt1 **(J)**, with two gene expressions increased consisted of GCH1**(L)** and eNOS **(M)**, whereas MMP9 **(C)** showed no significant difference. Quantification of IHC staining of MMP2 **(F)**, VEGF **(H)**, vWF **(I)**, and CD31 **(K)** also decreased in CCl_4-PTUPB group whereas MMP9 **(G)** showed no significant difference either. (*p <0.05, **p <0.01, ***p <0.001, ****p <0.0001 vs. CCl_4-VEH using Student's t-test, data are represented as mean with SD).

group (**Figures 6B,D**). After PTUPB treatment, vWF, VEGF, and CD68 decreased significantly (**Figures 6C–E**). As a contrast, only vWF reduced significantly in TPPU group (**Figure 6C**).

Molecular Mechanism of PTUPB in Alleviating Liver Fibrosis

A total of 4,679 genes related to liver fibrosis were predicted through the GeneCards database. Based on the pharmacophore model and the principle of structural similarity, a total of 366 PTUPB-related target genes were collected through the SwissTargetPrediction and the PharmMapper database. After intersecting and merging cirrhosis-related genes and PTUPB predicted targets, 230 overlapping targets were obtained as candidate genes, which were shown in the Venn diagram (**Figure 7A**). To clarify the interactions among common targets between liver fibrosis and PTUPB, a PPI network was constructed by String database and CytoHubba plugin of Cytoscape. Using MCC method, the hub genes with the highest DC values were identified based on three topological parameters (degree, betweenness, and closeness centrality). The top 10 hub genes included are MAPK1, CASP3, SRC, ALB, IGF1, EGFR, HSP90AA1, PTGS2, ESR1, and ANXA5 (**Figure 7B**).

Subsequently, enrichment analysis was conducted on common target genes. In GO analysis, the common targets mainly involved in regulation of inflammatory response, phosphatidylinositol 3-kinase signaling, response to reactive oxygen species, regulation of vasoconstriction, regulation of blood pressure, vasodilation, negative regulation of inflammatory response, tissue remodeling, nitric oxide metabolic process, regulation of tissue remodeling, nitric oxide synthase biosynthetic process, tube formation, VEGF receptor signaling pathway, macrophage chemotaxis, macrophage activation, regulation of fibroblast proliferation, fibroblast proliferation, collagen metabolic process, collagen catabolic process, and epoxygenase P450 pathway (**Figure 7C**).

Kyoto Encyclopedia of Genes and Genomes results suggested that the enrichment pathways included VEGF signaling pathway, tumor necrosis factor (TNF) signaling pathway, non-alcoholic fatty liver disease (NAFLD), lipid and atherosclerosis, hypoxia inducible factor (HIF)-1 signaling pathway, hepatitis B, drug metabolism—CYP450, chemokine signaling pathway, apoptosis, and ARA metabolism (**Figure 7D**).

As a dual COX-2/sEH inhibitor, we evaluated the inhibitory effect of PTUPB on COX-2/sEH pathways and proinflammatory cytokine TGF-β. In PHT rats, the hepatic expression of sEH, COX-2, and TGF-β increased significantly (**Figures 8B–D**). Besides, sEH staining in SMA also increased in PHT rats (A). Comparable results were found in western blot analysis (**Figure 8F**). After treatment with PTUPB, the expression of sEH, COX-2, and TGF-β in the liver decreased significantly (**Figures 8B,C**). Similar results were also seen in immunohistochemical analysis and western blot

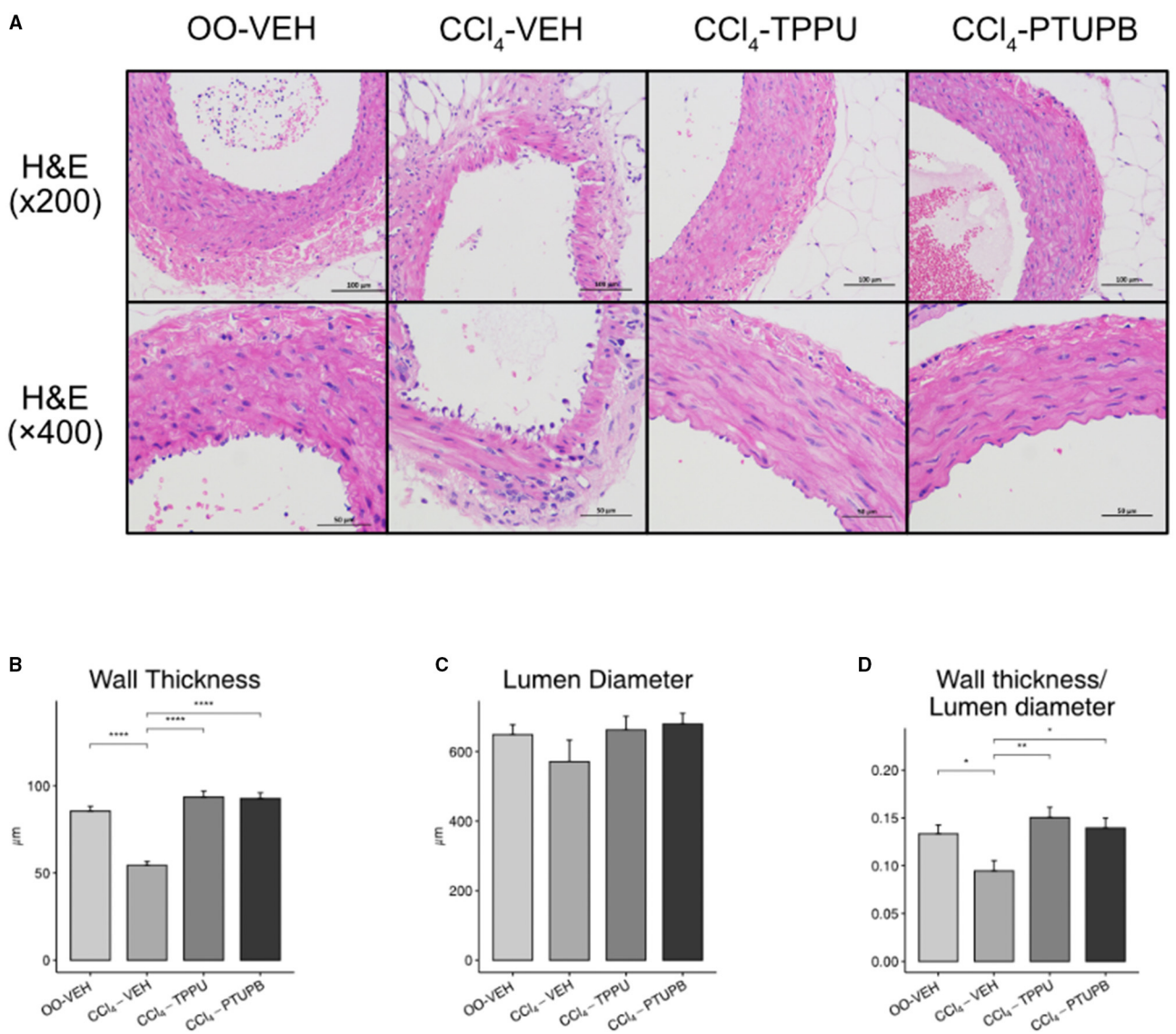


FIGURE 5 | PTUPB ameliorates the vascular remodeling in SMA. **(A)** H&E staining of SMA with magnification of × 200 and × 400. **(B)** The vessel wall thickness decreased in CCl_4-VEH rats and restored significantly in CCl_4-PTUPB group. **(C)** No significant difference was detected among the lumen diameters of SMA. **(D)** Similar to wall thickness, the ratio of wall thickness or lumen diameter deceased in CCl_4-VEH rats and restored significantly in CCl_4-PTUPB group. (*p <0.05, **p <0.01, ****p <0.0001 vs. CCl_4-VEH using Student's t-test, data are represented as mean with SD).

(**Figures 8A,E,F**). However, in the TPPU treatment group, only sEH and TGF-β decreased significantly, whereas COX-2 remained unchanged (**Figures 8B–D,F**).

DISCUSSION

As a dual sEH/COX-2 inhibitor, the beneficial effect of PTUPB in liver fibrosis and PHT was examined for the first time in our study. Several pharmacological effects of PTUPB were validated including antifibrosis, PP-lowering effect, antiinflammation, antiangiogenesis, sinusoidal vasodilation, and ameliorating vascular remodeling in sinusoids and SMA. The enrichment analysis indicated that PTUPB engaged in multiple biological functions related to liver fibrosis, including vasoconstriction, nitric oxide metabolic process, angiogenesis, blood pressure, tissue remodeling, immune inflammation, macrophage activation, fibroblast proliferation, and also collagen metabolism and CYP450 enzyme. The corresponding signaling pathways included VEGF, TNF, HIF, NAFLD, CYP450, hepatitis, etc. The inhibitory effects of PTUPB on sEH, COX-2, and TGF-β were also observed in our study. In summary, PTUPB has a substantial impact on liver fibrosis and PHT by suppressing inflammation, fibrosis, angiogenesis, and vascular remodeling.

In the treatment of cirrhosis, it is important to control tissue inflammation, fibrosis, and also vascular remodeling. As a critical

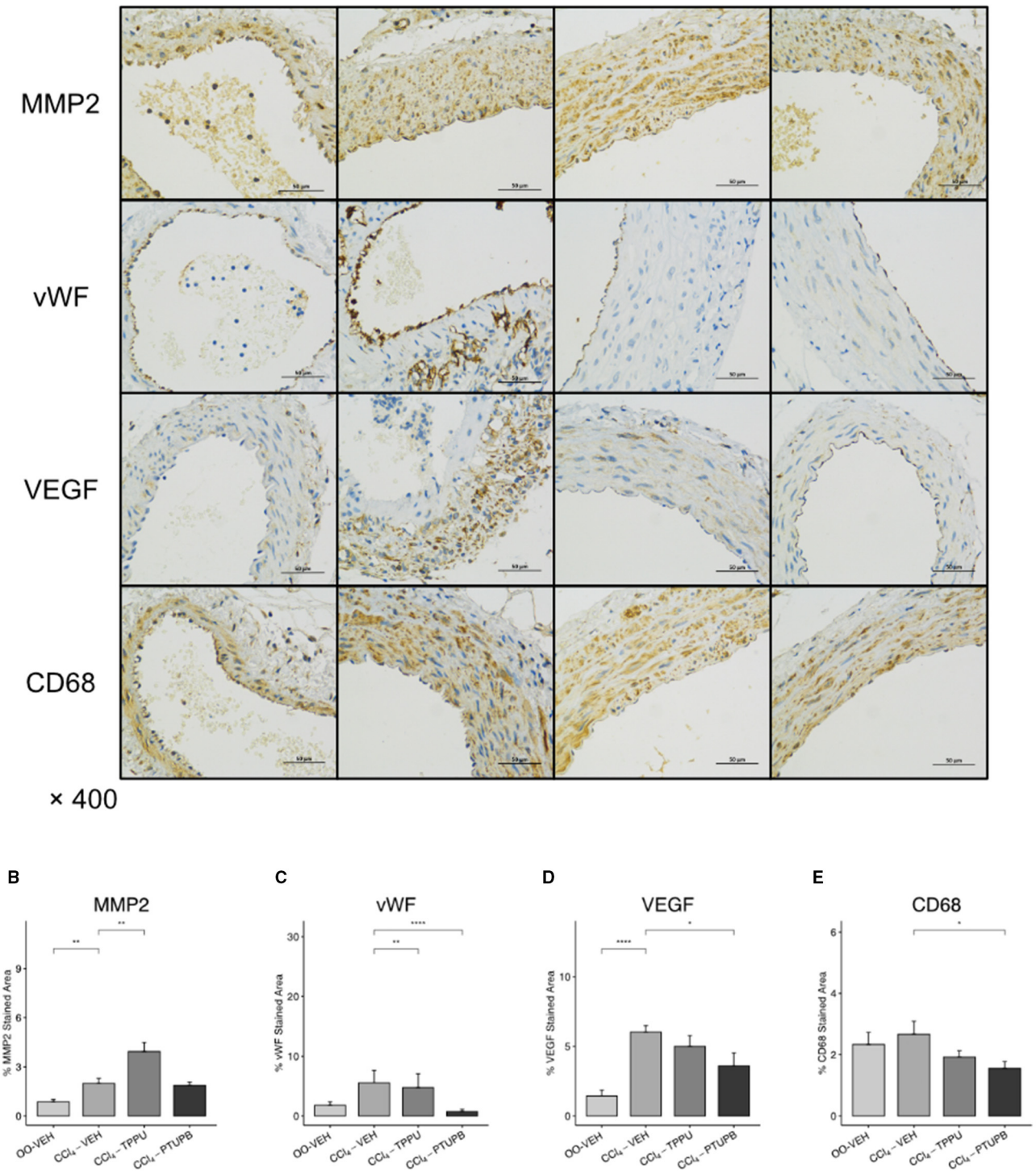


FIGURE 6 | PTUPB inhibits vascular remodeling and inflammation in SMA. **(A)** IHC staining for MMP2, vWF, VEGF, and CD68 in SMA. Quantification of IHC staining of vWF **(C)**, VEGF **(D)**, and CD68 **(E)** decreased in CCl_4-PTUPB group whereasMMP2 **(B)** showed no significant difference. (*p <0.05, **p <0.01, ****p <0.0001 vs. CCl_4-VEH using Student's t-test, data are represented as mean with SD).

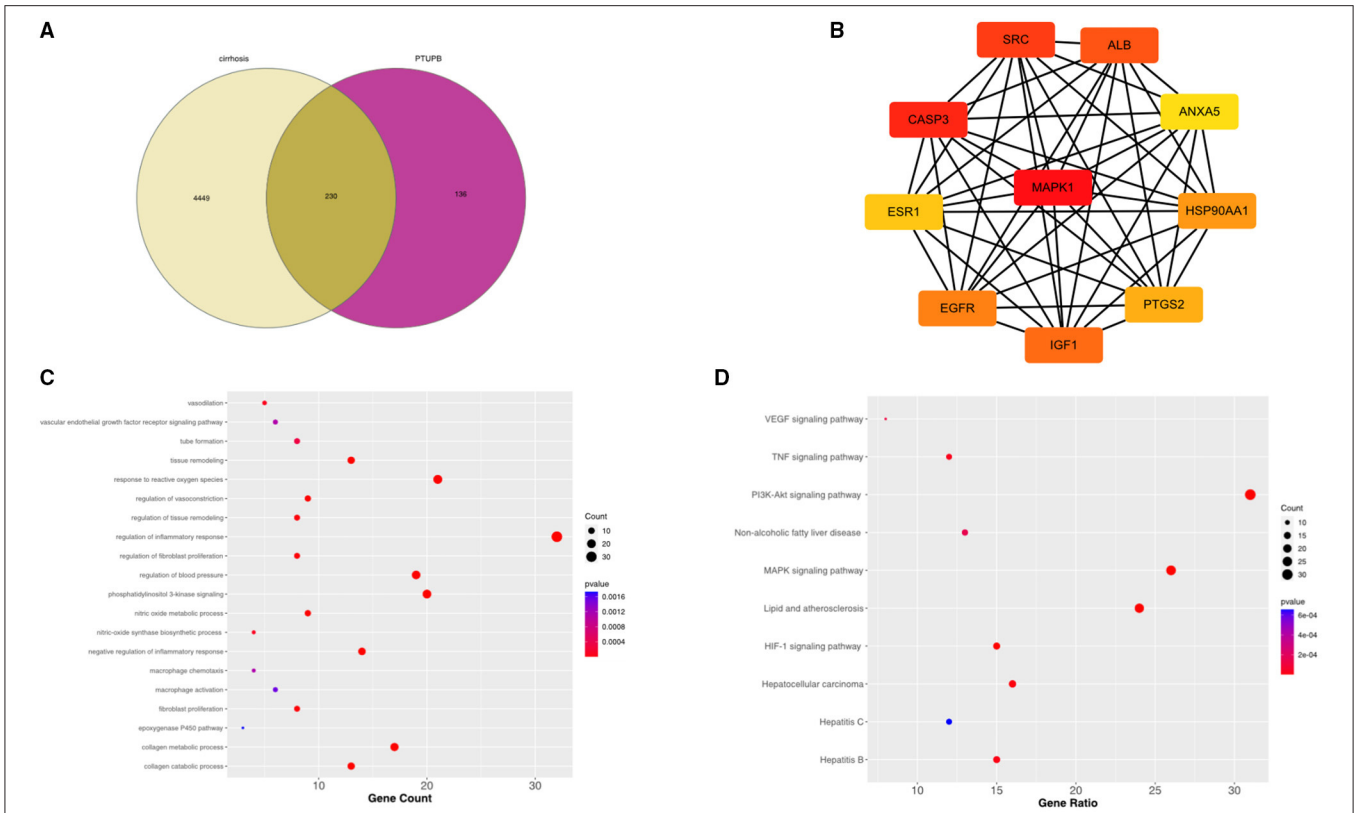


FIGURE 7 | Intersected targets between PTUPB and liver fibrosis and enrichment analysis. **(A)** The intersection of liver cirrhosis (yellow) and PTUPB targets (red). **(B)** Top ten genes among the common target genes determined by degree centrality. **(C)** Top 20 biological processes from GO enrichment. The *x*-axis represented the gene counts, *y*-axis indicated the different GO terms, and the color of dots represented the *p*-values. **(D)** Top 10 enriched KEGG signaling pathways. The *x*-axis represented the gene ratio, which indicated the number of genes enriched in one pathway compared with the total genes, *y*-axis indicated the different terms, and the color of dots represented the *p*-values.

component of the healing response to liver injury, inflammation is closely associated with the development of liver fibrosis. In this process, macrophage activation stimulates fibrosis by secreting a variety of proinflammatory cytokines such as IL-6 (21), which could further damage hepatocytes and exacerbate liver fibrosis (22). Our findings confirmed that IL-6 production, macrophage infiltration, and the number of bile ducts increased in liver fibrosis. Additionally, all those inflammation responses were alleviated effectively by PTUPB therapy.

The progression of liver fibrosis and inflammation causes intrahepatic resistance, hyperdynamic circulation, which leads to PHT eventually. In this process, the impact on hemodynamic parameters is divided into intra- and extrahepatic aspects. Similar to previous studies (23), multiple intrahepatic pathological alterations were observed in PHT including angiogenesis, vascular remodeling, and sinusoidal dysfunction. Our research revealed that PTUPB had a positive effect on hemodynamic parameters both structurally and functionally. Following PTUPB therapy, angiogenic and sinusoidal remodeling factors were found to be downregulated which include VEGF, vWF, CD31, and MMP2, whereas regulators of vascular tone such as GCH1 and eNOS were shown to be increased. According to previous study, VEGF, vWF, and MMP2 are major regulators of angiogenesis and vascular remodeling (24–26). CD31 is widely used to assess proliferation of endothelial cells and angiogenesis (27). GCH1 is a pivotal enzyme in the synthesis of eNOS. Both GCH1 and eNOS are important regulators of vascular tone (28).

Along with intrahepatic vascular in PHT, extrahepatic vascular undergoes structural alterations as well, including collateral angiogenesis, arterial vessel wall remodeling, etc. (2). A previous research indicated that cirrhotic PHT rats had a decrease in the wall thickness and total wall area of abdominal aorta (29), which was verified in SMA in our study. Our results showed that PTUPB treatment enhanced the thickness of the SMA wall, improved vascular remodeling, and decreased inflammatory markers including vWF, VEGF, and CD68. Furthermore, PTUPB decreased PPs in PHT rats as well. In conclusion, PTUPB had a favorable regulating effect on both intrahepatic and extrahepatic angiogenesis and vascular remodeling in cirrhotic PHT, which may explain its PP-lowering effect.

As previously stated, the ARA pathway plays a critical role in liver fibrosis, which was formerly characterized by two distinct pathways: COX-2 and sEH. COX-2 inhibitors

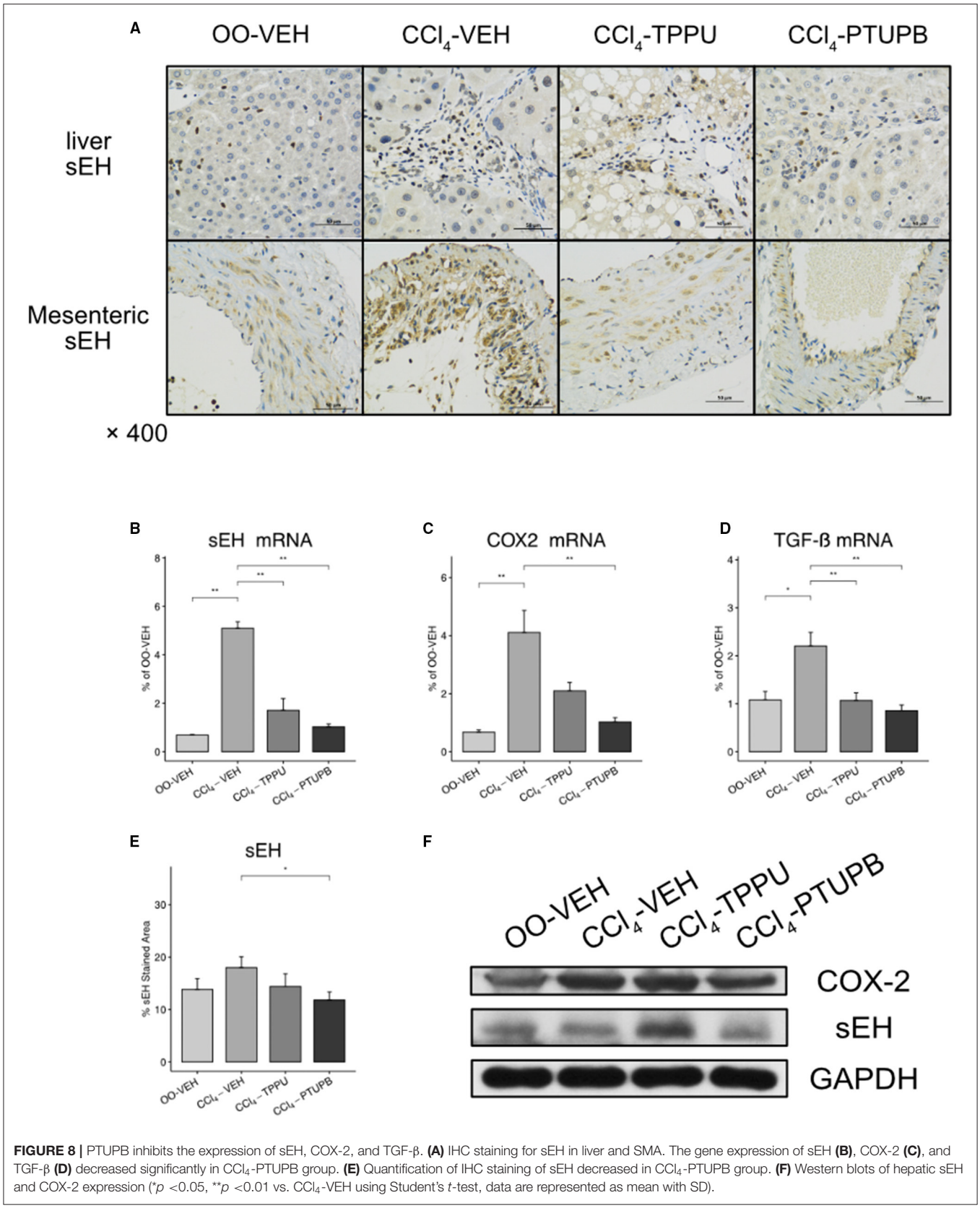


FIGURE 8 | PTUPB inhibits the expression of sEH, COX-2, and TGF-β. **(A)** IHC staining for sEH in liver and SMA. The gene expression of sEH **(B)**, COX-2 **(C)**, and TGF-β **(D)** decreased significantly in CCl_4-PTUPB group. **(E)** Quantification of IHC staining of sEH decreased in CCl_4-PTUPB group. **(F)** Western blots of hepatic sEH and COX-2 expression (*p <0.05, **p <0.01 vs. CCl_4-VEH using Student's t-test, data are represented as mean with SD).

such as SC-236 and meloxicam were shown to attenuate the development of liver fibrosis through cell apoptosis and TGF-β1 pathway, respectively (30, 31). However, Harris et al. (11) investigated the COX-2-selective inhibitor Celebrex in liver fibrosis and found no meaningful effect. Considering the mice model used in this study, COX-2 may have varying impacts on different stages of cirrhosis. Besides the COX-2, our group also found that sEH inhibition by t-TUCB increased eNOS levels, reduced inflammation, and alleviated cirrhotic PHT (32, 33). Furthermore, sEH-related pathways are involved in endothelial function, hypertension, and oxidative stress (6, 34, 35). As a key profibrotic factor, TGF-β is also engaged in sEH and COX-2 pathways (31, 36). This is consistent with our data, in which COX-2, she, and TGF-β were upregulated in liver fibrosis and decreased after the PTUPB treatment.

As a dual sEH/COX-2 inhibitor, PTUPB has been widely investigated in a variety of illnesses. Zhang et al. (9) discovered that PTUPB may decrease collagen deposition and ameliorate bleomycin-induced lung fibrosis through cellular senescence. Hye et al. (10) found that PTUPB can effectively alleviate renal injury, inflammation, and fibrosis in kidney injury models. In chronic liver disease, Sun et al. (37) found that PTUPB significantly reduced liver fibrotic deposition and inflammation in NAFLD mice induced by high-fat diet. These protective effects are mainly mediated through lipid metabolism, NLRP3 inflammasome, and steatosis. Furthermore, Harris et al. (11) revealed that PTUPB had a certain alleviating impact on liver fibrosis and inflammation in liver injury within CCl_4 injection for 5 weeks. However, the effect of PTUPB on advanced liver cirrhosis, intra- or extrahepatic angiogenesis, and vascular remodeling, and also PHT, still remains unknown. Here, our study confirmed its therapeutic effect using cirrhotic rat PHT model through CCl4 injection for 16 weeks.

The limitations of the study stemmed mostly from its phenotypic verification rather than in-depth research of its molecular mechanism. In future, further experiments *in vitro* will be required to establish PTUPB's cellular targets and validate the pathways. In terms of hemodynamics, we assessed the blood pressure but were unable to measure the hepatic blood flow due to equipment and experimental technical constraints. Additionally, CCl_4-induced liver cirrhosis is an artificial model that may be challenging to adapt to human disease prediction.

CONCLUSION

In conclusion, our findings showed that PTUPB had a significant protective effect on liver fibrosis and PHT by inhibiting hepatic fibrotic deposition and inflammation, suppressing angiogenesis and vascular remodeling in sinusoids and SMA, and inducing sinusoidal vasodilation. The mechanism may be mediated *via* the downregulation of the sEH/COX-2/TGF-β. Thus, PTUPB represents a promising approach in the treatment of cirrhosis-related PHT.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethical Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine.

AUTHOR CONTRIBUTIONS

ZZ and CZ were involved in the plan of program and drafted the manuscript. JL and LZ participated in data collection and analysis. HL and XQ performed the experiment. BH, HH, and XL provided reagents or materials or analysis tools. SH and BH designed and synthesized PTUPB. YB, ZZ, and ML participated in drafting or revising the work. All authors have given the final approval of the version to be published and accountable for all aspects of the manuscript.

FUNDING

This study is partially supported by NIH-NIEHS (RIVER Award) R35 ES030443-01, NIH-NIEHS (Superfund Award) P42 ES004699 (both to BH) and National Natural Science Fund of China (Project No. 82100639) (to LZ), (Project No. 81970526, 81770599) (to ML), (Project No. 81900550) (to HL).

ACKNOWLEDGMENTS

We are thankful to Manghua Xu, Tao Qin, Jian Li, and Dengyu Wang for providing excellent research condition.

REFERENCES

1. Borkham-Kamphorst E, Weiskirchen R. The PDGF system and its antagonists in liver fibrosis. *Cytokine Growth Factor Rev.* (2016) 28:53–61. doi: 10.1016/j.cytogfr.2015.10.002
2. Sacerdoti D, Pesce P, Di Pascoli M, Brocco S, Cecchetto L, Bolognesi M. Arachidonic acid metabolites and endothelial dysfunction of portal hypertension. *Prostaglandins Other Lipid Mediat.* (2015) 120:80–90. doi: 10.1016/j.prostaglandins.2015.05.008
3. Mohammed NA, Abd El-Aleem SA, El-Hafiz HA, McMahon RF, El-Aleem SA. Distribution of constitutive (COX-1) and inducible (COX-2) cyclooxygenase in postviral human liver cirrhosis: a possible role for COX-2 in the pathogenesis of liver cirrhosis. *J Clin Pathol.* (2004) 57:350–4. doi: 10.1136/jcp.2003.012120
4. Yang L, Ni L, Duan Q, Wang X, Chen C, Chen S, et al. CYP epoxygenase 2J2 prevents cardiac fibrosis by suppression of transmission of pro-inflammation from cardiomyocytes to macrophages. *Prostaglandins Other Lipid Mediat.* (2015) 116–117:64–75. doi: 10.1016/j.prostaglandins.2015.01.004
5. Imig JD. Epoxyeicosatrienoic Acids and 20-Hydroxyeicosatetraenoic Acid on Endothelial and Vascular Function. *Adv Pharmacol.* (2016) 77:105–41. doi: 10.1016/bs.apha.2016.04.003
6. Imig JD. Epoxyeicosanoids in hypertension. *Physiol Res.* (2019). doi: 10.33549/physiolres.934291

7. Panigrahy D, Kalish BT, Huang S, Bielenberg DR, Le HD, Yang J, et al. Epoxyeicosanoids promote organ and tissue regeneration. *Proc Natl Acad Sci USA*. (2013) 110:13528–33. doi: 10.1073/pnas.1311565110
8. Morisseau C, Hammock BD. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. *Annu Rev Pharmacol Toxicol.* (2013) 53:37–58. doi: 10.1146/annurev-pharmtox-011112-140244
9. Zhang C-Y, Duan J-X, Yang H-H, Sun C-C, Zhong W-J, Tao J-H, et al. COX-2/sEH dual inhibitor PTUPB alleviates bleomycin-induced pulmonary fibrosis in mice via inhibiting senescence. *FEBS J.* (2019) 287:1666–80. doi: 10.1111/febs.15105
10. Hye Khan MA, Hwang SH, Sharma A, Corbett JA, Hammock BD, Imig JD A. dual COX-2/sEH inhibitor improves the metabolic profile and reduces kidney injury in Zucker diabetic fatty rat. *Prostaglandins Other Lipid Mediat.* (2016) 125:40–7. doi: 10.1016/j.prostaglandins.2016.07.003
11. Harris TR, Kodani S, Rand AA, Yang J, Imai DM, Hwang SH, et al. Celecoxib does not protect against fibrosis and inflammation in a carbon tetrachloride-induced model of liver injury. *Mol Pharmacol.* (2018) 94:834–41. doi: 10.1124/mol.118.111831
12. Hwang SH, Wagner KM, Morisseau C, Liu J-Y, Dong H, Wecksler AT, et al. Synthesis and structure-activity relationship studies of urea-containing pyrazoles as dual inhibitors of cyclooxygenase-2 and soluble epoxide hydrolase. *J Med Chem.* (2011) 54:3037–50. doi: 10.1021/jm2001376
13. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.* (2019) 47:W357–64. doi: 10.1093/nar/gkz382
14. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. *Curr Protoc Bioinform*. (2016) 54:1.30.1–33. doi: 10.1002/cpbi.5
15. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* (2019) 47:D607–13. doi: 10.1093/nar/gky1131
16. Reimand J, Isserlin R, Voisin V, Kucera M, Tannus-Lopes C, Rostamianfar A, et al. Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. *Nat Protoc.* (2019) 14:482–517. doi: 10.1038/s41596-018-0103-9
17. Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol.* (2014) 8 Suppl 4:S11. doi: 10.1186/1752-0509-8-S4-S11
18. Wang X, Li H, Lu X, Wen C, Huo Z, Shi M, et al. Melittin-induced long non-coding RNA NONHSAT105177 inhibits proliferation and migration of pancreatic ductal adenocarcinoma. *Cell Death Dis*. (2018) 9:940. doi: 10.1038/s41419-018-0965-3
19. Da Huang W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* (2009) 37:1–13. doi: 10.1093/nar/gkn923
20. Da Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* (2009) 4:44–57. doi: 10.1038/nprot.2008.211
21. Popēna I, Ābols A, Saulite L, Pleiko K, Zandberga E, Jēkabsons K, et al. Effect of colorectal cancer-derived extracellular vesicles on the immunophenotype and cytokine secretion profile of monocytes and macrophages. *Cell Commun Signal.* (2018) 16:17. doi: 10.1186/s12964-018-0229-y
22. Jin X, Zimmers TA, Perez EA, Pierce RH, Zhang Z, Koniaris LG. Paradoxical effects of short- and long-term interleukin-6 exposure on liver injury and repair. *Hepatology.* (2006) 43:474–84. doi: 10.1002/hep.21087
23. Fernandez M. Molecular pathophysiology of portal hypertension. *Hepatology.* (2015) 61:1406–15. doi: 10.1002/hep.27343
24. Gana JC, Serrano CA, Ling SC. Angiogenesis and portal-systemic collaterals in portal hypertension. *Ann Hepatol.* (2016) 15:303–13. doi: 10.5604/16652681.1198799
25. Randi AM, Smith KE, Castaman G. von Willebrand factor regulation of blood vessel formation. *Blood.* (2018) 132:132–40. doi: 10.1182/blood-2018-01-769018
26. Barhoumi T, Fraulob-Aquino JC, Mian MO, Ouerd S, Idris-Khodja N, Huo K-G, et al. Matrix metalloproteinase-2 knockout prevents angiotensin II-induced vascular injury. *Cardiovasc Res.* (2017) 113:1753–62. doi: 10.1093/cvr/cvx115
27. Fernandez M, Mejias M, Angermayr B, Garcia-Pagan JC, Rodés J, Bosch J. Inhibition of VEGF receptor-2 decreases the development of hyperdynamic splanchnic circulation and portal-systemic collateral vessels in portal hypertensive rats. *J Hepatol.* (2005) 43:98–103. doi: 10.1016/j.jhep.2005.02.022
28. Antoniades C, Shirodaria C, van Assche T, Cunnington C, Tegeder I, Lötsch J, et al. GCH1 haplotype determines vascular and plasma biopterin availability in coronary artery disease effects on vascular superoxide production and endothelial function. *J Am Coll Cardiol.* (2008) 52:158–65. doi: 10.1016/j.jacc.2007.12.062
29. Fernández-Varo G, Ros J, Morales-Ruiz M, Cejudo-Martín P, Arroyo V, Solé M, et al. Nitric oxide synthase 3-dependent vascular remodeling and circulatory dysfunction in cirrhosis. *Am J Pathol.* (2003) 162:1985–93. doi: 10.1016/S0002-9440(10)64331-3
30. Planagumà A, Clària J, Miquel R, López-Parra M, Titos E, Masferrer JL, et al. The selective cyclooxygenase-2 inhibitor SC-236 reduces liver fibrosis by mechanisms involving non-parenchymal cell apoptosis and PPARgamma activation. *FASEB J.* (2005) 19:1120–2. doi: 10.1096/fj.04-2753fje
31. Kim SM, Park KC, Kim HG, Han SJ. Effect of selective cyclooxygenase-2 inhibitor meloxicam on liver fibrosis in rats with ligated common bile ducts. *Hepatol Res.* (2008) 38:800–9. doi: 10.1111/j.1872-034X.2008.00339.x
32. Deng W, Zhu Y, Lin J, Zheng L, Zhang C, Luo M. Inhibition of soluble epoxide hydrolase lowers portal hypertension in cirrhotic rats by ameliorating endothelial dysfunction and liver fibrosis. *Prostaglandins Other Lipid Mediat.* (2017) 131:67–74. doi: 10.1016/j.prostaglandins.2017.08.004
33. Zhang C, Zheng L, Gui L, Lin J, Zhu Y, Deng W, et al. Soluble epoxide hydrolase inhibition with t-TUCB alleviates liver fibrosis and portal pressure in carbon tetrachloride-induced cirrhosis in rats. *Clin Res Hepatol Gastroenterol.* (2018) 42:118–25. doi: 10.1016/j.clinre.2017.09.001
34. Fleming I. Epoxyeicosatrienoic acids, cell signaling and angiogenesis. *Prostaglandins Other Lipid Mediat.* (2007) 82:60–7. doi: 10.1016/j.prostaglandins.2006.05.003
35. Webler AC, Michaelis UR, Popp R, Barbosa-Sicard E, Murugan A, Falck JR, et al. Epoxyeicosatrienoic acids are part of the VEGF-activated signaling cascade leading to angiogenesis. *Am J Physiol Cell Physiol.* (2008) 295:C1292–301. doi: 10.1152/ajpcell.00230.2008
36. Zhou Y, Yang J, Sun G-Y, Liu T, Duan J-X, Zhou H-F, et al. Erratum to: Soluble epoxide hydrolase inhibitor 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea attenuates bleomycin-induced pulmonary fibrosis in mice. *Cell Tissue Res.* (2016) 364:675. doi: 10.1007/s00441-016-2379-9
37. Sun C-C, Zhang C-Y, Duan J-X, Guan X-X, Yang H-H, Jiang H-L, et al. PTUPB ameliorates high-fat diet-induced non-alcoholic fatty liver disease via inhibiting NLRP3 inflammasome activation in mice. *Biochem Bioph Res Co.* (2020) 523:1020–6. doi: 10.1016/j.bbrc.2019.12.131

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

CASE REPORT
published: 31 January 2022
doi: 10.3389/fmed.2021.818825

Successful Simultaneous Subtotal Splenectomy During Left Lobe Auxiliary Liver Transplantation for Portal Inflow Modulation and Severe Hypersplenism Correction: A Case Report

Guang-Peng Zhou[1,2†], *Wei Qu*[1,2], *Zhi-Gui Zeng*[1,2], *Li-Ying Sun*[1,2,3], *Ying Liu*[1,2,3], *Lin Wei*[1,2*†] *and Zhi-Jun Zhu*[1,2*†]

[1] *Liver Transplantation Center, National Clinical Research Center for Digestive Diseases, Beijing Friendship Hospital, Capital Medical University, Beijing, China,* [2] *Clinical Center for Pediatric Liver Transplantation, Capital Medical University, Beijing, China,* [3] *Department of Critical Liver Diseases, Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing, China*

Adult-to-adult living donor liver transplantation with small partial liver grafts often requires intraoperative portal inflow modulation to prevent portal hyperperfusion and subsequent small-for-size syndrome (SFSS). However, there are concerns about the specific morbidity of these modulation techniques. This study aims to lower post-perfusion portal venous pressure and correct severe hypersplenism in a patient with end-stage liver cirrhosis by simultaneous subtotal splenectomy during auxiliary partial orthotopic liver transplantation (APOLT). A 29-year-old man was diagnosed with cryptogenic cirrhosis and severe portal hypertension suffered recurrent acute variceal bleeding, severe thrombocytopenia, and massive ascites before admission to our hospital. After the recipient's left liver was resected, we performed APOLT using his 51-year-old father's left lobe graft with a graft-to-recipient weight ratio of 0.55%. Intraoperatively, simultaneous subtotal splenectomy was performed to lower graft post-perfusion portal vein pressure below 15 mmHg and correct severe hypersplenism-related pancytopenia. The recipient's postoperative hospital course was uneventful with no occurrence of SFSS and procedure-related complications. Platelet and leukocyte counts remained in the normal ranges postoperatively. The living donor was discharged 6 days after the operation and recovered well-with no complications. After a follow-up period of 35.3 months, both the recipient and donor live with good liver function and overall condition. This is the first case report of simultaneous subtotal splenectomy during APOLT using small-for-size living-donated left liver lobes, which is demonstrated to be a viable procedure for modulating portal inflow and correcting severe hypersplenism in selected adult patients with end-stage liver cirrhosis. APOLT using a small-for-size liver graft may be a safe and feasible treatment option for selected adult patients with end-stage liver cirrhosis.

Keywords: subtotal splenectomy, auxiliary liver transplantation, portal inflow modulation, hypersplenism, thrombocytopenia

OPEN ACCESS

Edited by:
Jonel Trebicka,
Goethe University Frankfurt, Germany

Reviewed by:
Michael Praktiknjo,
University Hospital Bonn, Germany
Maximilian Joseph Brol,
University Hospital Frankfurt, Germany

***Correspondence:**
Zhi-Jun Zhu
zhu-zhijun@outlook.com
Lin Wei
weilin@ccmu.edu.cn

†ORCID:
Guang-Peng Zhou
orcid.org/0000-0002-3063-4528
Lin Wei
orcid.org/0000-0002-0435-3829
Zhi-Jun Zhu
orcid.org/0000-0001-7031-2083

Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *20 November 2021*
Accepted: *28 December 2021*
Published: *31 January 2022*

Citation:
Zhou G-P, Qu W, Zeng Z-G, Sun L-Y, Liu Y, Wei L and Zhu Z-J (2022) Successful Simultaneous Subtotal Splenectomy During Left Lobe Auxiliary Liver Transplantation for Portal Inflow Modulation and Severe Hypersplenism Correction: A Case Report. Front. Med. 8:818825. doi: 10.3389/fmed.2021.818825

INTRODUCTION

Due to a critical shortage of donor organs, living donor liver transplantation (LDLT) has been increasingly used in adult recipients. In general, a graft ≥0.8% of the recipient's body weight or 30–40% of standard liver volume (SLV) is necessary to meet the recipient's functional demands, whereas procuring such a graft from a living donor may subject a healthy individual to a significantly high operative risk (1). To ensure donor safety and expand the donor pool, small-for-size grafts (SFSGs), defined as grafts with a graft-to-recipient weight ratio (GRWR) <0.8%, are being widely used in clinical practice. However, SFSGs are thought to be the leading cause of small-for-size syndrome (SFSS) associated with poorer prognosis (1, 2).

Auxiliary partial orthotopic liver transplantation (APOLT), which is implanting a normal partial liver allograft while preserving part of the native liver, has emerged as an attractive alternative to liver transplantation (LT) for the treatment of selected patients (3, 4). The implanted partial liver graft and residual partial native liver will co-function and support each other, which can balance the risk of donors and the need of recipients. In fulminant liver failure, the auxiliary liver graft is implanted to temporarily provide emergent support for the loss of native liver function, offering a chance to the potential spontaneous recovery of the native liver, and then allowing for possible progression to immunosuppression withdrawal (5, 6). In non-cirrhotic inherited metabolic liver disease, the implanted small graft can compensate for the recipient's enzyme deficiency without the complete removal of the native liver. The implanted partial liver graft and the residual non-cirrhotic native liver can permanently cooperate to maintain normal liver function (7, 8). In chronic cirrhotic liver disease, the residual native liver with relatively preserved liver function can temporarily support the implanted small volume graft during the immediate postoperative period until sufficient regeneration (3, 9–11). Recently, Brunner et al. reported two successful cases of auxiliary two-staged partial resection LT using living-donor left lobes (GRWR 0.65 and 0.43%, respectively) for end-stage liver cirrhosis to prevent SFSS (12). Together, APOLT using a small volume liver graft may be a feasible therapeutic option for adult patients with end-stage liver cirrhosis.

Post-transplant portal hyperperfusion is believed to be a primary contributor to the pathophysiology of SFSS. In this setting, intraoperative portal inflow modulation techniques, including splenectomy, splenic artery ligation, and portosystemic shunts, can be used to optimize the post-perfusion portal vein pressure (PVP) and thus prevent SFSS-related graft loss (13, 14). Yoshizumi et al. found that simultaneous total splenectomy should be recommended for patients with severe portal hypertension or high portal pressure (>20 mmHg) after reperfusion in adult LDLT (15). Thrombocytopenia in the immediate postoperative period after LDLT is a common phenomenon in patients with end-stage liver cirrhosis (16). Prolonged thrombocytopenia can expose liver transplant recipients to a high risk of severe and even life-threatening hemorrhagic complications, thereby complicating the postoperative clinical course and even resulting in increased morbidity and mortality (16, 17). A recent study by Pamecha et al. showed that postoperative severe thrombocytopenia was associated with a high risk of early graft dysfunction, prolonged ascites drainage, and sepsis in LDLT recipients (17). It has been previously reported that simultaneous splenectomy has a beneficial role in adult LDLT to correct hypersplenism-related pancytopenia (18). However, splenectomy-related lethal complications, including hemorrhage from the splenectomy bed, thromboembolic and septic complications, pancreatic fistula and/or abscess, might overshadow the benefits of splenectomy (15, 19, 20).

The present case describes an adult patient diagnosed with cryptogenic cirrhosis and severe portal hypertension who underwent APOLT using a small volume living-donated left liver lobe. Simultaneous subtotal splenectomy was performed intraoperatively to lower graft PVP and correct severe hypersplenism-related pancytopenia.

CASE REPORT

A 29-year-old man was admitted to our hospital because of recurrent variceal bleeding. One year ago, he presented with sudden melena and was diagnosed with cryptogenic cirrhosis and severe portal hypertension at the local hospital. Since then, he had experienced seven episodes of acute variceal bleeding despite multiple endoscopic therapies (including endoscopic injection sclerotherapy and endoscopic variceal ligation) and regular administration of non-selective beta-blockers (carvedilol). Two months ago, he suffered from a hemorrhagic shock caused by gastrointestinal bleeding. On this admission, he had melena and his blood routine examination revealed moderate anemia (hemoglobin 75 g/l) and severe hypersplenism (platelet count 13,000/mm^3 and white blood cell count 600/mm^3). Serum biochemical examinations showed slightly elevated total bilirubin (2.0 mg/dl) and conjugated bilirubin (0.57 mg/dl), normal aspartate aminotransferase (26.6 IU/l) and alanine aminotransferase (6 IU/l), normal serum creatinine (67 μmol/l) and electrolytes (sodium 139.8 mmol/l, potassium 4.16 mmol/l, chloride 112 mmol/l) and low albumin level (2.91 g/dl). The blood ammonia level was 33 μmol/l, and the patient did not experience any episodes of hepatic encephalopathy previously. His international normalized ratio (INR) was slightly prolonged (1.49). Abdominal computed tomography (CT) showed atrophic liver, severely dilated gastroesophageal varices and short gastric veins, umbilical vein recanalization, splenomegaly, massive volume of free peritoneal fluid, and small volume of bilateral pleural effusion. The Child–Pugh classification was B (score 9), and the Model for End-Stage Liver Disease (MELD) score was 12. After a discussion between the patient and his

Abbreviations: SFSS, small-for-size syndrome; APOLT, auxiliary partial orthotopic liver transplantation; LDLT, living donor liver transplantation; LT, liver transplantation; SLV, standard liver volume; SFSG, small-for-size graft; GRWR, graft-to-recipient weight ratio; PVP, portal vein pressure; GV, graft volume; POD, postoperative day; INR, international normalized ratio; CT, computed tomography; MELD, Model for End-Stage Liver Disease; GSVR, graft-to-spleen volume ratio.

parents and the multidisciplinary team, including radiologists, hepatologists, and liver transplant surgeons, the patient refused to undergo partial splenic embolization, surgical shunt or transjugular intrahepatic portosystemic shunt because the patient thought that abovementioned procedures were just temporary therapeutic options but not a cure, with the potential high risk of procedure-related complications including portal vein thrombosis, substantial infectious risk, and occurrence of hepatic encephalopathy, which may adversely influence his quality of life and the subsequent chance of LT. On the other hand, the indication for LT in this patient was relatively clear, namely decompensated liver cirrhosis, including portal hypertension, recurrent variceal hemorrhage, splenomegaly, hypersplenism, and moderate to severe ascites. In such a situation, considering the patient's own wish regarding the LT and the donor's safety being the priority during LDLT, as well as our transplant team's previous extensive experience with APOLT, and thus APOLT using a small-for-size living-donated left liver lobe was considered for this patient.

The patient's 51-year-old father was evaluated to be a suitable living donor. Considering the advanced age of the donor, we planned procurement of the left lobe liver to ensure the donor's safety. Using the three-dimensional imaging reconstruction analysis system, the estimated left lobe graft volume was 476 ml, representing an estimated GRWR of 0.65%, and graft volume (GV)/SLV was 36.71%. Thus, APOLT using a left lobe liver graft was designed to decrease the morbidity of SFSS in the recipient. Given the presence of severe portal hypertension, hypersplenism, and massive splenomegaly, simultaneous subtotal splenectomy was planned, which served as a portal inflow modulation strategy and treatment for severe hypersplenism. The calculated volume of the whole spleen and the designed preserved upper pole of the spleen were 1,445 and 503 ml, respectively (**Figure 1a**).

The donor underwent a left hemihepatectomy with preservation of the middle hepatic vein, and the intraoperative course was uncomplicated. The actual graft weight was 405 g, GRWR was 0.55%, and GV/SLV was 31.23%. The recipient underwent APOLT with preservation of the native right lobe liver (**Figure 1b**). Before hepatectomy, PVP was measured with a needle inserted directly in the portal vein trunk; the value of which was 26 mmHg. The graft was implanted using a modified piggyback technique with an end-to-end anastomosis between the left hepatic vein of the donor graft and the common left/middle hepatic vein orifice of the recipient. The end-to-end portal anastomosis was performed between the graft's left portal vein and the recipient's left portal vein stump. The graft's left hepatic artery was anastomosed to the recipient's left hepatic artery (**Figure 1c**). Graft PVP measurement was 19 mmHg after reperfusion and decreased to 12 mmHg upon temporary clamping of the splenic artery trunk. Subtotal splenectomy was performed by ligating the corresponding segmental arteries and veins of the lower two-thirds of the spleen, as previously described (21). About 70% of the splenic volume was resected (**Figure 1d**), and the final graft PVP was 14 mmHg. Biliary anastomosis was performed between the graft left bile duct and the recipient left bile duct (**Figure 1e**). The warm and cold ischemia time of the graft was 23 and 276 min, respectively. The total operating time was 770 min (the time of subtotal splenectomy was about 39 min). Intraoperatively, the patient remained hemodynamically stable without unexpected complications. The intraoperative blood loss was ∼750 ml.

Based on our and other centers' experience, vaccination against pneumococci and any other encapsulated bacteria was not performed before or after transplantation (18, 21). To prevent vascular thrombosis, unfractionated heparin sodium (10,000 U/day) was started from the postoperative day (POD) 3–7. Then anticoagulation using warfarin was added to maintain INR 1.5–2.0 until postoperative month 3 (**Figure 2**). Postoperative abdominal Doppler ultrasonography was performed to examine flow in the graft vessels at least once daily until POD 7 and once every 2 days between POD 8 and 14. Abdominal dynamic contrast-enhanced CT was performed if ultrasound abnormalities were noticed. Postoperative immunosuppressive therapy consisted of methylprednisolone, mycophenolate mofetil, and tacrolimus. Pathological analysis of the excised liver specimen suggested cirrhosis.

Apart from massive ascites production, the postoperative hospital course was uneventful, and graft function recovered rapidly (**Figure 2**). It took 6 weeks to achieve disappearance of abdominal drainage, and then the drainage tube was removed. The platelet and leukocyte counts increased immediately during the perioperative period and remained in the normal ranges during long-term follow-up (**Figure 2**). There was no occurrence of splenectomy-related complications such as bleeding from splenectomy bed, portal venous thrombosis, pancreatic fistula and abscess, or septic complications. The recipient was discharged from the hospital on day 43 post-transplant. He was followed up regularly according to his clinical and laboratory data. The anatomical and functional volume of the implanted liver graft and residual native liver were evaluated using a dynamic contrast-enhanced CT scan and 99 mTc-GSA scintigraphy. The results showed gradually increased volume and enhanced function of the liver graft (**Figure 3**). The volume of the remnant spleen was also monitored, indicating no occurrence of splenic regrowth (**Figure 3A**). Moreover, there were no procedure-related complications throughout the follow-up period. To date (35.3 months post-transplant), the patient's graft function is good, and peripheral platelet and leukocyte counts remain in the normal ranges (**Figure 2**). Meanwhile, the living donor recovered well-with no postoperative complications. He was discharged in good condition 6 days after the operation, and the clinical course after discharge was uneventful. This study was conducted in accordance with the Declaration of Helsinki. It was approved by the Ethical Committee of Beijing Friendship Hospital, Capital Medical University (No. 2018-P2-127-01). The patient has signed informed consent.

DISCUSSION

With a critical shortage of donated organs and increased mortality for those on the waiting list, living donors have become an increasingly accepted potential alternative source of organs for adult patients with end-stage liver disease. Due

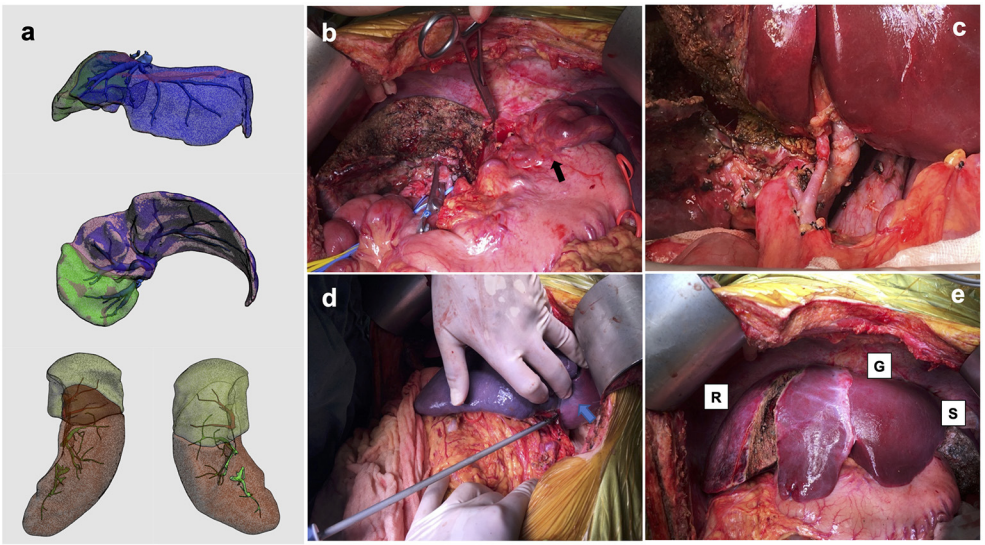


FIGURE 1 | Estimated hepatic and splenic volumes of the recipient and photographs of the operating field. **(a)** Calculated hepatic volume was 905 ml (volume of planned preserved right hemi-liver was 270 ml), and splenic volume was 1,445 ml (volume of designed preserved upper pole was 503 ml; **(b)** remnant right hemi-liver and large varicose veins (black arrow); **(c)** hepatic artery and portal vein to auxiliary partial graft; **(d)** ischemic demarcation line on spleen (blue arrow) during simultaneous subtotal splenectomy; **(e)** native remnant liver (R), partial liver graft (G) and residual spleen (S).

to the assurance of donor's safety being the priority in LDLT, SFSGs have been used to minimize risks to donors in adult-to-adult LDLT. However, SFSGs are generally considered unsafe in terms of the risk of SFSS (22). Notably, the occurrence of SFSS is not only attributed to graft size mismatch but also likely to be associated with other factors, including graft quality (donor age and steatosis), recipient conditions (portal hypertension and severity of original liver disease), and technical issues (venous reconstruction) (23, 24). In the present study, insufficient graft parenchyma, advanced donor age, and severe portal hypertension may have induced excessive portal blood flow through the small-volume graft, leading to a high risk of postoperative SFSS and early allograft dysfunction. APOLT, which was initially explored for potentially reversible fulminant liver failure (temporary support by the auxiliary graft), has been described in patients with liver-based metabolic diseases, chronic liver disease and small liver grafts (temporary or permanent support by the native liver remnant) (3, 4, 8, 10). Therefore, APOLT with the preservation of the right lobe, which can lower the volume requirement for the graft, was performed in our recipient. It was anticipated that the residual native liver with relatively preserved liver function could temporarily support the function of the implanted small-for-size left liver lobe during the immediate postoperative period until the small graft had regenerated sufficiently. Then the graft can be expected to fully meet the hepatic functional demands of the recipient. More recently, a similar approach known as auxiliary two-staged partial resection LT using living-donor left lobes has been reported as a safe and technically feasible treatment for end-stage liver disease to avoid the small-for-size situation in adults/adolescents (12). Here, our case further demonstrated the clinical feasibility of APOLT using small-for-size living-donated left liver lobes for selected adult patients with end-stage liver cirrhosis, allowing increasing the pool of liver grafts and decreasing the risk of SFSS.

Notably, the functional competition of the portal vein flow between the remnant native liver and the implanted graft is a significant concern of APOLT, leading to progressive growth of one-side conquering liver and chronic atrophy of the defeated liver (4, 25). In our patient, due to the pre-existence of cirrhosis contributing to consequent higher intrahepatic vascular resistance in the remnant native liver, the portal blood flow to the soft implanted graft had an advantage over that to the cirrhotic residual liver. By virtue of the superiority of portal vein blood flow, the small liver graft was expected to expand its function in proportion to volume growth until it met the hepatic functional demand of the recipient with sufficient volume, while the native liver would shrink gradually. In fact, the results of postoperative abdominal dynamic contrast-enhanced CT scan and 99 mTc-GSA scintigraphy showed a progressive increase in the volume of the implanted liver graft and a gradual decrease in that of the residual native liver (**Figure 3**). Due to the inherent benign nature of the disease, namely cryptogenic cirrhosis, issues of disease transmission from the remnant native liver to graft can be ignored; therefore, removing the native cirrhotic liver was not considered in this recipient. Notably, however, given the potential risk of carcinogenicity of the remnant native liver, the patient underwent a hepatocellular carcinoma surveillance program including abdominal ultrasound, serum alpha-fetoprotein and prothrombin induced by vitamin K absence-II during his regular follow-up.

Previous studies have suggested that preoperative graft-to-spleen volume ratio (GSVR) at a ratio of <0.6 g/ml is a

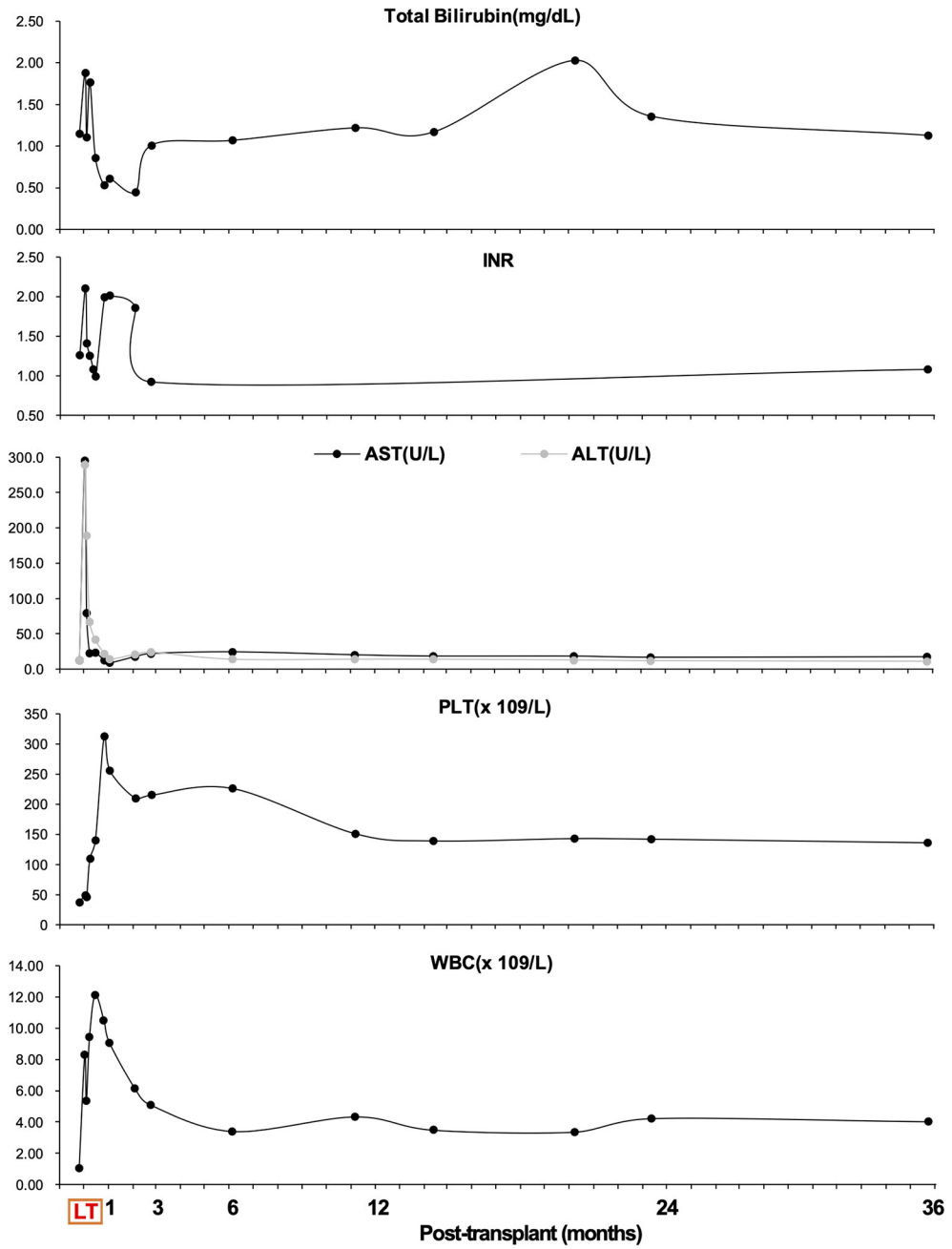


FIGURE 2 | Overview of total bilirubin (mg/dl), international normalized ratio (INR), aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), platelet (PLT, 10^9/L) count, and white blood cell (WBC, 10^9/L) count during follow-up.

good predictor of the development of post-transplant portal hyperperfusion; therefore, it can be used to indicate whether splenectomy is required before reperfusion (26, 27). Yao et al. reported that PVP >15 mmHg was associated with poor prognosis in grafts from donors aged ≥45 years, and lowering PVP to ≤15 mmHg was a key to prevention of SFSS and consequent early graft loss (28). Given the high PVP after reperfusion and advanced donor age, intraoperative portal inflow modulation was necessary for our recipient. Currently, there are several available strategies for portal inflow modulation, including splenic artery ligation, splenectomy, and portosystemic shunts, while concerns about these procedures remain (29, 30). Splenic artery ligation has limited effect on PVP control and carries a risk of sepsis and insufficient recovery of pancytopenia (29, 31); simultaneous splenectomy could be associated with the risk of portal hypoperfusion, portal venous thrombosis, infectious complications, and postoperative bleeding (20, 32); portosystemic shunts carry a risk of the development of portal

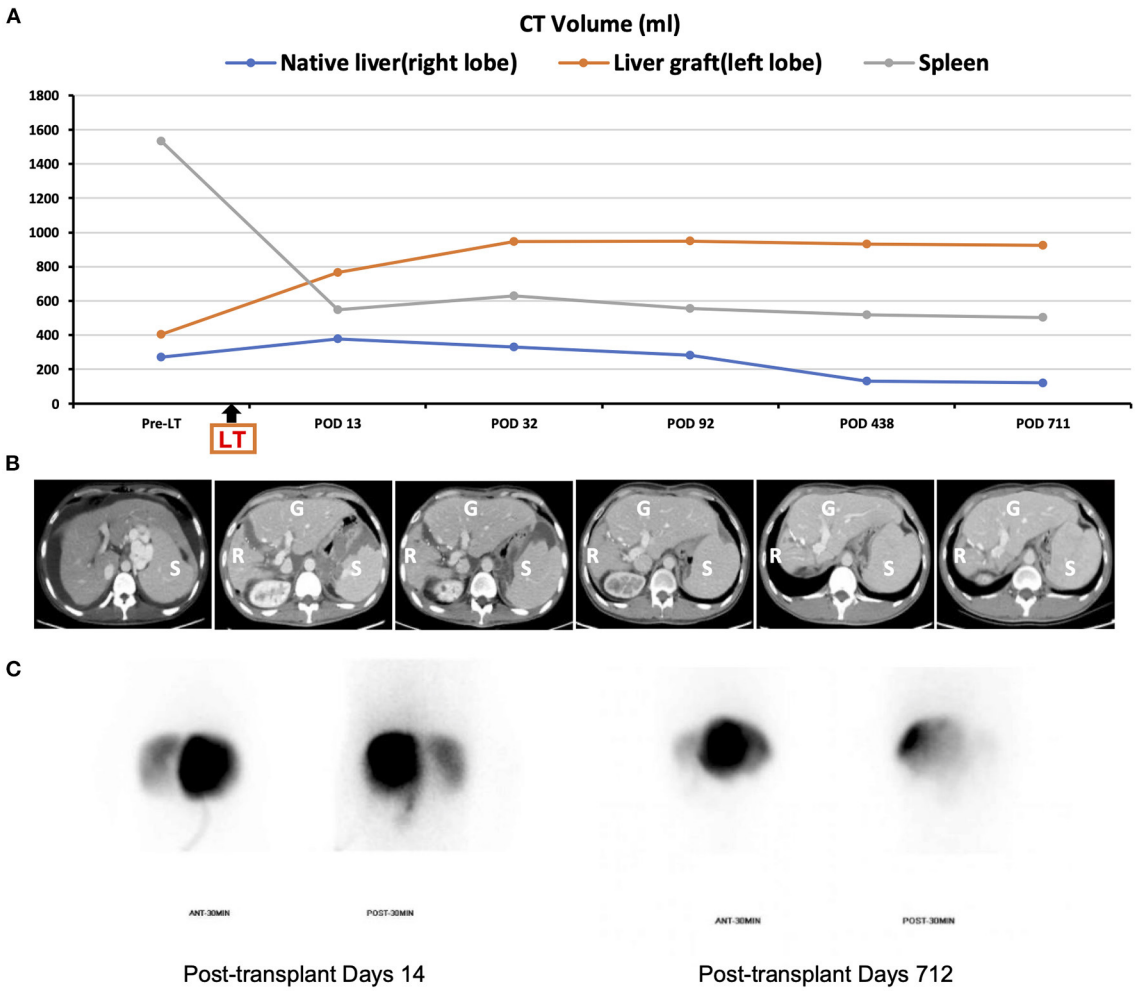


FIGURE 3 | Pre- and postoperative imaging. **(A)** Changes in CT-simulated volume of the native residual liver, graft, and spleen throughout follow-up; **(B)** representative abdominal CT images (R, liver remnant; G, graft; S, spleen); **(C)** Tc-99 m hepatobiliary scintigraphy on post-transplant day 14 and 712 (left liver graft: right native liver = 88%: 12 and 97.9%: 2.1%, respectively).

steal phenomenon, causing excessive diversion of the portal flow to the systemic circulation (27, 31).

More recently, Zhou and Wei et al. performed simultaneous partial splenectomy during LT in patients with severe hypersplenism, which achieved a satisfactory long-term hematological response but avoided untoward complications of total splenectomy (21, 33). A recent study by Yao et al. found that an extremely low GSVR (≤0.7 g/ml) was associated with persistent hypersplenism, impaired graft function, and consequent early graft loss after LDLT in patients with spleen preservation (34). Furthermore, it has been proved that preoperative spleen volume and low platelet count may contribute to post-transplant persistent thrombocytopenia, complicating the postoperative course among liver transplant recipients (16, 35, 36). Given our recipient's high graft PVP (19 mmHg) after reperfusion, large spleen (GSVR = 0.28 g/ml) and severe hypersplenism, we performed subtotal splenectomy at the time of transplantation, aiming to decompress graft portal hyperperfusion and correct hypersplenism-related pancytopenia. Apart from a long period of abdominal drainage, our recipient showed no noticeable signs of post-reperfusion portal hypertension and consequent complications in the early postoperative period. Platelet and leukocyte counts remained in the normal ranges throughout follow-up, suggesting severe pancytopenia related to hypersplenism had also been completely corrected.

CONCLUSION

This case suggests that simultaneous subtotal splenectomy during APOLT using small-for-size living-donated left liver lobes is not only an efficient intraoperative portal inflow modulation strategy but also a feasible treatment for severe hypersplenism in selected adult patients with end-stage liver cirrhosis. It could be a viable alternative to splenectomy, which preserves splenectomy's beneficial effects but avoids splenectomy-related

lethal complications. Furthermore, APOLT using a small-for-size liver graft may be a safe and feasible therapeutic option for selected adult patients with end-stage liver cirrhosis, which can further expand the pool of donor grafts.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Beijing Friendship Hospital, Capital Medical University (No. 2018-P2-127-01). The patients/participants provided their written informed consent to participate in this study and for the publication of this case report.

AUTHOR CONTRIBUTIONS

Z-JZ and LW: conception and design of the research, analysis and interpretation of the data, and preformation of the operations. G-PZ: acquisition of data, statistical analysis, and writing of the initial draft of manuscript. WQ, Z-GZ, YL, and L-YS: clinical management and follow-up of the patient. All authors involved in the critical revision of the manuscript for important intellectual content and approved the final version of the manuscript.

FUNDING

The present work was supported by the Capital's Funds for Health Improvement and Research (Grant/Award Number: 2020-1-2024) and Beijing Municipal Science and Technology Commission (No. Z211100002921026). The funding body had no role in the design of the study and collection, analysis, and interpretation of data, and in writing the manuscript.

ACKNOWLEDGMENTS

We are particularly grateful to all the people who have given us help on our article.

REFERENCES

1. Dahm F, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transpl.* (2005) 5:2605–10. doi: 10.1111/j.1600-6143.2005.01081.x
2. Yoichi T, Takayashiki T, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, et al. Protective effects of simultaneous splenectomy on small-for-size liver graft injury in rat liver transplantation. *Transpl Int.* (2014) 27:106–13. doi: 10.1111/tri.12223
3. Wang SF, Chen XP, Chen ZS, Wei L, Dong SL, Guo H, et al. Left lobe auxiliary liver transplantation for end-stage hepatitis b liver cirrhosis. *Am J Transpl.* (2017) 17:1606–12. doi: 10.1111/ajt.14143
4. Kasahara M, Takada Y, Egawa H, Fujimoto Y, Ogura Y, Ogawa K, et al. Auxiliary partial orthotopic living donor liver transplantation: Kyoto University experience. *Am J Transpl.* (2005) 5:558–65. doi: 10.1111/j.1600-6143.2005.00717.x
5. Quadros J, Piedade C, Lopes MF. Auxiliary liver transplantation for management of acute liver failure in children - Systematic review. *Transpl Rev.* (2021) 35:100631. doi: 10.1016/j.trre.2021.100631
6. Rela M, Kaliamoorthy I, Reddy MS. Current status of auxiliary partial orthotopic liver transplantation for acute liver failure. *Liver Transpl.* (2016) 22:1265–74. doi: 10.1002/lt.24509
7. Kasahara M, Sakamoto S, Horikawa R, Fukuda A. Auxiliary partial orthotopic liver transplantation for noncirrhotic metabolic liver disease: reigniting interest in an old but new technique. *Liver Transpl.* (2019) 25:12–3. doi: 10.1002/lt.25388
8. Qu W, Wei L, Zhu ZJ, Sun LY, Liu Y, Zeng ZG. Considerations for use of domino cross-auxiliary liver transplantation in metabolic liver diseases: a review of case studies. *Transplantation.* (2019) 103:1916–20. doi: 10.1097/tp.0000000000002602
9. Wei L, Zhang HM, Wan CD, Qu W, Zeng ZG, Liu Y, et al. Auxiliary liver graft can be protected from HBV infection in hbsag positive blood circulation. *Front Med.* (2021) 8:726502. doi: 10.3389/fmed.2021.726502
10. Dokmak S, Elkrief L, Belghiti J. Auxiliary liver transplantation with a small deceased liver graft for cirrhotic liver complicated by hepatocellular carcinoma. *Transpl Int.* (2013) 26:e102–4. doi: 10.1111/tri.12173
11. Kasahara M, Kiuchi T, Uryuhara K, Takakura K, Egawa H, Asonuma K, et al. Auxiliary partial orthotopic liver transplantation as a rescue for small-for-size grafts harvested from living donors. *Transpl Proc.* (1998) 30:132–3. doi: 10.1016/s0041-1345(97)01210-4
12. Brunner SM, Brennfleck FW, Junger H, Grosse J, Knoppke B, Geissler EK, et al. Successful auxiliary two-staged partial resection liver transplantation (ASPIRE-LTx) for end-stage liver disease to avoid small-for-size situations. *BMC Surg.* (2021) 21:166. doi: 10.1186/s12893-021-01167-6
13. Ohno Y, Mita A, Ikegami T, Masuda Y, Urata K, Nakazawa Y, et al. Temporary auxiliary partial orthotopic liver transplantation using a small graft for familial amyloid polyneuropathy. *Am J Transpl.* (2012) 12:2211–9. doi: 10.1111/j.1600-6143.2012.04061.x
14. Singh Soin A, Yadav SK, Saha SK, Rastogi A, Bhangui P, Thiagarajan S, et al. Is portal inflow modulation always necessary for successful utilization of small volume live donor liver grafts? *Liver Transpl.* (2019) 25:1811–21. doi: 10.1002/lt.25629
15. Yoshizumi T, Itoh S, Shimokawa M, Inokuchi S, Harada N, Takeishi K, et al. Simultaneous splenectomy improves outcomes after adult living donor liver transplantation. *J Hepatol.* (2021) 74:372–9. doi: 10.1016/j.jhep.2020.08.017
16. Takahashi K, Nagai S, Safwan M, Liang C, Ohkohchi N. Thrombocytopenia after liver transplantation: should we care? *World J Gastroenterol.* (2018) 24:1386–97. doi: 10.3748/wjg.v24.i13.1386
17. Pamecha V, Mahansaria SS, Kumar S, Bharathy KG, Sasturkar SV, Sinha PK, et al. Association of thrombocytopenia with outcome following adult living donor liver transplantation. *Transpl Int.* (2016) 29:1126–35. doi: 10.1111/tri.12819
18. Moon DB, Lee SG, Hwang S, Ahn CS, Kim KH, Ha TY, et al. Splenic devascularization can replace splenectomy during adult living donor liver transplantation - a historical cohort study. *Transpl Int.* (2019) 32:535–45. doi: 10.1111/tri.13405
19. Ito K, Akamatsu N, Ichida A, Ito D, Kaneko J, Arita J, et al. Splenectomy is not indicated in living donor liver transplantation. *Liver Transpl.* (2016) 22:1526–35. doi: 10.1002/lt.24489
20. Kurata N, Ogura Y, Ogiso S, Onishi Y, Kamei H, Kodera Y. Splenectomy in living donor liver transplantation and risk factors of portal vein thrombosis. *Hepatobil Pancreat Dis Int.* (2019) 18:337–42. doi: 10.1016/j.hbpd.2019.06.011
21. Wei L, Zhou GP, Qu W, Zeng ZG, Sun LY, Liu Y, et al. Is simultaneous partial splenectomy during pediatric liver transplantation safe and effective for severe

hypersplenism? A prospective cohort study. *Int J Surg.* (2021) 88:105926. doi: 10.1016/j.ijsu.2021.105926
22. Line PD, Hagness M, Berstad AE, Foss A, Dueland S. A novel concept for partial liver transplantation in nonresectable colorectal liver metastases: the rapid concept. *Ann Surg.* (2015) 262:e5–9. doi: 10.1097/SLA.0000000000001165
23. Raut V, Alikhanov R, Belghiti J, Uemoto S. Review of the surgical approach to prevent small-for-size syndrome in recipients after left lobe adult LDLT. *Surg Today.* (2014) 44:1189–96. doi: 10.1007/s00595-013-0658-6
24. Macshut M, Kaido T, Yao S, Yagi S, Ito T, Kamo N, et al. Older donor age is a risk factor for negative outcomes after adult living donor liver transplantation using small-for-size grafts. *Liver Transpl.* (2019) 25:1524–32. doi: 10.1002/lt.25601
25. Ikegami T, Shimada M, Imura S, Arakawa Y, Nii A, Morine Y, et al. Current concept of small-for-size grafts in living donor liver transplantation. *Surg Today.* (2008) 38:971–82. doi: 10.1007/s00595-008-3771-1
26. Cheng YF, Huang TL, Chen TY, Concejero A, Tsang LL, Wang CC, et al. Liver graft-to-recipient spleen size ratio as a novel predictor of portal hyperperfusion syndrome in living donor liver transplantation. *Am J Transpl.* (2006) 6:2994–9. doi: 10.1111/j.1600-6143.2006.01562.x
27. Gyoten K, Mizuno S, Kato H, Murata Y, Tanemura A, Azumi Y, et al. A novel predictor of posttransplant portal hypertension in adult-to-adult living donor liver transplantation: increased estimated spleen/graft volume ratio. *Transplantation.* (2016) 100:2138–45. doi: 10.1097/TP.0000000000001370
28. Yao S, Kaido T, Uozumi R, Yagi S, Miyachi Y, Fukumitsu K, et al. Is portal venous pressure modulation still indicated for all recipients in living donor liver transplantation? *Liver Transpl.* (2018) 24:1578–88. doi: 10.1002/lt.25180
29. Hernandez-Alejandro R, Sharma H. Small-for-size syndrome in liver transplantation: new horizons to cover with a good launchpad. *Liver Transpl.* (2016) 22:33–6. doi: 10.1002/lt.24513
30. Ikegami T, Balci D, Jung DH, Kim JM, Quintini C. Living donor liver transplantation in small-for-size setting. *Int J Surg.* (2020) 82s:134–7. doi: 10.1016/j.ijsu.2020.07.003
31. Wang H, Ikegami T, Harada N, Yoshizumi T, Soejima Y, Uchiyama H, et al. Optimal changes in portal hemodynamics induced by splenectomy during living donor liver transplantation. *Surg Today.* (2015) 45:979–85. doi: 10.1007/s00595-014-0999-9
32. Badawy A, Hamaguchi Y, Satoru S, Kaido T, Okajima H, Uemoto S. Evaluation of safety of concomitant splenectomy in living donor liver transplantation: a retrospective study. *Transpl Int.* (2017) 30:914–23. doi: 10.1111/tri.12985
33. Zhou GP, Wei L, Zhu ZJ, Qu W, Zeng ZG, Sun LY, et al. Successful simultaneous anatomic subtotal splenectomy during pediatric living-donor liver transplantation: a case report. *Transpl Proc.* (2020) 52:2767–72. doi: 10.1016/j.transproceed.2020.01.170
34. Yao S, Kaido T, Yagi S, Uozumi R, Iwamura S, Miyachi Y, et al. Impact of imbalanced graft-to-spleen volume ratio on outcomes following living donor liver transplantation in an era when simultaneous splenectomy is not typically indicated. *Am J Transpl.* (2019) 19:2783–94. doi: 10.1111/ajt.15337
35. Chen TY, Chen CL, Huang TL, Tsang LL, Ou HY, Yu CY, et al. Predictive factors for persistent splenomegaly and hypersplenism after adult living donor liver transplantation. *Transpl Proc.* (2012) 44:752–4. doi: 10.1016/j.transproceed.2012.01.044
36. Stanca CM, Fiel MI, Aledort L, Cohen E, Del Rio Martin J, Schiano TD. Factors associated with persistent thrombocytopenia after liver transplantation. *Transpl Proc.* (2010) 42:1769–73. doi: 10.1016/j.transproceed.2010.02.075

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership