Thesis

The Impact of Portosystemic Shunts on Bile Acid Metabolism in Patients with Liver Cirrhosis

A Pilot Study

submitted by

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Graz, 19.03.2025

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Graz, at 19.03.2025 Florian Hinterhölzl m.p.

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Zusammenfassung

Hintergrund: Während erhöhte Gallensäurekonzentrationen im Serum als Hauptmerkmal cholestatischer Lebererkrankungen gelten, wurden Veränderungen der Gallensäurekonzentrationen und -profile bei Leberzirrhose ohne zugrunde liegende Cholestase weniger beachtet. Da solche Veränderungen sowohl eine Folge als auch ein potenzieller Auslöser für fortgeschrittene Lebererkrankungen und deren Komplikationen sein können, war es Ziel dieser Studie, die Veränderungen im Gallensäureprofil bei Patient*innen mit Leberzirrhose genauer zu charakterisieren. Außerdem sollen weitere Veränderungen im Gallensäureprofil beschrieben werden, die nach dem Einsetzen eines transjugulären portosystemischen Shunts (TIPS) auftreten.

Methoden: Zum einen wurden die Gallensäurekonzentrationen und die Zusammensetzung des Gallensäurepools im Serum von 274 Patient*innen mit Leberzirrhose und 33 gesunden Kontrollpersonen retrospektiv analysiert und mit klinischen Daten korreliert. Zum anderen wurden prospektiv die Gallensäurespiegel vor und nach einem TIPS-Eingriff sowie die Spiegel in Leber- und Pfortaderblut gemessen, um den Einfluss eines iatrogenen Shunts auf das Gallensäureprofil zu bestimmen.

Ergebnisse: Die Konzentrationen der Gallensäuren im Serum waren bei Patient*innen mit Leberzirrhose signifikant höher als bei gesunden Kontrollen (67.1 ± 72.9 vs. 3.2 ± 2.6 µmol/L; $p<0.0001$). Sowohl der MELD ($r=0.63$; $p<0.0001$) als auch der Child-Pugh Score ($r=0.61$; $p<0.0001$) korrelierten mit den Gesamtgallensäurekonzentrationen. Ebenso ging das Auftreten von Komplikationen der Leberzirrhose wie hepatische Enzephalopathie ($p=0.001$) und Aszites ($p<0.0001$) mit erhöhten Gallensäurekonzentrationen einher. Patient*innen mit alkoholassoziierter Leberzirrhose wiesen auch nach Korrektur für den Schweregrad der Zirrhose die höchsten Gesamtgallensäurekonzentrationen auf. Der Anstieg der Gesamtgallensäuren bei Zirrhose war größtenteils auf eine Zunahme der Taurin- und Glycin-Konjugate der primären Gallensäuren Chenodeoxycholsäure (CDCA) und Cholsäure (CA) zurückzuführen. Das Verhältnis von sekundären zu primären Gallensäuren verschob sich von etwa 0.6 bei Gesunden zu 0.2 bei Patient*innen mit Zirrhose ($p=0.012$) deutlich. Im TIPS-Teil dieser Arbeit waren die Gallensäure-Konzentrationen im peripheren Blut höher als in der Lebervene und Pfortader und sanken innerhalb von 24h nach dem TIPS

tendenziell ab (mediane Reduktion 10.4 µmol/L, p=0.63). Die hepatische Extraktionsrate von Gallensäuren lag zwischen 14 und 71% und scheint negativ mit dem Child-Pugh-Score (r=-0.9, p=0.0119) und dem hepatisch-venösen Druckgradienten (HVPG) vor TIPS (r=-0.8, p=0.33) zu korrelieren. Im Langzeit Follow-up (n=2) 90 Tage nach TIPS zeigte sich keine weitere relevante Änderung der Serumgallensäuren.

Schlussfolgerung: Diese Arbeit zeigt, dass die Serumgallensäuren bei Patient*innen mit Leberzirrhose mit dem Schweregrad der Erkrankung und klinischen Scores wie dem MELD-Score korrelieren, was darauf hindeutet, dass Gesamt-Gallensäure-Messungen als prognostische Indikatoren bei Zirrhose dienen könnten. Mit dem Fortschreiten der Zirrhose kommt es zu einem Anstieg konjugierter primärer Gallensäuren und zu einem Rückgang der sekundären Gallensäuren. Die Serumgallensäure-Spiegel nahmen am Tag nach dem TIPS ab, obwohl der portosystemische Shunt zunahm. Dies könnte auf eine höhere hepatische Extraktionsrate von Gallensäuren hinweisen. Die Auswirkungen des TIPS auf die Gallensäuren-Dynamik unterstreichen aber die Komplexität der Gallensäure-Regulation bei Zirrhose und bedürfen weiterer Forschung.

Abstract

Background: While elevated bile acid concentrations in serum are considered a key characteristic of cholestatic liver diseases, changes in bile acid concentrations and profiles in liver cirrhosis without underlying cholestasis have received less attention. Since such changes can be both a consequence and a potential driver of advanced liver diseases and their complications, this study aims at characterizing the deviations in the bile acid profile in patients with liver cirrhosis more precisely. Additionally, we attempt to explore fluctuations in the bile acid profile, following the placement of a transjugular portosystemic shunt (TIPS).

Methods: (i) Serum bile acid concentrations and composition of the bile acid pool of 274 patients with liver cirrhosis and 33 healthy controls were retrospectively analyzed and correlated with clinical data. (ii) We prospectively measured bile acid levels during TIPS implantation in peripheral, portal, and hepatic venous blood, to calculate the hepatic bile acid extraction rate and compared peripheral venous bile acid levels pre and post TIPS, to determine the influence of an iatrogenic shunt on the bile acid profile.

Results: Bile acid concentrations in serum were considerably higher in liver cirrhosis than in healthy controls (67.1 ± 72.9 vs. 3.2 ± 2.6 µmol/L; $p<0.0001$). Both the MELD score ($r=0.63$; $p<0.0001$) and the Child-Pugh score ($r=0.61$; $p<0.0001$) correlated with total bile acid concentrations. Likewise, the occurrence of complications of cirrhosis, such as hepatic encephalopathy ($p=0.001$) and ascites ($p<0.0001$), was associated with increased bile acid concentrations. Patients with alcohol-associated liver cirrhosis had the highest total bile acid concentrations even after taking the severity of cirrhosis into account. The increase in total bile acids in cirrhosis was primarily caused by an increase in taurine and glycine conjugates of the primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA). The ratio of secondary to primary bile acids shifted markedly from about 1 in healthy individuals to 0.2 in patients with cirrhosis ($p=0.012$). Bile acid concentrations measured during TIPS implantation were heightened in the peripheral blood in comparison to liver and portal venous blood, and peripheral bile acid concentrations tended to decrease within 24 hours after TIPS (median reduction 10.4 µmol/L, $p=0.63$). The percentage of hepatic bile acid extraction ranged from 14 to 71% and appeared to correlate negatively with Child-Pugh

score (r=-0.9, p=0.0119) and pre-TIPS hepatic venous pressure gradient (HVPG) (r=-0.8, p=0.33). Long-term follow-up (n=2) 90 days after TIPS showed no further relevant changes in serum bile acids.

Conclusion: This thesis demonstrates that bile acid levels in patients who battle liver cirrhosis significantly correlate with disease severity and clinical scores like the MELD score, suggesting that total bile acid measurements could serve as prognostic indicators in cirrhosis. In addition, cirrhosis progression was linked to specific changes in bile acid composition, including an increase in conjugated primary bile acids and a decline in secondary bile acids. Serum bile acid levels decreased the day after TIPS, despite increased portosystemic shunting after shunt placement indicating increased hepatic bile acid extraction after this procedure. The influence of TIPS on bile acid dynamics warrants further research but underlines the complexity of bile acid regulation in cirrhosis.

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List of Abbreviations

ALT	Alanine-Aminotransferase
ASBT	Apical Sodium-dependent Bile Acid Transporter
AST	Aspartate-Aminotransferase
BA	Bile Acid
BAT	Brown Adipose Tissue
BCS	Budd-Chiari Syndrome
BSEP	Bile Salt Export Pump
BSH	Bile Salt Hydrolase
CA	Cholic Acid
CAR	Constitutive Androstane Receptor
CCM	Cirrhotic Cardiomyopathy
CDCA	Chenodeoxycholic Acid
CPS	Child-Pugh-Score
CRP	C-Reactive-Protein
CYP	Cytochrome-P
DCA	Deoxycholic Acid
EHC	Enterohepatic Cycle
eNOs	endothelial Nitric Oxide synthase
FGF 15/19	Fibroblast-Growth-Factor 15/19
FGFR4	Fibroblast Growth Factor Receptor 4
FXR	Farnesoid X Receptor
GGT	Gamma-Glutamyl-Transferase
GLC	Gas-Liquid-Chromatographic method
GLP-1	Glucagon Like Peptide 1
H2S	Hydrogen Sulfide
HE	Hepatic Encephalopathy
HH	Hepatic Hydrothorax
HPS	Hepatic Pulmonary Syndrome
HRS	Hepatorenal Syndrome
IBABP	Ileal Bile Acid Binding Protein
IJV	Internal Jugular Vein

IQR	Interquartile Range
IVC	Inferior Vena Cava
LCA	Lithocholic Acid
LRH-1	Liver Receptor Homolog-1
MASLD	Metabolic dysfunction-Associated Steatotic Liver Disease
MELD	Model for End-stage Liver Disease
MRP2	Multidrug Resistance-associated Protein 2
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NTCP	Natrium-Taurocholate Cotransporting Polypeptide
OATP	Organic Anion Transporting Protein
OST	Organic Solute Transporter
OSTα-OSTβ	Organic Solute Transporter alpha-beta
PH	Portal Hypertension
PKA	Protein Kinase A
PLT	Platelet count
PVT	Portal Vein Thrombosis
PXR	Pregnane X Receptor
RIA	Radioimmunoassay method
RXRα	Retinoid X Receptor alpha
RYGB	Roux-en-Y Gastric Bypass
SBA	Serum Bile Acids
SD	Standard Deviation
SHP	Small Heterodimer Partner
SPSS	Spontaneous Porto-Systemic Shunts
TBA	Total Bile Acids
TGR5	Takeda G protein-coupled Receptor 5
TIPS	Transjugular Intrahepatic Portosystemic Shunt
UDCA	Ursodeoxycholic Acid
VDR	Vitamin D Receptor
WBC	White-Blood-Cell count

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1 Introduction

Liver Cirrhosis, an incurable illness characterized by progressive scarring of liver tissue, stands in association with considerable fluctuations in bile acid metabolism, as well as the initiation of portosystemic shunts (1). In healthy individuals, bile acids demonstrate a major factor in digestion, particularly in the emulsification and absorption of fats (2). However, in cirrhosis, the liver´s capability to synthesize and process bile acids is severely impaired. Portosystemic shunts represent irregular interconnections between the portal and systemic circulation, aggravating this problem (3) by shifting portal blood aside from the liver. This, in turn, presents a reduction in delivery of bile acids to hepatocytes with subsequent changes in bile acid synthesis and excretion (4).

Consequently, this can lead to an accumulation of bile acids (BAs) in the systemic circulation. In addition to alterations in hepatic bile acid metabolism, portosystemic shunts can also have a considerable impact on the microbial composition, which in turn leads to alterations in bile acid profiles (5). This can cause malabsorption of fats and fat-soluble vitamins, worsening the nutritional status in patients who are already predisposed to malnutrition, frailty, and sarcopenia (6). Moreover, the pathogenesis of pruritus is related to the variations in the bile acid pool and contributes to the suffering among liver cirrhosis´ patients (7).

The presence of portosystemic shunts directly correlates with an elevated potential of complications, including hepatic encephalopathy. Altered hemodynamics and the shunting of gut-derived substances, such as bile acids, into the systemic circulation, bypassing hepatic detoxification, are key factors in the onset of these complications (8).

The main objective of this thesis is twofold: (i) To elucidate the alterations in bile acid metabolism in liver cirrhosis through a retrospective analysis and (ii) by a prospective evaluation after TIPS placement.

1.1 Bile Acid Metabolism

Bile acids are derivatives of cholesterol, which are being produced in the liver, and are of the utmost importance for both absorption and digestion of cholesterol, fats, and fat-soluble vitamins. In addition, BAs are highly relevant for the activation of pancreatic enzymes (2, 9,

10). With over 60 species identified, BAs exhibit a structurally diverse nature, with each species serving different bioactive functions. Since several diseases are linked to higher levels of bile acids and alterations regarding BA pool composition, studies on BA metabolism are required to understand the mechanism behind these changes (11). Liver cirrhosis leads to perturbations in bile acid metabolism. Therefore, this chapter aims to discuss the physiological metabolism of bile acids.

1.1.1 Bile Acid Synthesis

1.1.1.1 Primary Bile Acids

A significant proportion of cholesterol undergoes conversion to BAs daily, involving various chemical reactions which, in turn, leads to the formation of primary bile acids. In the human liver cholic acid (CA) and chenodeoxycholic acid (CDCA) are the two primary bile acids synthesized (9, 11). Four sequential steps subsequently result in the synthesis of primary BAs, including initiation, changes to the ring structure, subsequent oxidation and shortening of the side chain, and conjugation. Several enzymes of the cytochrome P450 group participate in the process of incorporating hydroxyl groups to the ring structure of cholesterol. Two pathways can be differentiated in the synthesis of primary bile acids (11), the first one being the classic pathway, known to be responsible for over 90% of total primary BA creation in humans (12). To be exact, cholesterol 7α-hydroxylation is the initiative step and is catalyzed by the rate-limiting enzyme CYP7A1 (11). The gene encoding for CYP7A1 is subject to stringent regulation at the transcriptional step by bile acids, glucagon, certain cytokines, and FGF15 in rodents and FGF19 in humans (10). Another critical enzyme of the classic pathway is the sterol 12α-hydroxylase (CYP8B1), controlling the CA/CDCA ratio. In comparison to the pathway described above, the alternative pathway is started by 27-hydroxylation through CYP27A1 and followed by 7α-hydroxylation, catalyzed by CYP7B1. It is only marginally active under physiological conditions and contributes to BA production under certain conditions, namely, exposure to the cold, a diet high in fat and/or cholesterol, or liver disease (11). Following the synthesis of primary bile acids, they are conjugated with glycine or taurine, which increases water solubility and polarity, therefore making them non-permeable to cell walls leading to their high concentrations in bile and intestinal content (13). Human BAs exhibit a conjugation pattern of 70% to glycine and 30% to taurine, which is influenced by dietary components (13, 14).

1.1.1.2 Secondary Bile Acids

BAs are powerful surfactants from which bacteria in the large bowel must protect themselves. The hydroxyl group and the tauryl or glycyl conjugate are crucial elements in this regard, therefore removal or modification of these structures diminishes potential toxic features of BAs and renders them largely apolar. Bacteria have an enzymatic system for BA conversion to cope with that (15), ultimately expanding the molecular diversity of the intestinal microbiome (2). Approximately 15% of conjugated primary BAs are not reabsorbed by the terminal ileum, instead, they enter the colon, which is the place where gut microbiota deconjugate and bio-transform primary BAs into secondary BAs. Namely, these are deoxycholic acid (DCA) and lithocholic acid (LCA) as well as the tertiary BA ursodeoxycholic acid (UDCA) (16). Deconjugation, referring to the removal of taurine or glycine, is mediated through bile salt hydrolases (BSHs) (15). Dehydroxylation refers to the removal of 7α or 7β-hydroxyl groups from primary BAs. 7α/β-dehydroxylation seems to be reduced to the freeing of bile acids, making the removal of glycine and taurine via BSH enzymes a vital step (17). The extensive metabolism of microbiota leads to secondary BAs having a higher frequency than primary ones in stool. About 50% of DCA, in contrast to small amounts of UDCA and LCA, are getting reabsorbed by the terminal ileum and colon, and subsequently enter the liver over the portal vein, where secondary BAs are reconjugated with glycine or taurine (16).

1.1.2 Enterohepatic Circulation Recovers Most Bile Acids

The enterohepatic cycle refers to the circulation of primary and secondary bile acids and other solutes. It comprises biliary excretion and intestinal reabsorption of BAs (18). The liver produces around 0.5g of BAs daily, with CA and CDCA being the two major primary bile acids excreted into the canaliculi as conjugates via active transport mediated by the bile salt export pump (BSEP) (11). Secreted BAs pervade the canaliculi, interlobular bile ducts, the left or right hepatic duct, and the common hepatic duct (19). Bile gets formed along the way and is then consequently kept in the gallbladder, until its delivery to the small intestine via the major duodenal papilla, following the ingestion of food. Along with food, BAs enter the small intestine and are processed by the local gut bacteria, forming the secondary BAs LCA, DCA, and UDCA. After completing their physiological function in the intestine, BAs reach their final destination in the distal ileum, where they are efficiently reabsorbed by an

active transport system involving sodium. The apical sodium-dependent BA transporter (ASBT) is expressed in the luminal membrane of enterocytes and represents the main transporter for the reabsorption of BAs. Following its passage through the luminal membrane, the so-called ileal bile acid binding protein (IBABP), enables the intracellular diffusion of BAs to the basolateral membrane, where the organic solute transporter is responsible for the mediation of their exit into the portal blood circulation (19, 20). Once returned to the liver, BAs are actively reabsorbed by sinusoidal blood via basolateral transporter systems on hepatocytes (21). Conjugated BAs are gathered by the Na+/taurocholate cotransporting polypeptide (NTCP) and unconjugated BAs by the organic anion transporting proteins (OATP2s). Subsequently, the BSEP and partly the multidrug resistance-associated protein 2 (MRP2) reintroduce BAs into the biliary ductal system. This cycle is repeated numerous times daily, efficiently recovering about 95% of the BA pool. Only tiny amounts of BAs tend to get lost in feces and, as a result, have to be reproduced by de novo synthesis, in order to ensure the maintenance of bile acid homeostasis (19, 20).

1.2 Bile Acid Receptors at the Heart of Metabolic Control

BAs serve as endogenous ligands for multiple receptors, which are involved in signaling and regulating of bile acid metabolism. The so-called gut-to-liver axis plays a crucial role in modulating the EHC, in addition to the composition and pool size of BAs. BA-responsive receptors present promising drug-developing opportunities, aiming at addressing metabolic disorders (20). The first identified bile acid receptor was the farnesoid X receptor (FXR), which is known to adjust bile acid transport, synthesis and detoxification at the transcriptional stage. But this is only one of many different bile-acid-activated nuclear receptors involved in bile acid homeostasis (22).

1.2.1 FXR: The Central Bile Acid Receptor

The farnesoid X receptor (FXR), which is a nuclear receptor that forms heterodimers with retinoid X receptor α (RXRα) and is predominantly expressed in the liver and distal ileum, where BAs are actively reabsorbed. Its expression extends to other tissues, such as the kidney, adrenal gland (23), adipose tissue and heart, although the functional significance in these locations remains incompletely characterized (24). FXR can be triggered by most natural BA species with CDCA displaying the highest potency (CDCA > DCA > LCA >

CA). FXR is essential for the regulation of BA transport from enterocytes to the EHC in the ileum, thereby maintaining intestinal barrier integrity (23). BA activation of FXR initiates a negative feedback regulation of the apical sodium-dependent bile acid transporter (ASBT) via the activation of small heterodimer partner (SHP), which leads to the subsequent inhibition of retinoic acid receptor/retinoid X receptor (RAR/RXR) activity within enterocytes, consequently suppressing BA absorption from the intestinal tract (25). The expression of ileal bile acid binding protein (IBABP) is inducted by FXR, promoting the transport of BAs through the enterocyte to the basolateral membrane (26). Here, BA transport through the basolateral membrane of the enterocyte is also regulated by FXR. The expression of the intestinal organic solute transporter alpha-beta (OSTα-OSTβ) is regulated through both positive and negative mechanisms involving BAs, mediated through pathways involving FXR and liver receptor homolog-1 (LRH-1) elements. Ultimately this leads to a predominant positive regulation by BAs, which promotes the onward transport of absorbed BAs. Conversely, a decline in FXR activity has the potential to induce ileal enterocyte toxicity.

Another important ileal gene targeted by FXR is fibroblast growth factor 15 (in rodents) / 19 (in humans) (FGF15/19). The responsiveness of FGF15 expression upon BA stimulation was completely abolished when FXR was disrupted, although basal FGF15 expression persists. Thus, the production of FGF15/19 is critically controlled by ileal FXR activity (23). FGF19 subsequently binds to a cell surface receptor complex, to repress bile acid synthesis and gluconeogenesis and stimulates gall bladder filling (27). This cell surface receptor complex, namely FGFR4-KLβ heterodimer, is mainly found in the liver and displays the main target site for FGF15/19. Both, FXR activation by BA and activation of FGFR4 by FGF15/19 induce the expression of SHP, which in turn binds to the transcriptional factors HNF4α and LRH-1, suppressing their activity and subsequently decreasing CYP7A1 expression (23). Some data also suggests alternative SHP-independent pathways for FGF15/19-mediated CYP7A1 suppression (23, 26).

Furthermore, FXR activation upregulates genes involved in BA secretion, such as BSEP and MRP2, as well as phospholipid secretion, specifically multidrug resistance protein 3 (MDR3), across the bile canalicular membrane (26, 28). On the sinusoidal membrane, FXR activation inhibits the expression of sodium taurocholate cotransporting polypeptide (NTCP)

and organic anion transporting peptides, thereby maintaining low intrahepatic BA concentration to prevent cholestatic liver injury.
This bile acid-FXR-FGF19 signaling pathway provides multiple opportunities, with FXR agonists and FGF19 analogs, for treatment of bile acid-related disorders and metabolic diseases (27).

1.2.2 TGR-5 Signaling: Key Mediator of Systemic Bile Acid Actions

Besides the influence of nuclear FXR in regulating the BAs, accumulated scientific references hint to a pivotal function of Takeda G protein-coupled receptor (TGR5), in the process of balancing systemic actions of BAs. It is activated by several BAs, with conjugated lithocholic acid (LCA) representing the most potent natural ligand. Other than LCA, TGR5 is also activated by conjugated and unconjugated DCA, CDCA, and CA with lower potency respectively. TGR5 is widely represented in diverse tissues, which include the gallbladder epithelium, colon and ileum, brown adipose tissue, human spleen, skeletal muscle, and in certain areas of the central nervous system (29). In the liver, it is expressed in varying non-parenchymal cells, including macrophages, biliary epithelial cells, activated hepatic stellate cells (HSCs), and sinusoidal endothelial cells (LSECs) (30).

The receptor is activated by the compounding process of BAs to the ligand-binding pocket, triggering intracellular cAMP-production and subsequent activation of cAMP-dependent protein kinase A (PKA) (29). TGR5 not only protects the intestinal barrier function, but also leads to the reduction of inflammation processes, as well as the stimulation of gallbladder filling and secretion of GLP-1, originating from enteroendocrine L-cells. The inducement of TGR5 gene expression in the mouse intestine has been suggested for FXR, indicating that both receptors participate in glucose-induced GLP-1 secretion. In brown adipose tissue thyroid hormone deiodinase 2 (D2) is activated and stimulates energy metabolism, showing positive impact in mice obesity and hepatic steatosis (31).

1.2.3 Other Receptors Signaling

In addition to the FXR, three closely related xenobiotic nuclear receptors, namely vitamin D receptor (VDR), pregnane X receptor (PXR), and in addition constitutive androstane receptor (CAR), among others, contribute to the gut-liver crosstalk regulation of BAs. The above mentioned receptors are known to be decisively entangled with the triphasic metabolic

process of drugs and BAs in the liver and intestine. The activation of PXR, for instance, has been proven to result in the repression of CYP7A1 transcription, by inhibiting the nuclear receptor (22). LCA is identified as the most potent activator of PXR, whereas FXR does not respond to LCA stimulation. This suggests that PXR may function as a protective mechanism to reduce LCA-induced hepatotoxicity by reducing LCA concentrations (32). Both PXR and CAR are identified as key modulators in phase I and II detoxification processes and influence the expression of enzymes such as CYP3A4, SULT2A1, glutathione S-transferase, and UDP-glucuronosyltransferase. Additionally, CAR is pivotal in the regulation of BA sulfation and their basolateral transport. These protective pathways are particularly active under conditions with high intracellular BA concentrations (33). VDR, activated by its natural ligand's vitamin D and LCA, plays a protective role in human hepatocytes by inhibiting CYP7A1 gene transcription, thus safeguarding against damage in cholestatic liver injuries (34). Beyond its involvement in BA metabolism, transport, and detoxification, markedly through activation of CYP3A4 (35), VDR also regulates calcium homeostasis, immune responses, and cellular differentiation (35, 36). Another receptor worth mentioning is the sphingosine-1-phosphate receptor 2 (S1PR2) which is predominantly activated by primary BAs, most efficiently by TCA, in hepatocytes. This receptor emerged as a potential key player in hepatic lipid metabolism. However, the extent of its involvement necessitates further investigation (37).

1.3 Disrupted Bile Acid Metabolism in Liver Cirrhosis: A Closer Look

In healthy individuals the bile acid pool circulates enterohepatically about 5-10 times per day with a total of 2-5g BAs. The primary BAs CA and CDCA account for about 40% of the pool each, DCA around 20%, while LCA and UDCA only account for a few percent. Approximately one third are taurine conjugates and two thirds are glycine conjugates. In liver cirrhosis there is a shift towards taurine-conjugates (38). While in healthy subjects the ratio of CA:CDCA ranges from 0.6 to 1, in cirrhosis it is reduced to 0.5-0.1 further decreasing by cirrhosis severity (21). The above-mentioned differential affinities of different BAs to FXR and TGR5 underlines the significance of the compounding of the BA pool (39, 40).

The overall bile acid pool decreases in dependency of disease severity (38), while serum concentrations of total BAs increase by the degree of decompensation in alcohol associated cirrhosis (21). In healthy individuals, the ratio of BA concentrations between portal venous and peripheral venous blood ranges from 3:1 (41) to 7-10:1 in a fasting state (42). As for liver cirrhosis and cholestasis, systemic and portal BA concentrations equilibrate (41), partly caused by the systemic spillover of BAs through portosystemic shunts (43). The accumulation of BAs in hepatocytes might promote the inhibition of CYP7A1, thereby contributing to the depletion of the BA pool (38). However, it remains unclear so far, whether bile acid concentrations in hepatocytes are decreased or increased in liver cirrhosis

Cirrhosis leads to decreased levels of fecal BAs (44) and dysbiosis in the gut with overgrowth of pathogenic bacterial species. The observed dysbiosis is associated with low levels of BAs passing into the intestine and manifests itself in an accumulation of *Enterobacteriaceae* and a decrease in *Autochthonous* genera. This leads to a reduced converting from primary to secondary BAs, which in turn, lowers the ratio of secondary/primary BAs in feces with the progression of liver cirrhosis severity (45). DCA is a means to suffocate unwanted microbial populations, a reduction in this species therefore results in bacterial overgrowth and inflammation (44, 46). This is accompanied by an uprise of conjugated and unconjugated primary BAs in serum of cirrhotic patients with subsequent reduction of serum DCA (45). Also, in bile-rich duodenal fluid of patients with cirrhosis, the percentage of DCA and LCA has been shown to be at a level which is significantly lower than in clinically unremarkable individuals (47).

The dysregulated bile acid metabolism and dysbiosis in cirrhosis are therefore in a multidirectional relationship to each other. On top of that, recent studies have risen awareness to distinct changes in bile acid profiles, according to the prospect cause of liver disease (44).

1.4 Effects of Altered Bile Acid Metabolism in Liver and Extrahepatic Tissues

Liver cirrhosis results in significant alterations in hepatic blood flow, cellular and extrahepatic hemodynamics, leading to the development of collateral vessels bypassing the liver. This results in a reduced hepatic uptake of many substrates (18, 21). The hyperdynamic

circulatory state caused by splanchnic and systemic vasodilation, along with central hypovolemia, contributes to dysfunction of other organs. Liver cirrhosis causes distortion in the intestinal barrier, thus allowing molecules and microorganisms to translocate into the blood stream, where they act as drivers of systemic inflammation (21). Various molecules contribute to the above, with this section focusing on BAs and their potential influence as signaling molecules.

1.4.1 Metabolic Regulation: The Role of Bile Acids in Glucose and Lipid Homeostasis

Bile acids have a systemic endocrine function and play an important role in the coordination of lipid, glucose, and energy homeostasis (33, 48). There is compelling evidence that nutrition exerts a direct regulatory influence on BA synthesis. The enzyme CYP7A1, which is considered the rate-limiting enzyme in BA synthesis, exhibits increased activity during postprandial periods and a decrease during fasting and nocturnal states (49).The other way around, BAs arriving in the liver through enterohepatic circulation provide a signal that coordinates lipid and glucose homeostasis in the liver and extrahepatically by spillover into systemic circulation (33).

1.4.1.1 Bile Acids Stimulate GLP-1 and Improve Insulin Resistance

Glucose plays a pivotal role in the transcriptional upregulation of CYP7A1, which displays the cross-regulation between glucose and bile acid metabolism (48, 50). Additionally, insulin exerts a stimulatory effect on CYP7A1 gene expression, implying that glucose can also induce CYP7A1 through insulin-mediated mechanisms (48). This intriguing relationship between glucose metabolism and bile acid regulation raises the question whether interventions targeting bile acid metabolism could control the impaired glucose metabolism observed in diabetes. Bile acids mainly modulate glucose metabolism via the activation of two crucial receptors, namely FXR and TGR5. By activating intestinal FXR, FGF19 is produced and released, thus stimulating insulin secretion. Released FGF19 subsequently enhances glucose tolerance and insulin sensitivity, primarily over the extracellular signal-regulated kinase (ERK) pathway. A mouse experiment showed that gut microbiota, especially *Acetatifactor* and *Bacteroides*, was induced upon activation of FXR signaling. This leads to increased conversion of UDCA and CDCA to LCA, which in turn triggers

TGR5 signaling from enteroendocrine L-cells to stimulate GLP-1 secretion and improve glucose tolerance (51).

Activation of TGR5 in enteroendocrine L-cells of the intestine increases the secretion of glucagon-like peptide 1 (GLP-1), in turn promoting glucose-dependent insulin secretion from pancreatic ß-cells postprandially. (33, 50, 52). For people with type 2 diabetes, FGF19 concentrations were found to be significantly lower accompanied with an elevation of BA levels, suggesting a potential impairment within the FGF19-BA metabolic pathway. A plausible explanation for this could be a lack of response to BAs and FGF19 in the liver. Following Roux-en-Y gastric bypass (RYGB) surgery, which typically induces diabetes remission in a majority of patients, FGF19 and BA levels exhibited an increase. This underscores the putative beneficial impact of BAs on the management of glucose metabolism disorders (53). Other authors have confirmed the increase in BAs following RYGB surgery and suggest that the increased BAs may contribute to improved glucose metabolism (54, 55).

Anomalies in glucose tolerance are commonly observed among cirrhotic patients, primarily manifesting as peripheral insulin resistance. High concentrations of circulating BAs in these patients may impact glucose metabolism by counteracting peripheral insulin resistance, thereby targeting glucose, glucagon, and insulin metabolism (56). Supporting this, an inverse correlation between total BAs and glucose levels measured at two hours postprandially has been demonstrated in liver cirrhosis patients, suggesting a role for BAs in human glucose homeostasis (54, 56). While improved peripheral insulin resistance generally serves as a protective mechanism, in the context of liver cirrhosis, it may represent an adaptive response. This adaptation potentially directs glucose to the liver, reducing hepatic gluconeogenesis and enabling glycogenesis and other regenerative processes (56).

1.4.1.2 Lipid Metabolic Disorders are Positively Influenced by Bile Acids

Formerly known as non-alcoholic fatty liver disease (NAFLD), now more precisely termed metabolic dysfunction-associated steatotic liver disease (MASLD), is an increasingly common health problem worldwide (57). Its more severe form, non-alcoholic steatohepatitis (NASH), has become an important cause of chronic liver disease. While lifestyle interventions and management of comorbidities such as T2DM and dyslipidemia remain foundational to MASLD treatment, BA-related signaling pathways are gaining recognition

as candidate therapeutic targets, largely due to their role in regulating lipid metabolism. FXR plays a decisive role in regulation here.

Studies on FXR knockout (FXR-KO) mice have shown distinctly elevated serum and hepatic cholesterol and triglyceride levels. The application of FXR agonists has been found to reduce triglyceride and plasma cholesterol levels as well as free fatty acid levels. FXR activation stimulates fatty acid β-oxidation, suppresses hepatic de novo lipogenesis, and regulates multiple other key genes related to the triglyceride metabolism (58). In illnesses such as diabetes and MASLD, de novo lipogenesis is powered by hyperinsulinemia-induced activation of sterol regulatory element-binding protein-1 (SREBP-1) and carbohydrate response element-binding protein (CREBP), both of which have been shown to be repressed by FXR activation (59, 60). Additionally, peroxisomal proliferator-activated receptor α (PPARα) (61) and fibroblast growth factor 21 (FGF-21), a hormone that stimulates lipid oxidation and ketogenesis, are induced by FXR (62). Beyond its direct hepatic effect, FXR-mediated induction of FGF-19 is also crucial for controlling hepatic lipid metabolism. Activation of FGF-19 seemingly improves the metabolic rate and decreases adiposity by increasing brown adipose tissue (BAT) in mice (33). In addition, FGF-19 has been shown to both reduce the development of steatosis and improve insulin sensitivity, another mechanism associated with FXR (63). Notably, circulating FGF19 concentrations are reduced in NASH, suggesting dysregulated FGF-19 expression contributing to NASH pathogenesis (64).

Considering these mechanisms, obeticholic acid, an FXR agonist, has shown a positive effect on liver histology in NASH patients during Phase 2 clinical trials (65). However, its utilisation is accompanied by a number of adverse effects, such as hypercholesterolemia, an increased chance of cardiovascular disease, pruritus, and concerns regarding tumorigenesis due to FGF-19 induction (63). Therefore, direct administration of FGF-19 or FGF-21 could effectively treat various metabolic disorders, while potentially reducing the broader spectrum side effects of FXR and PPARα agonists (33). For instance, the non-tumorigenic FGF-19 analogue NGM282 has shown rapid and significant reductions in liver fat content and improvements in alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), and non-invasive serum fibrosis biomarkers in NASH patients. NGM282 also increased LDL-C levels, a side effect also observed with obeticholic acid (66). However, concomitant lipid-lowering therapies like statins have been shown to mitigate the cholesterol increase

seen with NGM282 (67). Notably, administration of SGLT-2 inhibitors also increases LDL-C despite the beneficial aspects on cardiovascular outcomes (68).

1.4.2 Bile Acids and the Immune System: A Double-Edged Sword in Inflammation

BAs can interact with diverse immune cells and modulate cytokine production. Innate and adaptive immune cells both express BA receptors, with an increasing agreement that BAs exert pro-inflammatory as well as anti-inflammatory responses. (69, 70)

FXR is expressed in macrophages, monocytes, natural killer T cells (NKT), and dendritic cells (DCs). The triggering of FXR in monocytes and macrophages inhibits toll-like receptor 9 (TLR9), thereby exerting anti-inflammatory effects by repressing NF-κB, decreasing the production of proinflammatory cytokines. In NKT cells, FXR activation inhibits the manufacture of osteopontin, interleukin 1β (IL-1 β), and interferon γ (IFN- γ) (70). Bile acid-dependent FXR activation combats bacterial overgrowth, thus ensuring the integrity of the mucous membranes and alleviating experimentally induced colitis (40). TGR5 in monocytes, macrophages, and DCs inhibits the pro-inflammatory NF-κB signaling pathway, reducing the production of interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor α (TNFα) (70). Studies have demonstrated the potential of TGR5 activation to mitigate gastric inflammation and prevent colitis development (40). Besides the interactions with innate immune cells, there is growing evidence of BA signaling interactions with adaptive immune cells. Although there had previously been an assumption that T cells lacked FXR and TGR5 expression, recent research highlights the role of BAs via VDR and FXR in inflammatory bowel disease. For example, stimulation of VDR and FXR in T helper cells inhibits their pro-inflammatory actions and proliferation (69). Due to their broad immune-metabolic anti-inflammatory actions, BAs and FXR/TGR5 agonists hold considerable promise in tackling inflammation in autoimmune diseases (40). Conversely, BAs can activate the innate immune system, contributing to the pathogenesis of for example cholestatic liver disease. They can damage mitochondria in hepatocytes, releasing damage-associated molecular patterns (DAMPs) that activate TLR9, stimulating inflammatory cytokine expression. This process recruits cytotoxic cells to the inflammation site and induces additional tissue damage (71).

The effects of BAs on immune cells vary depending on the BA profile, as different BAs have varying potencies on receptors such as FXR and TGR5. Therefore, the outcomes of BA changes are difficult to predict (72). Even a higher receptor affinity nor always ensures a stronger effects (69).

Innate and adaptive immune dysfunction is a key component of chronic liver disease, contributing to the progression of liver fibrosis. The immune status of patients is subject to variation. Alterations to the balance between pro- and anti-inflammatory processes result in dynamic responses during the course of the disease (73). Disrupted immune homeostasis is partly caused by impaired gut barrier function and the presence of LPS from microbiota, that both dysregulate macrophage function (69). Inflammatory mediators such as ethanol have been shown to compromise the functional integrity of the intestinal barrier, leading to a state known as “leaky gut”. This causes endotoxins, LPS, and gram-negative bacteria to enter the portal system. These inflammatory initiators are thought to be involved in the onset of chronic liver disease (74). The gut microbiota also modulates BA metabolism, influencing intestinal permeability and PH through FXR. At the same time, cirrhosis and PH influence microbiota composition and promote bacterial translocation (75). In patients with cirrhosis DCA and HDCA are further dysregulated by infection. These two BA, synergizing with LPS, induce proinflammatory cytokines (72). In vitro, a BA mix mimicking the BA composition in cirrhosis significantly induced ROS production by unstimulated neutrophils, primarily due to the binding and activation of TGR5 by LCA (76).

1.4.3 Heart and Liver Interplay: The Cardiovascular Impact of Bile Acids

1.4.3.1 Bile Acids Role in Cirrhotic Cardiomyopathy

Cirrhotic cardiomyopathy (CCM) occurs in about 50% of cases of liver cirrhosis (77) and is characterized by specific alterations in cardiac function and structure, where alternative cardiac pathologies are absent. The mechanisms initiating CCM remain partially understood (78), but in part electrophysiological abnormalities contribute, including altered pharmacological responsiveness, disrupted contraction response to electrical stimuli, and prolonged QT intervals. Notably, prolongation of QT interval was the most commonly seen abnormality in cirrhotic versus non-cirrhotic controls, elevating arrhythmia risk and sudden

cardiac death likelihood, irrespective of the underlying cause of cirrhosis. Whether the severity of cirrhosis correlates with prolonged QT intervals differs in several studies conducted (77, 79, 80).

The increased prevalence of cardiac dysfunction in liver cirrhosis has spurred research into the impact of BA metabolism abnormalities on that finding (77). However, human studies investigating the association of BAs and CCM are scarce (78). A recent study involving 86 cirrhotic patients demonstrated a correlation between elevated serum concentrations of conjugated BAs and several cardiac parameters, including an echocardiogram and cardiac MRI indicators of diffuse myocardial fibrosis. These findings suggest BAs´ involvement in hyperdynamic circulation and cardiac dysfunction in cirrhosis (81).

In animal studies, bile duct ligation in mice induced significant hemodynamic changes, leading to increased cardiac output, hypotension, and basal bradycardia, which cholestyramine administration reversed. This implies BAs´ direct influence on these parameters. In vitro, BA exposure to cardiomyocytes slowed down cardiomyocyte contraction, also seen with isolated DCA administration. Further negative inotropic effects were shown. Injection of CA also induced negative chronotropic effects. Cardial mitochondrial function is also affected by BAs. In vitro, varying hydrophobicity levels of BAs influenced mitochondrial energetics differently. Relatively more hydrophobic BAs resulted in alterations in mitochondrial energetics, while relatively hydrophilic BAs had less effect. GUDCA showed the least mitochondrial toxicity (77). It has been demonstrated that the most hydrophilic BA, UDCA, has the potential to improve peripheral blood flow in patients with chronic heart failure and safeguard the heart from reperfusion injury (82, 83).

The role of receptors that mediate actions of BAs, has gained attention in the context of CCM. Receptors such as FXR were found to be expressed in the tissue of heart and vasculature. FXR has been identified as a novel apoptotic mediator and stimulates myocyte apoptosis dose- and time-dependently. Also, mitochondria are disrupted upon FXR activation. A low expression of FXR was found in the hearts of adult mice, which increased significantly after myocardial ischemia or reperfusion of the heart muscle (84). In FXR knockout mice cardiac functionality was better and infarct sizes were smaller. These results support the role in cardiac apoptosis and the development and progression of heart injury (85). The FXR receptor could also play a role here via indirect mechanisms by affecting lipid and glucose metabolism, inflammation, oxidative stress, vascular remodeling and cell death,

Also, VDR and PXR are relevant regulators in cardiac disease. In animal model's vitamin D prevents cardiac dysfunction and ventricular hypertrophy, while PXR controls inflammatory responses, targeting the activity of enzymes and transporters associated with oxidative stress (86).

1.4.3.2 Bile Acids, Vascular Function and Extrahepatic Hemodynamics

Liver cirrhosis is associated with significant changes in extrahepatic hemodynamics, resulting in peripheral systemic vasodilation, overall hyperdynamic circulation, and central hypovolemia. The contribution of BAs in the pathogenesis of these changes and their impact on vascular function will be discussed in the following section (21).

The vasodilatory properties of BAs have been well documented in the recent decades and have gained renewed attention due to the identification of BA receptors in cardiovascular tissues (87). Two receptors, FXR and TGR5, are also expressed in endothelial cells and are believed to mediate these properties. Research has demonstrated that TLCA-induced activation of TGR5 leads to the phosphorylation of endothelial nitric oxide synthase (eNOs) through an increase in [CA2+] and AKT activation, rather than the cAMP/PKA mechanism. This results in increased production of nitric oxide (NO), which in turn leads to higher conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). In the development of atherosclerosis, adhesion of monocytes to endothelial cells is a crucial step that is suppressed by NO through TGR5-mediated NO production (88). Ligands of TGR5 also modulate the activity of enzymes participating in the formation of hydrogen sulfide (H2S), a potent vasodilator (87).

Additionally, FXR plays a crucial part in adjusting vascular reactivity. Immunohistochemical evidence has confirmed FXR expression in the vasculature, and activation by CDCA has been shown to significantly increase NO levels in the vasculature, suggesting regulation by NO mechanisms (89). FXR expression has also been detected in pulmonary endothelial cells in both humans and rats. Upon activation the expression of endothelin-1 (ET-1), a vasoconstrictor, was downregulated (90). Moreover, FXR seems to modulate eNOS at the transcriptional level, as both pharmacological and genetic activation of FXR has been shown to significantly enhance eNOS promoter activity (91). There is also evidence that eNOS activity can be regulated post-transcriptionally via phosphorylation (87). These mechanisms may explain the association between BA retention and hepatic

pulmonary syndrome (HPS) and gas exchange abnormalities. In patients meeting the criteria for HPS, both bilirubin and serum BA levels were significantly elevated (92). But also, endothelium-independent mechanisms seem to cause vasodilation through bile acids. Ca^{2+}-activated K^+ (BK_{Ca}) channels, which are abundantly expressed in vascular smooth muscle cells, lead to hyperpolarization of the cell membrane, counteracting vasoconstriction (93). One study found a reduction in vascular tone and blood pressure in mice and dogs. This effect was reversed by iberiotoxin, an inhibitor of BK_{Ca}, which supports the role of TGR5 stimulated activation of BK_{Ca} channels in peripheral arterial vasodilation (94).

Portal hypertension leads to an augmentation of hepatic resistance to blood influx into the liver and is associated with hepatic endothelial dysfunction. The dysfunction of the cirrhotic liver to generate NO contributes largely to the decreased ability to respond to changes in portal blood flux by vasodilation. This deficiency in eNOS activity is associated with eNOS binding to caveolin-1, whose levels are elevated in the cirrhotic liver (87, 95). By activating TGR5, it may be possible to modulate the expression of CSE and H_2S production, thus rescuing the transsulfuration pathway in cirrhotic livers and potentially treating portal hypertension (87).

1.4.4 The Gut Microbiome and Bile Acid Dynamics

The diverse microbial communities in the digestive tract are important for regulating various pathophysiological processes in humans, including maintaining intestinal homeostasis. In this bidirectional relationship, bile acid homeostasis and variety are influenced and modulated by the efforts of the host and the gut microflora, while the bile acid pool and composition conversely influence the diversity and stability of the gut microflora. Disturbances in bile acid and gut flora have been linked to various medical conditions, including inflammatory bowel disease (IBD), type 2 diabetes (T2DM), hepatocellular carcinoma (HCC), colorectal cancer (CRC), and polycystic ovary syndrome (PCOS) (96).

Bile acids possess antimicrobial properties, with their concentration and hydrophobicity playing key roles in their antimicrobial actions. Resistance to BAs is a major selective pressure shaping the microbiome structure in the gut (97). Gram-negative bacteria are broadly resistant to BA-induced membrane and DNA defects, whereas BA-susceptible bacteria – such as *Staphylococci*, *Spirochetes*, *Enterococci,* and *Pneumococci* – are inhibited in their growth and proliferation, preserving gut flora homeostasis and barrier function (96).

High concentrations of DCA in the intestine, often resulting from high dietary fat contents, can shift the bacterial population from *Firmicutes* to *Bacteroidetes*. This shift includes increased abundances of *Parabacteroides* and *Bacteroides* and decreased BSH-expressing bacteria, such as *Lactobacillus*, *Clostridium XI*, and *Clostridium XIV* at the genus level. These alterations in microbial communities could induce and promote inflammatory responses in the intestinal epithelium (98).

1.5 Transjugular Intrahepatic Portosystemic Shunt (TIPS)

The leading cause of complications related to liver cirrhosis is portal hypertension (PH). That is the result of increased intrahepatic resistance and subsequent activation of vasodilatory pathways, leading to splanchnic vasodilation and resultant effective hypovolemia. This pathophysiological cascade contributes to cirrhotic cardiomyopathy and renal dysfunction through circulatory dysfunction and organ hypoperfusion, further exacerbating clinical manifestations like ascites and hyponatremia. Moreover, PH facilitates bacterial translocation, triggering systemic inflammation and oxidative stress, which contribute to a hyperdynamic circulatory state and increase the risk of further cardiac complications (99). Complications stemming from liver cirrhosis associated with portal hypertension include ascites, bleeding from esophageal varices, hepatic encephalopathy, hepatic hydrothorax, hepatorenal-, and hepatopulmonary syndrome. TIPS is an established intervention to reduce portal pressure, thereby addressing the key upstream factor driving many of the severe complications connected with liver cirrhosis (99, 100).

1.5.1 TIPS-Intervention

The procedure is usually performed by an interventional radiologist under general anesthesia or deep sedation. Initially, the internal jugular vein (IJV), preferably the right IJV as it allows a direct path to the IVC, is punctured under sonographic guidance. A catheter is then navigated through the IVC past the right atrium to access one of the hepatic veins, with the right hepatic vein often being the vein of choice. This preference is due to its facilitation of an anterior inferior transhepatic puncture of the right portal vein (101). The utilization of real-time ultrasound guidance facilitates visualization of the portal vein, thus enabling navigation through the liver parenchyma until one of the primary branches of the portal vein is located. The optimal entry point is 1 to 2 cm above the main bifurcation to reduce the risk

of extra-hepatic puncture and potential hemoperitoneum. Following this, an angiographic catheter is advanced to confirm access to the portal vein. The channel is then widened to 8 to 10 millimetres. Finally, a semi-covered stent is placed between portal venous end and the hepatic vein (102).

1.5.2 Indications

TIPS is increasingly recognized not merely as a bridging therapy to liver transplantation but as a viable treatment option for various complications associated with portal hypertension, notably refractory variceal bleeding and refractory ascites (103).

Variceal Bleeding:
For cirrhotic patients at high danger of treatment failure in acute variceal bleeding, early intervention with TIPS within 72h (ideally ≤ 24 h), is crucial. It is also recommended for patients experiencing persistent bleeding despite concomitant pharmacological and endoscopic therapies, or for those with severe rebleeding episodes. In case of failure of the standard secondary prophylaxis, TIPS also represents the preferred option. Although TIPS has demonstrated efficacy in reducing variceal rebleeding rates and rebleeding-related mortality compared to endoscopic procedures, it is associated with an enlarged incidence of hepatic encephalopathy and offers no advantage for overall survival (104). The utility of TIPS in managing cases of bleeding from isolated gastric varices (IGV) or gastroesophageal varices (GOV) seems promising but warrants further research (103).

Refractory Ascites:
TIPS is a common indication for managing refractory ascites in cirrhotic patients. While large-volume paracentesis with concomitant albumin infusion is a well-established treatment, its use is restricted due to side effects on hemodynamics, renal function, and nutritional status. Previous concerns regarding an uncertain effect of TIPS on patient´s survival (103) have been alleviated by meta-analyses that suggest better survival rates with TIPS compared to large volume paracentesis. Even further, the prognosis improved when TIPS was placed earlier in the course of the disease (105, 106). This suggests the potential for earlier TIPS intervention in the disease course for patients with recurrent, though not yet refractory, ascites (103, 107). Still there are indefinite suggestions for the best timing of TIPS laying and the ideal stent diameter to minimize the chance of shunt-related adverse-effects

(107). Further, several non-hepatic factors, including heart and kidney function, must be considered when initiating the utilization of TIPS for managing ascites (108).

Hepatic Hydrothorax:

In cases of hepatic hydrothorax (HH), a less common complication of cirrhosis characterized by repeated pleural effusions in patients with end-stage liver disease, TIPS may offer symptomatic relief. Proposed mechanisms for hydrothorax are the transdiaphragmatic passage of peritoneal fluid through a porous diaphragm, decreased oncotic pressure by hypoalbuminemia, and leakage of the thoracic duct. The primary management of HH is diuresis in conjunction with fluid and salt restriction, with other treatment options comprising TIPS, thoracentesis, and liver transplantation (109). TIPS has been shown to be efficacious in achieving symptomatic relief, leading to a reduction in the frequency of thoracocentesis (110), however, with only complete resolution of HH in some patients (111). Also, it was demonstrated that TIPS for HH does not raise the possibility of death in cirrhosis patients, indicating safety of use in carefully selected patients with low expected post-TIPS mortality (111, 112).

Portal Vein Thrombosis:

In liver cirrhosis the prevalence of portal vein thrombosis (PVT) is higher ranging between 4.4 and 15.8% and increases proportionally with the severity of portal hypertension and liver disease (113). In liver transplant recipients, the existence of PVT at the moment of transplant is associated with heightened posttransplant mortality. Apart from liver transplant candidates, it is unclear whether PVT is independently causative of increased mortality (114).While the standard approach to anticoagulation in a no-cirrhotic portal vein thrombosis is to administer the medication, in cirrhosis it is necessary to undertake comprehensive evaluation of the risks and benefits of anticoagulation. This is due to the complex hemostatic profile, which features both bleeding and procoagulant features. In accordance with established guidelines though, the administration of anticoagulation for PVT in cirrhosis should not be considered intimidating, and may indeed be endorsed, particularly in cases that are acute or subacute. In cases where progression or complications of portal hypertension such as recurrent bleeding or recurrent ascites, are encountered and remain unmanageable medically or endoscopically, TIPS can be considered a viable option (113, 114). TIPS has been shown to be more effective than endoscopic band ligation plus propranolol in reducing the incidence of variceal bleeding and decreased the incidence of

death due to acute gastrointestinal bleeding, without increasing the risk of HE (115, 116). The benefit did not translate to better overall survival, though endoscopic therapy plus propranolol may still be preferable (116).

Primary Budd-Chiari Syndrome:

Budd-Chiari syndrome (BCS) is a rare condition affecting hepatic vein or inferior vena cava with obstructed hepatic venous outflow and requires a step by step therapeutic approach with anticoagulation, angioplasty, TIPS and liver transplantation as available options (117). TIPS in BCS is indicated with remaining ascites or further related complications when anticoagulation and mechanical revascularization fail. The successful implementation of TIPS in BCS may be more challenging due to hepatic vein obstruction, so these patients should only be treated at centers of high expertise. But when correctly performed TIPS offers particularly good long-term outcomes, symptomatic resolution, and prevents chronic liver damage and cirrhosis (118).

Hepatorenal Syndrome:

Hepatorenal syndrome (HRS) is defined as renal dysfunction in cirrhosis patients, characterized by renal vasoconstriction with reduced blood flux and glomerular filtration rate. The prognosis of HRS is poor, with high mortality rates. Besides pharmacological treatment, invasive procedures, including plasma exchange, renal replacement therapy, TIPS and liver transplantation can be considered. Liver transplantation remains the most helpful treatment consideration for HRS and end-stage liver disease, yet the efficacy of TIPS in enhancing renal function has also been demonstrated (119). In cases of diuretics refractory ascites and uncontrolled variceal hemorrhage TIPS is especially beneficial (120). Overall, there is very low convincing evidence on the effect of TIPS compared with conventional treatment yet (121).

1.5.3 Contraindications

Table 1. TIPS contraindications

Absolute Contraindications	Relative Contraindications
Sever liver failure (CPS > 13, MELD > 20)	Anatomical/technical contraindications
Heart failure (in particular right heart failure)	Extensive (hepatic) malignancy.

Pulmonary hypertension	History of recurrent spontaneous episodes of hepatic encephalopathy grade III/IV[a]
Unrelieved biliary obstruction	Severe coagulopathy and/or thrombocytopenia
Severe sepsis	
Extensive hepatic cysts	

Note. [a]Acute HE at the time of acute variceal bleeding does not represent a contraindication for a bleeding TIPS. Adapted from (122) and (102).

Severe Cardiopulmonary Disease:

Placement of a TIPS radically changes central hemodynamics by increasing central blood volume, cardiopulmonary pressure, cardiac output, and stroke volume (123). In long-term follow-up the median left atrial diameter, left ventricular end-diastolic diameter, and pulmonary artery systolic pressure were increased. Therefore, pre-existing congestive heart failure, labeled as left ventricular ejection fraction <50%, as well as pulmonary hypertension or severe valvular diseases are a contraindication to TIPS implantation (124). In mild to moderate diseases a TIPS can be placed if the benefits outweigh the risks that can be stratified with some tests. For example, a low E/A ratio of less than or equal to one, indicating advanced diastolic dysfunction, is a predictor of higher mortality after TIPS. Also, patients with a n-terminal pro-BNP (NT-pBNP) above the threshold of 125 pg/ml are at higher risk of cardiac decompensation (123). So, heart failure and presence of high pulmonary pressures are absolute contraindications to TIPS (118).

History of Hepatic Encephalopathy:

Hepatic encephalopathy after TIPS is one of the most common complications following the procedure. The pathophysiology is multifactorial, with inefficient hepatic metabolism of ammonia by the urea cycle in liver cirrhosis and bypassing of hepatic perfusion via the TIPS leading to serum hyperammonemia (125). The incidence of HE is increased by 30-50% post-procedure, which develops within the first three months in 90% of affected patients (126). Poor nutritional status or sarcopenia, large spontaneous portosystemic shunts, proton pump inhibitor use, hyponatremia, diabetes, impaired renal function, poor liver function and an age of >70 are contributory risk factors for the manifestation of post-TIPS HE. Also, procedural risk factors may contribute to post-TIPS HE. For example, the stent diameter matters to the post-TIPS HE risks, with stents bigger than 10mm associated with a higher incidence. Additionally, a history of HE is a highly established predictor of HE after TIPS in most cases. Consequently, the presence of recurrent and persistent HE is an absolute

contraindication for TIPS, unless it is performed as a salvage procedure for a planned liver transplant (127).

1.5.4 Complications

Complications from TIPS can be categorized into immediate procedure-related clinical complications and long-term complications:

Table 2. TIPS complications

Immediate complications	Long-term complications
Puncture related bleeds, including hemoperitoneum	Intimal hyperplasia within the TIPS -> reduced blood flow
Stent malposition/migration	TIPS thrombosis
Early shunt thrombosis	
Shunt-related encephalopathy (30% to 46%)	
Deterioration of hepatic function	
Shunt stenosis	

Note. Adapted from (102).

Hepatic Infarction:

Hepatic infarction can arise as a consequence of the aforementioned complications. However, it is a rare occurrence following TIPS due to the liver´s dual blood supply. Diagnosis is challenging, as ultrasound findings are often nonspecific. Potential mechanisms contributing to its development include portal vein thrombosis, hepatic arterial injuries (such as needle puncture or compression by the expanded stent graft), overall hemodynamic instability, or a low portosystemic gradient. (128).

Endotipsitis

A uncommon but dangerous complication of TIPS, with an estimated infection rate of 1-1.7% (123, 129, 130) is endotipsitis, defined as systemic infection accompanied by shunt occlusion, vegetation on the shunt, or bacterial growth originating from the stent (123). Since endotipsitis is always a diagnosis of exclusion unless the pathogen is identified within the thrombus, a high degree of clinical suspicion is necessary. Consequently, its true rate is likely underestimated.(131).

2 Patients and Methods

2.1 Study Design and Study Plan

A prospective, open-label, single-center study was conducted to evaluate bile acid metabolism in patients admitted to the department of gastroenterology and hepatology for elective TIPS placement between March 2023 and March 2024. This investigation was a sub-study within a larger TIPS study entitled “Optimizing selection and periinterventional management of patients undergoing a transjugular intrahepatic portosystemic shunt implantation”. Exclusion criteria were partly based on TEG-controlled coagulation substitution requirements, but our bile acid sub-study primarily involved control group patients, who did not require coagulation substitution. The principal investigator of this study is Univ.-Prof. Priv.-Doz. Dr. Vanessa Stadlbauer-Köllner. Our trial aimed to investigate alterations in bile acid metabolism and profile after TIPS implantation in cirrhotic patients. Approval was obtained from the ethics committee of the medical university of Graz, with all study procedures adhering to good clinical practice and the declaration of Helsinki.

2.2 Eligibility and Enrollment

Eligible participants were selected based on inclusion and exclusion criteria. The inclusion criteria required participants to be willing and able to provide informed consent, have histological/clinical/radiological evidence of cirrhosis, be over 18 years of age, and have an indication for elective TIPS implantation. Exclusion criteria comprised contraindications to TIPS implantation, ongoing bleeding, hepatic encephalopathy grade 3 or 4, hepatocellular carcinoma BCLC D, pre-existing anticoagulant therapy at the time of enrollment, administration of blood products within one week prior to enrollment, other malignancies leading to an impaired 90-day survival, inherit coagulation disorders, and any other condition or circumstance interfering with protocol adherence. Patients fulfilling the inclusion criteria were recruited during their initial visit (Day 0). Written informed consent was obtained before conducting any study-related procedures from all subjects.

2.3 Protocol and Procedures

Baseline characteristics were obtained, and patients were either randomized or assigned to the control group in alignment with the criteria of the larger TIPS study. The cohort subdivision was irrelevant to our sub-study; however, it is important to note that patients in our analysis were mostly part of the control group. Fasting bile acids were collected from a peripheral vein puncture at admission on Day 0 (T0) in all patients, regardless of their assigned cohort. During TIPS-procedure, blood samples were taken from the hepatic vein (T1) before portal vein puncture and from the portal vein (T2) immediately after the puncture but before stent placement. Additional sampling occurred 12-72 hours post-TIPS (T3) and at a follow-up visit 90±7 days (T4) after TIPS (**Figure 1**).

In addition to this prospective study part, we retrospectively analyzed bile acid parameters in two liver cirrhosis cohorts. This was meant to elucidate the physiological changes that occur due to liver cirrhosis itself, making it easier to discern post-TIPS parameters from already existing changes. The preexisting two datasets were provided by Univ.-Prof. Priv.-Doz. Dr. Vanessa Stadlbauer-Köllner and Priv.Doz. DDr. Elisabeth Tatscher.

2.4 Data Collection and Analysis

Data was documented and collected via the medical information system "openMEDOCS" or the Case Report Form (CRF). The following data was assessed:

- **Demographic patient data**: Sex, age, and the clinical history, including the diagnosis of cirrhosis, the etiology, previous decompensations, past and present medical therapy
- **Clinical examination**: The presence of ascites and hepatic encephalopathy
- **Laboratory parameters**: The day before TIPS, 12-72 hours after TIPS, in the follow-up visits and additionally during the TIPS-procedure full blood count, liver function tests (bilirubin, AP, GGT, AST, ALT), renal function (creatinine, urea), PT, PT-INR, fibrinogen, activated prothrombin time (aPTT), antithrombin (AT III), thrombelastography, creatine kinase, C-reactive protein, HDL cholesterol and bile acid parameters (total BA, total UDCA, BA-derivates) were routinely assessed in the central laboratory of the University of Graz.
- **Post-TIPS**: 12-72 hours after TIPS and at the follow up visits' complications of the TIPS procedure and the amount of blood products administered were captured. Also, the current medication was obtained.

2.5 Influence of TIPS Placement on Bile Acid Levels

The primary objective of this study was to assess the effect of TIPS procedure on total bile acid (TBA) concentrations. Limited by the timeframe of a diploma thesis, only seven patients who received elective TIPS were included from March 2023 to March 2024. Blood samples were taken before, during and after TIPS-intervention (**Figure 1**). The first two patients were excluded in some comparisons since BAs were measured postprandially before TIPS but in a fasted state after TIPS. On the day of TIPS and after the intervention fasting BAs were collected in all seven patients. The study protocol was changed accordingly, and the subsequent participants had fasting BAs measured consistently.

The determination of bile acid levels and bile acid composition was achieved by using a high-resolution mass spectrometry technique employing a full scan method (QExactive Orbi Trap, Thermo Scientific) as previously methodically outlined in references (132, 133).

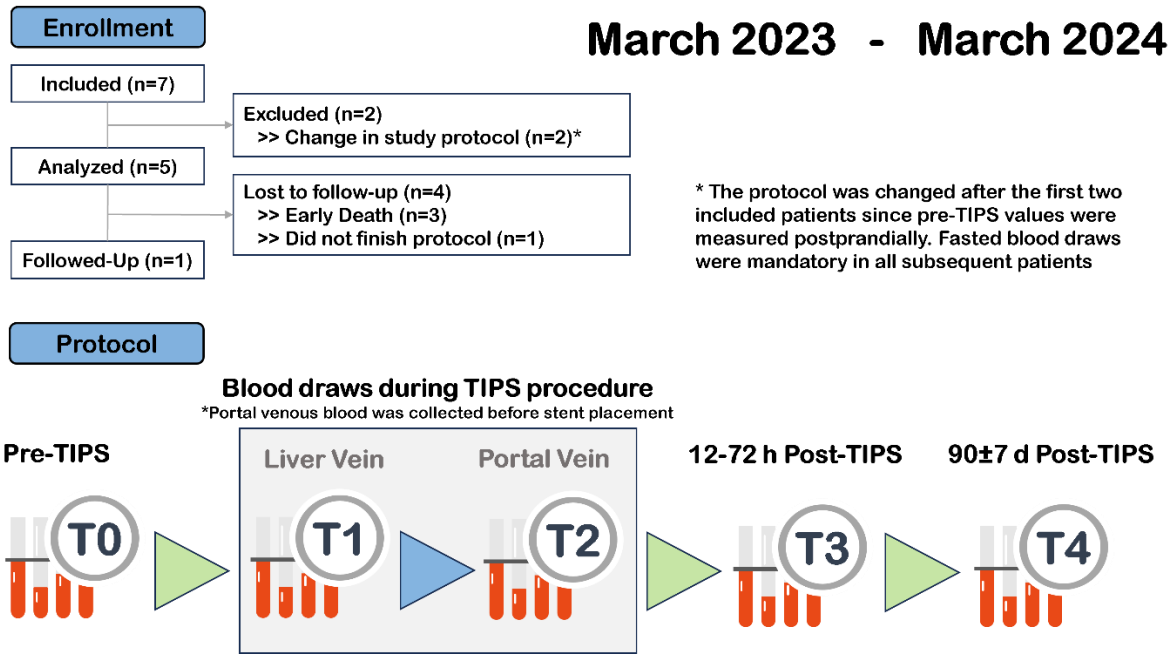


Figure 1. Flowchart of enrolled TIPS patients and protocol of the study plan.

2.6 Retrospective Analysis

Two different datasets were analyzed retrospectively regarding the bile acid composition: Cohort 1 is displayed in **Figure 2A** and cohort 2 in **Figure 2B.** Since we aimed to determine the effects of cirrhosis, we excluded all patients with uncertain cirrhosis and cholestatic liver

disease. In Cohort 1 13 patients with Cholestasis were excluded as well as six more patients, two with PSC, three with ASH, and one with PBC, consequently including 173 cirrhotic patients and 23 healthy controls for the analysis (**Figure 2A**). Patients with PSC/ASH/PBC related cirrhosis were excluded from the study due to the vastly different pathophysiology, behavior, and course of cholestatic liver diseases regarding the bile acid metabolism. Also, in cohort 2 a total of 30 patients were excluded leading to the inclusion of 101 cirrhotic patients and 10 healthy controls. (**Figure 2B**).

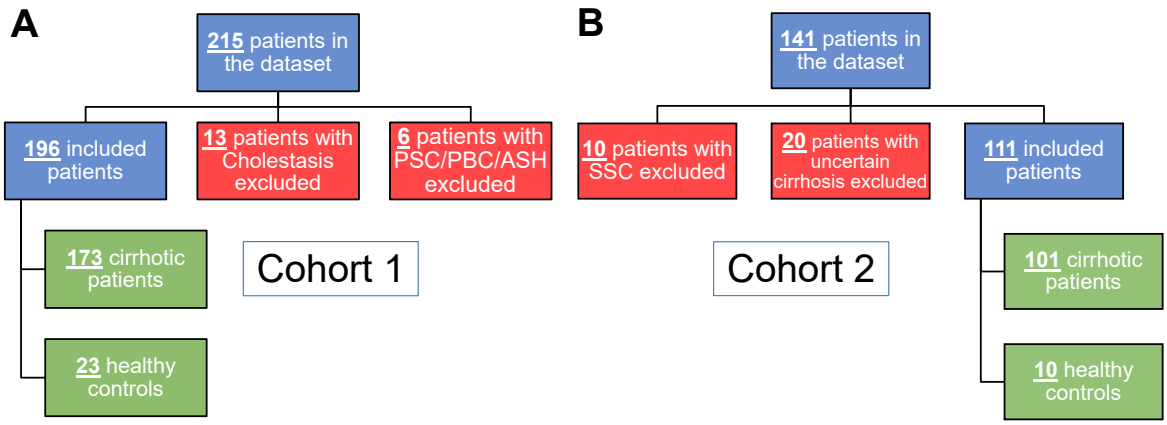


Figure 2. Flowchart of patients in the retrospective analysis. (A) Cohort 1 and (B) Cohort 2. PSC, Primary Sclerosing Cholangitis; PBC, Primary Biliary Cholangitis; ASH, Alcoholic Steatohepatitis; SSC, Secondary Sclerosing Cholangitis.

2.7 Statistical Analysis

Statistical analysis was conducted using SPSS version 28-29, GraphPad Prism 10, and Microsoft Excel 365. Nonparametric variables were expressed as median (interquartile range, IQR) and were compared using Mann-Whitney's U test. Parametric variables were expressed with mean (standard deviation, SD) and were compared using two-tailed paired t-test for paired variables and two-tailed unpaired t-test for unpaired variables. Unmatched or unpaired data with more than two groups was analyzed using ordinary one-way ANOVA for normally distributed variables or Kruskal-Wallis test for nonparametric data. Matched or paired data with more than two groups was analyzed with RM one-way ANOVA for parametric data or Friedman test for nonparametric data. Post-hoc analysis was conducted using Tukey´s multiple comparison test in parametric data or Dunn´s multiple comparison test in nonparametric data. Categorical variables were expressed as absolute numbers or

percentages and were compared using Pearson´s chi-squared test or Fisher´s exact test. For all statistical tests, a p-value less than 0.05 was considered statistically significant.

3 Results

3.1 Bile Acid Levels Decrease Pre- to Post-TIPS

The majority of the cohort were male patients (n=5), with the predominant etiology being alcohol-associated liver disease (n=4). Some liver parameters were elevated, with a mean gamma-glutamyl transferase (GGT) of 223 U/L and a mean alkaline phosphatase (AP) of 217 U/L. Markers of hepatic synthetic function were altered in certain patients, with four exceeding the upper limit of the International Normalized Ratio (INR) and a mean albumin level below the normal limit at 3.4 g/dl. Renal function was compromised in two patients (**Table 3**).

Table 3. Baseline Characteristics of the TIPS-Cohort

	TIPS-Cohort
Variable	**n=7**
Sex (n)	m - 5, w - 2
Age (years)	62.4±6.8
Etiology (Alcoholic/NAFLD/PBC/Cryptogenic; n)	4/1/1/1
Indication (Refractory ascites/Hepatic hydrothorax; n)	6/1
Child-Pugh-Score	8±1.2
Child-Pugh-Stage (n)	A – 1, B - 6
MELD-Score	10.6±3.6
ALT (U/L)	30 (18)
AST (U/L)	37 (21)
GGT (U/L)	222.7±241.9
AP (U/L)	217.1±174.6
Bilirubin total (mg/dl)	0.9±0.5
Ammoniac (µmol/L)	24±8.2
INR (ratio)	1.2±0.1
Albumin (g/dl)	3.4±0.5
Creatinine (mg/dl)	1 (0.8)
GFR (ml/min)	70.1±29

Note. Data are mean±SD or median (IQR).

Total bile acid levels were lower in 4 out of 5 patients 12-72 hours after the TIPS procedure. The median reduction was 10.4 µmol/L (p=0.63), which did not reach statistical significance due to the small number of patients (**Figure 3)**.

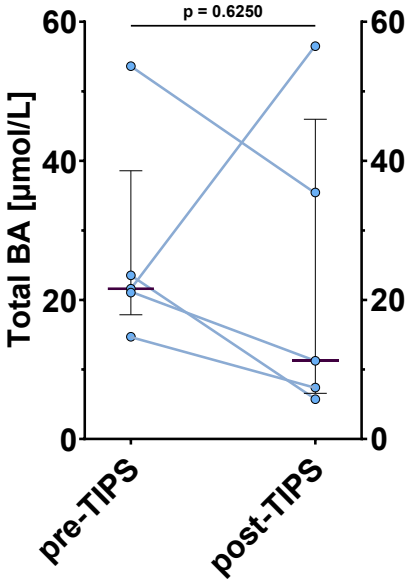


Figure 3. Impact of TIPS procedure on total bile acid levels. Measured before and after TIPS from a peripheral vein. Individual patient data points are connected by lines. Statistical analysis was conducted with Wilcoxon matched-pairs signed rank test.

3.2 Hepatic Extraction of Bile Acids is Decreased in Cirrhosis

TBA concentrations were measured in samples from liver and portal venous blood. The hepatic extraction fraction (HEF) of BAs was calculated using the formula:

$$HEF = \frac{Portal\ vein - Liver\ vein}{Portal\ vein} \quad (1)$$

One outlier with higher concentrations in the hepatic vein than in the portal vein, giving a negative HEF of -1.38, was excluded from this analysis due to probable measurement error. The HEF for the remaining six patients ranged from 0.14 to 0.71 (**Table 4**). Spearman correlation analysis was performed to evaluate if HEF depends on liver cirrhosis severity or the grade of portal hypertension, with negative correlations indicating that higher Child-Pugh-Scores (r=-0.91; p=0.0119) or HVPG pre-TIPS (r=-0.8, p=0.33) are associated with a lower HEF of bile acids (**Figure 4**).

Table 4. Individual total bile acid levels at different locations and calculated HEF

ID	Liver vein [µmol/L]	Portal vein [µmol/L]	Child-Pugh score	HVPG pre-TIPS (mmHg)	Hepatic extraction fraction
1	3.22	10.96	6	15	0.71
4	36.5	42.5	9	*	0.14
5	7.6	12.25	7	*	0.38
6	*	3.82	9	*	*
7	12.6	14.64	9	17	0.14
9	32.69	38.25	8	18.5	0.15
10	11.54	38.01	8	16	0.7
Median (IQR)	12.1 (27.1)	14.6 (27.3)	8 (2)		

Note. Hepatic extraction fraction = Portal vein - Liver vein / Portal vein; * Measurement not performed or censored due to potentially incorrect measurement

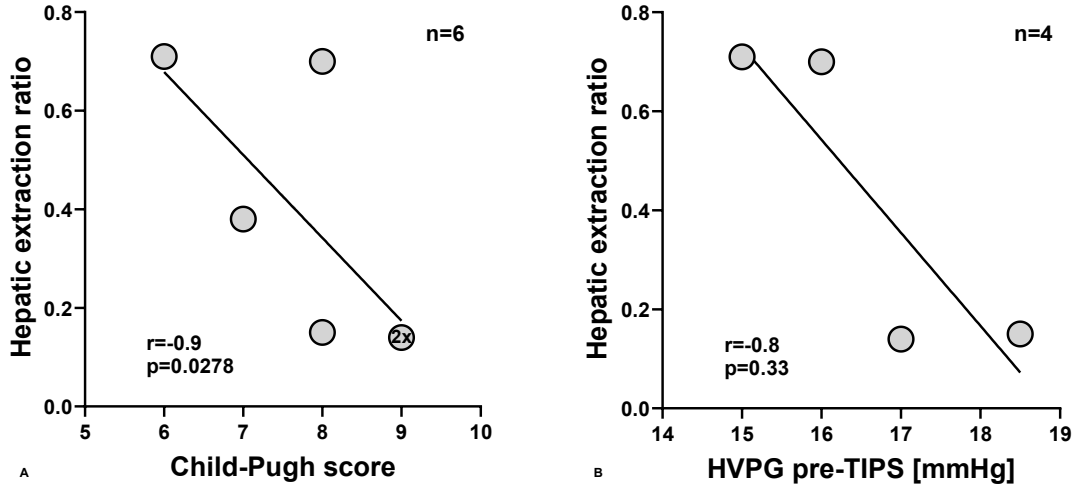
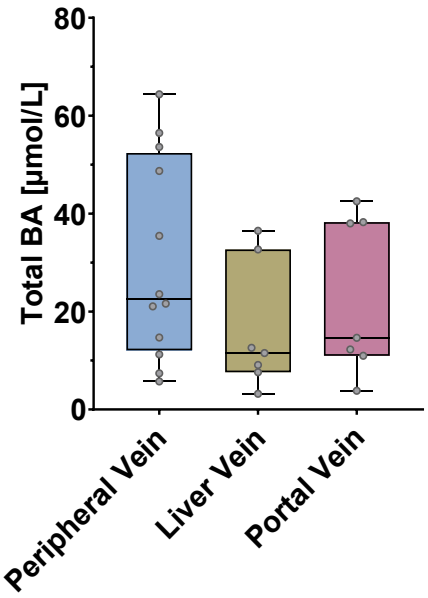


Figure 4. Correlation between hepatic extraction fraction and (A) Child-Pugh score or (B) HVPG pre-TIPS. The data point at a Child-Pugh score of 9 is labeled "2x" to denote that this value represents two patients with identical scores and extraction ratios.

3.3 Peripheral Bile Acids are Consistently Higher than in Liver and Portal Vein

Peripheral venous TBA levels were compared against centrally measured liver and portal venous levels. Bile acid levels were higher in peripheral blood compared to liver and portal

venous blood. Ratios of TBA concentrations across different sampling sites were calculated to further elucidate the distribution of bile acids. Peripheral vein pre-TIPS TBA concentrations were consistently higher than those in the portal vein (mean ratio: 0.7; IQR: 0.4) and liver vein (median ratio: 0.5; IQR: 0.2), as summarized in **Figure 5**.



Ratios	
PV/PeV-pre-TIPS	**LV/PeV-pre-TIPS**
0.52	0.15
0.40	0.35
0.57	0.35
0.26	0.62
0.70	0.60
0.71	0.61
1.61	0.49
Mean ± SD	
0.68±0.44	**0.45±0.18**

Figure 5. Total bile acid levels between anatomical measure sites and calculated ratios of portal and liver vein concentrations to pre-TIPS peripheral vein samples. PV, Portal Vein; PeV, Peripheral Vein.

3.4 Bile Acid Pool Composition does not Change Significantly

Individual bile acid profiles were assessed at different timepoints and locations as shown in **Figure 6**. No changes were observed in the composition of the bile acid profile pre- to post-TIPS. Despite the small sample size, a general consistency in the concentrations of most bile acids was noted across measurements, with the error bars (SD) overlapping in most pairs of measurement, signifying high individual variability. Some specific BAs, such as GCDCA, GCA and TCDCA appeared to have slightly higher median levels in the portal vein than in the liver vein, while other BAs were relatively comparable (median GCDCA 3.9 vs. 2.7 and TCDCA 3.7 vs. 2 µmol/L). Pre-TIPS levels to liver vein levels showed that taurine-conjugated primary BAs were higher in pre-TIPS blood (median TCDCA 4.9 vs. 2 and TCA 2.5 vs. 0.8 µmol/L).

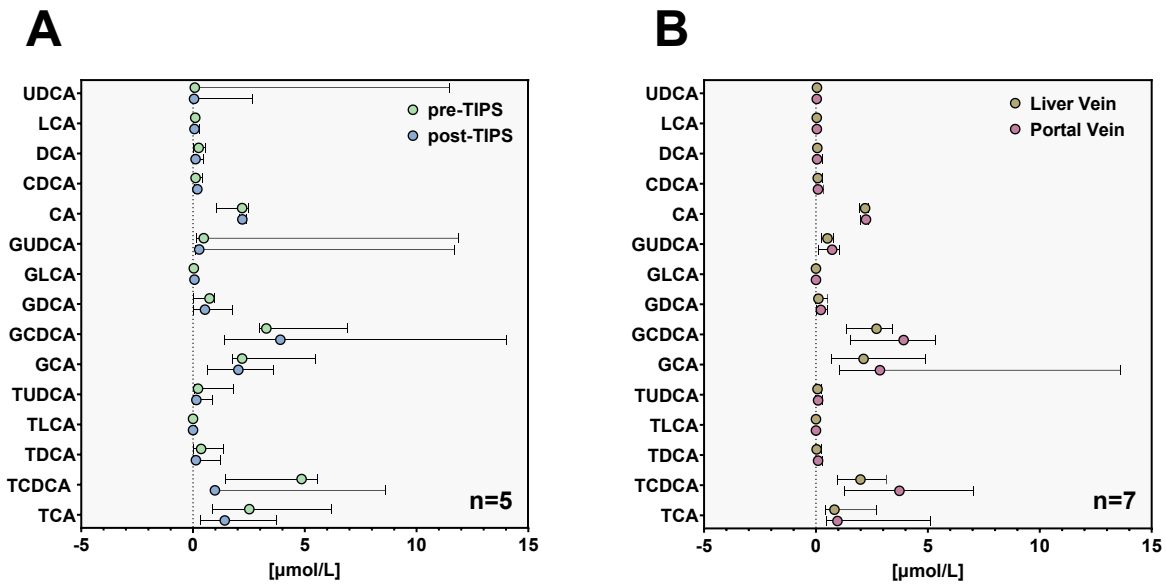


Figure 6. Median abundance of various bile acids in side-to-side comparison with (A) pre- to post-TIPS and (B) liver to portal vein, with error bars representing the interquartile range. Wilcoxon matched-pairs signed rank test with Holm-Šídák multiple comparison correction revealed no statistically significant differences.

We also calculated percentual abundances of bile acids from the calculated medians of total bile acids. Secondary conjugated bile acids like GDCA and TDCA were higher in peripheral venous samples (pre/post-TIPS 5.9%/6.1% vs. liver/portal vein 3.7%/2.7%) (**Table 7**). Other than that, we could not capture any further consistent differences in percentual abundances across location sites or timepoints.

Table 5. Percentual abundance of unconjugated bile acids between location sites and timepoints

	Unconjugated bile acids					
Location/Timepoint	**CA**	**CDCA**	**DCA**	**LCA**	**UDCA**	**Sum**
Pre-TIPS	11.66%	0.95%	1.44%	0.44%	0.35%	**14.85%**
Post-TIPS	18.19%	1.60%	0.94%	0.57%	0.40%	**21.70%**
Liver Vein	20.51%	0.74%	0.55%	0.35%	0.43%	**22.59%**
Portal Vein	18.77%	0.77%	0.42%	0.39%	0.31%	**20.65%**

Note. The percentage of calculated total bile acids from medians.

Table 6. Percentual abundance of primary conjugated bile acids between location sites and timepoints

	Primary conjugated bile acids				
Location/Timepoint	**GCA**	**GCDCA**	**TCA**	**TCDCA**	**Sum**

Pre-TIPS	12.96%	20.12%	18.68%	24.69%	**76.45%**
Post-TIPS	16.71%	32.18%	11.69%	8.10%	**68.67%**
Liver Vein	19.33%	24.60%	7.51%	18.06%	**69.50%**
Portal Vein	9.42%	24.35%	8.03%	31.11%	**72.92%**

Note. The percentage of calculated total bile acids from medians.

Table 7. Percentual abundance of secondary conjugated bile acids between location sites and timepoints

	Secondary conjugated bile acids				
Location/Timepoint	**GDCA**	**GLCA**	**TDCA**	**TLCA**	**Sum**
Pre-TIPS	3.76%	0.16%	1.93%	0.00%	**5.85%**
Post-TIPS	4.43%	0.57%	1.07%	0.00%	**6.07%**
Liver Vein	2.76%	0.00%	0.97%	0.00%	**3.73%**
Portal Vein	1.84%	0.00%	0.90%	0.00%	**2.74%**

Note. The percentage of calculated total bile acids from medians.

3.5 Follow-up TIPS Data

In the revised protocol, follow-up was successfully conducted for one patient. Among the two initially excluded patients, one was lost to follow up, while the other one was subsequently included in the follow-up analysis, having completed all subsequent assessments after pre-TIPS in a fasted state. The hypothesis was that bile acid levels do not immediately increase post-TIPS but might rise after a certain period. Data presented in **Table 8** illustrates that no significant difference in total bile acid concentrations was found between the early TIPS phase and three months after TIPS.

Table 8. TIPS follow-up data

	Follow-up	
ID	**12-72h post-TIPS (µmol/L)**	**90±7d post-TIPS (µmol/L)**
1	48.72	50.36
5	56.49	56.61

Note. The values are total bile acids expressed in micromoles per liter (µmol/L)

As of 13.05.2024, three out of seven patients were still alive after TIPS. Three Patients died within 100 days after the TIPS intervention and one a year after. The one-year survival rate was 29% and median survival was 356 days in our small cohort.

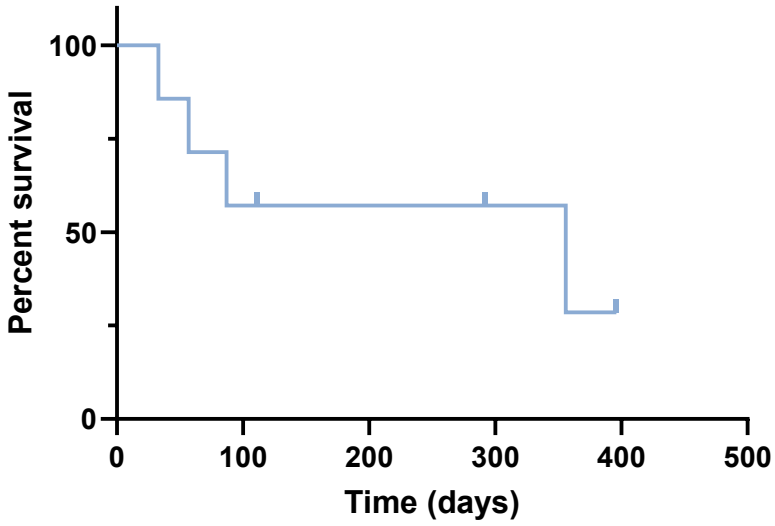


Figure 7. Kaplan-Meier curve of patient's survival after TIPS placement.

3.6 Retrospective Analysis of a Liver Cirrhosis Cohort

3.6.1 Demographic Data

In cohort 1 the most frequent cause of cirrhosis was alcoholic cirrhosis (68%), followed by hepatitis C (14.8%) and cryptogenic cirrhosis (7.1%). The entire distribution of etiology groups is shown in **Figure 8*A***. The age did not differ significantly between healthy controls and patients with Child-Pugh-Stage (CPS) A or B+C. Liver parameters were higher in cirrhotic patients, but only AST and bilirubin were significantly different between CPS A and CPS B+C. Furthermore, INR and albumin displayed deterioration with a higher CPS classification, as summarized in **Table 9**. Similar observations pertaining to demographic data and laboratory markers were made in cohort 2, as outlined in **Table 10**.

To investigate potential differences in the observed and expected frequency of Child-Pugh stages across various etiology groups of liver cirrhosis in cohort 1, a chi-squared test of independence was conducted. To satisfy the assumption of this test, each cell was required to have an expected frequency of at least 5. To enhance the test's power, etiology categories with an expected frequency of less than 5 were combined into a new category labeled "Others", which comprised Hepatitis B, Hemochromatosis, AIH and NASH-associated liver cirrhosis. With these adaptations the Pearson-Chi-Quadrat-value was 10.88 with a p-value of 0.012, indicating that a statistically significant difference exists in the distribution of CPS among the various etiology groups. The data displayed in the graph suggests that discrepancy primarily stems from the higher prevalence of Child B+C stages in alcoholic liver cirrhosis. Specifically, about 57% of alcoholic cases were classified as Child B or Child C, while the other etiology groups only had 22-36% of Child B+C cases. The severity of liver cirrhosis classified through Child-Pugh-Stages seems to be significantly higher in alcohol-related cirrhosis than in other groups in the present dataset (**Figure 8*B***).

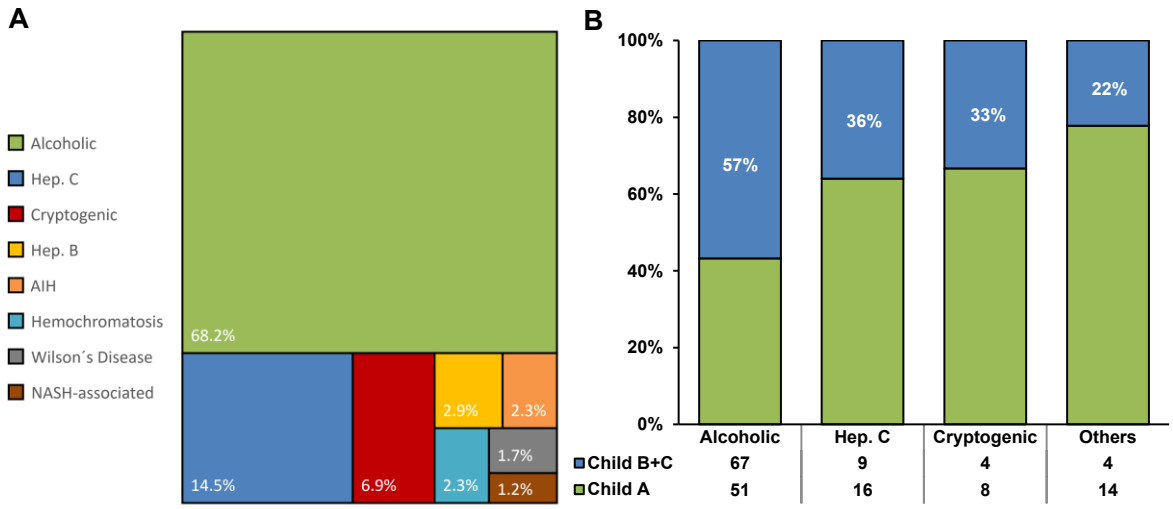


Figure 8. Cohort 1: (A) Distribution of cirrhosis etiology and (B) percentual prevalence of Child-Pugh-Stages between etiology groups. AIH, Autoimmune hepatitis; NASH, Nonalcoholic steatohepatitis.

Table 9. Cohort 1: Demographic characteristics and liver function parameters

	Controls	Child A	Child B+C	p-value
Variable	**n=23**	**n=89**	**n=84**	
Age (years)	52±11	56±14	55±12	n.s.
Etiology (Alcoholic/HCV/Others; n)	-	51/16/22	67/9/8	-
Child-Pugh-Stage (n)	-	A - 89	B - 55, C - 29	-
Child-Pugh-Score	-	5±1	8±3	-
MELD Score	6±1	9±3	14±6	$p<0.001^{\&,£,©}$
AST (U/L)	23±10	42±25	59±44	$p<0.001^{\&,£,©}$
ALT (U/L)	18±13	34±28	31±24	$p<0.001^{£,©}$
GGT (U/L)	16±13	103±124	88±125	$p<0.001^{£,©}$
AP (U/L)	63±17	104±61	121±59	$p<0.001^{£,©}$
Bilirubin total (mg/dL)	0.4±0.2	0.9±0.7	2.5±2.61	$p<0.001^{\&,£,©}$
INR (ratio)	1±0.1	1.2±0.2	1.4±0.33	$p<0.001^{\&,£,©}$
Kreatinin (mg/dL)	0.8±0.1	0.8±0.3	0.8±0.22	n.s.
Albumin (g/dl)	4.4±0.5	4.3±0.6	3.2±0.7	$p<0.001^{\&,©}$

Note. Median±IR. [&]Significant difference between Child-Pugh A and Child-Pugh B+C; [£]Significant difference between Child-Pugh A and Controls; [©]Significant difference between Child-Pugh B+C and Controls; Differences calculated via Mann-Whitney-U-Test; n.s.= not significant.

Table 10. Cohort 2: Demographic characteristics and liver function parameters

	Child A	Child B+C	p-value

Variable	n=72	n=29	
Age (years)	58±14	55±8	n.s.
Etiology (Alcoholic/HCV/others; n)	37/14/21	17/7/5	-
Child-Pugh-Stage (n)	A - 72	B - 25, C - 4	-
Child-Pugh-Score	5±1	8±1	-
MELD Score	9.2±3.6	15.3±4.5	p<0.001
AST (U/L)	43±25	62±46	p<0.001
ALT (U/L)	36±35	33±20	n.s.
GGT (U/L)	130±164	73±123	n.s.
AP (U/L)	107±75	115±66	n.s.
Bilirubin total (mg/dL)	1±0.7	2.8±2.8	p<0.001
INR (ratio)	1.2±0.2	1.4±0.2	p<0.001
Kreatinin (mg/dL)	0.8±0.3	0.8±0.2	n.s.
Albumin (g/dl)	4.3±0.7	3.2±0.2	p<0.001

Note. Median±IR. p-value = Significance of the difference between Child-Pugh A and Child-Pugh B+C; Differences calculated via Mann-Whitney-U-Test; n.s.= not significant.

3.6.2 Bile Acids are Elevated and Higher in Presence of Ascites and Hepatic Encephalopathy

Regardless of the underlying etiology, concentrations of TBA were observed to be elevated in liver cirrhosis. In the group of cirrhotic patients, TBA concentrations in systemic circulation were distinctly higher than in healthy individuals, irrespective of the severity of their cirrhotic condition (67.1±72.9 vs. 3.2±2.6 µmol/L). As the disease progressed, characterized by a higher CPS, concentrations exhibited a corresponding increase. Specifically, mean TBA concentrations in patients with CPS A were 32.6 µmol/L compared with 103.6 µmol/L in patients with CPS B or C (p<0.0001) (**Figure 9A**). A Kruskal-Wallis test was performed to clarify whether the medians between etiology groups varied significantly, which showed a p-value of 0.0001, suggesting a significant difference in TBA levels. Dunn´s multiple comparison test showed a significant difference between alcoholic and others etiology groups (p=0.0004) as evident in **Figure 9B**. When examining the data from cohort 2 in **Figure 10**, similar mean concentrations of TBA were observed in the control group, as well as in patients with CPS A and CPS B+C stages. A significant difference in TBA levels between etiology groups is noticeable as well. Again, the discrepancy primarily stems from higher TBA concentrations in alcoholic liver cirrhosis, as

confirmed by a Kruskal-Wallis test (p=0.0021). Importantly, it was noted that unlike cohort 1, the distribution of Child-Pugh-Stages across different etiologies in cohort 2 was balanced, as confirmed by a chi-squared test (Pearson-chi-squared value of 1.563; p=0.458) (**Figure 10C**). This collectively suggests that the etiology of liver cirrhosis influences the magnitude of TBA elevation, independent of the liver cirrhosis severity represented by Child-Pugh-Stages.

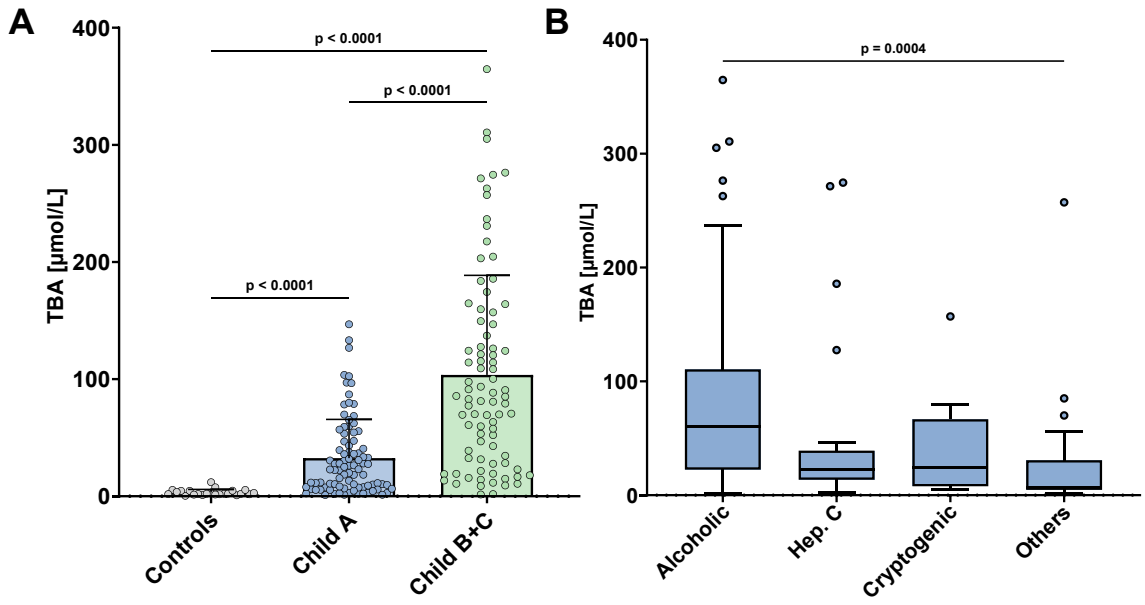


Figure 9. Cohort 1: Total bile acids in dependency of (A) Child-Pugh-Stage and (B) etiology of liver cirrhosis. Data is displayed in (A) Mean±SD and (B) Box with whiskers after Tukey. Statistical analysis was conducted using a Kruskal-Wallis test combined with Dunn's multiple comparison test.

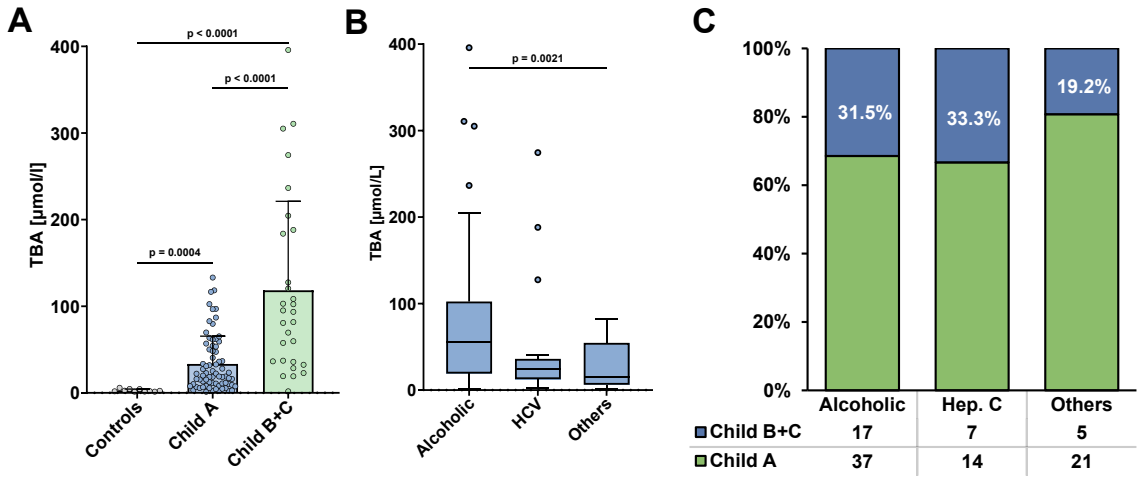


Figure 10. Cohort 2: Total bile acids in dependency of (A) Child-Pugh-Stage and (B) etiology of liver cirrhosis; (C) Percentual prevalence of Child-Pugh-Stage in etiology groups. (A) Mean+SD. (B) Box with whiskers after

Tukey. Statistical analysis was conducted using a Kruskal-Wallis test combined with Dunn's multiple comparison test.

Next, we investigated whether clinical features such as portal hypertension (PH), hepatic encephalopathy (HE) and ascites had an influence on TBA concentrations in cohort 1. As shown in **Figure 11**, TBA levels averaged 117.2 µmol/L in patients with HE, and 60.6 µmol/L in patients without HE (p=0.0007). The same relation has been noted for Ascites (p<0.0001) and PH (0.0038), as TBA levels were also clearly higher in presence of these clinical complications of cirrhosis (**Figure 11**).

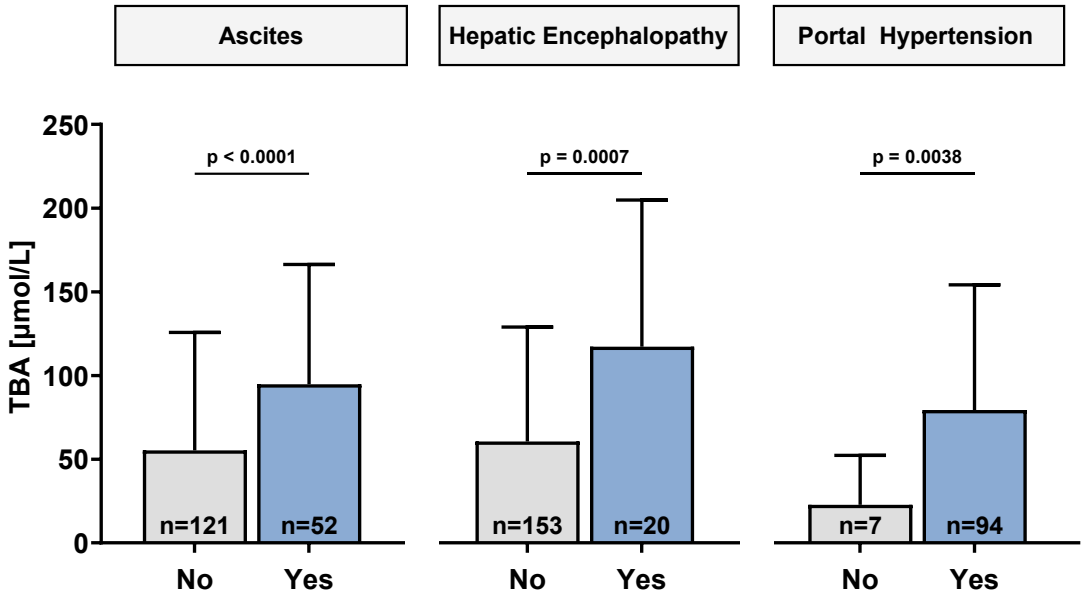


Figure 11. Cohort 1: Differences in total bile acids by clinical complications. Bars are mean and error bars are standard deviation. Differences between the groups were calculated by multiple Mann-Whitney tests with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli for multiple comparisons.

3.6.3 Bile Acids Correlate with MELD and FIB4 Score, and Laboratory Parameters

Correlation analyses on data in cohort 1 showed a statistically significant positive linear correlation between Model for End-Stage Liver Disease (MELD) score (calculated with creatinine, bilirubin, and INR) and total bile acids (TBA) with a Spearman correlation coefficient of 0.63 (p<0.0001; 95%CI [0.53,0.72]) in patients with liver cirrhosis. The correlation coefficient squared indicates that 43.5% of variability is shared between the MELD score and TBA. A similar correlation with the Child-Pugh score was demonstrated by a Spearman correlation coefficient of 0.61 (p<0.0001; 95%CI [0.50,0.70]). The

correlation between the Fibrosis-4 (FIB4) score (calculated with age, AST, ALT, and platelet count) and total bile acids also demonstrated a significant positive linear correlation. However, with a Spearman correlation coefficient of 0.38 ($p<0.0001$; 95%CI [0.24,0.50]) the correlation was comparatively weaker. Only 7.2% of the variability in BAs is coupled with the FIB4 score (**Figure 12**). Based on these findings, it can be inferred that the MELD score demonstrates a more robust correlation with TBA levels in patients with liver cirrhosis when compared to the FIB4 score. To validate the above observations, a correlation analysis was further performed between the MELD-Score and TBA in cohort 2, demonstrating a Spearman correlation coefficient of 0.54 ($p<0.0001$; 95%CI [0.38,0.67]). The correlation between FIB4-Score and TBA was weaker with a Spearman correlation coefficient of 0.44 ($p<0.0001$; 95%CI [0.26, 0.59]). The Spearman correlation between the Child-Pugh score and TBA in cohort 2 was 0.35 ($p=0.0003$; 95%CI [0.16, 0.52]).

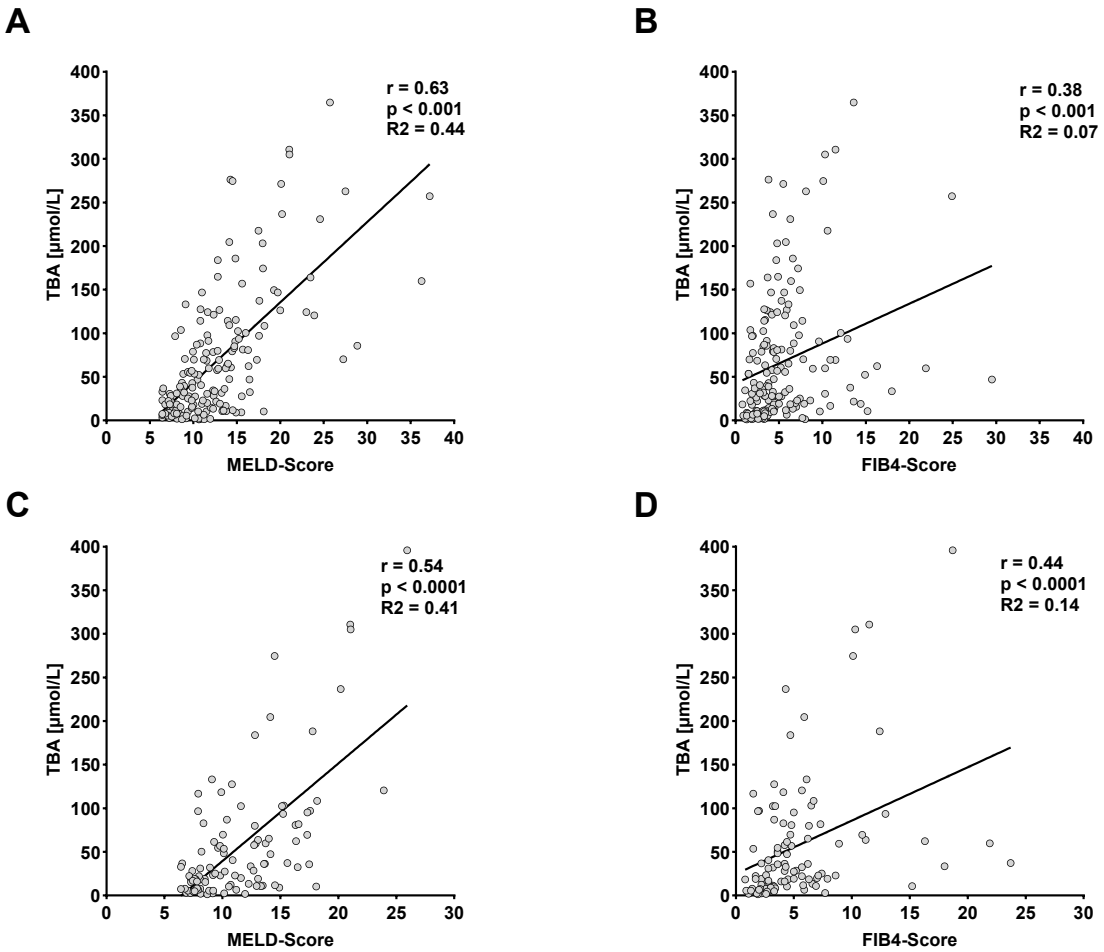


Figure 12. Cohort 1: Scatterplots of (A) TBA and MELD-Score as well as (B) TBA and FIB4-Score. Cohort 2: Scatterplots of (C) TBA and MELD-Score and (D) TBA and FIB4-Score. MELD, Model for End-Stage Liver Disease; FIB4, Fibrosis-4.

Additionally, Spearman correlation analysis was conducted on clinical laboratory markers and BA profile of cirrhotic patients. Various laboratory values were correlated with BA profile, with direct-bilirubin (dBIL), internationalized-ratio (INR), and albumin (ALB) showing especially strong correlations. Out of individual bile acids TCDCA and GCDCA correlated best with laboratory parameters (**Figure 13**).

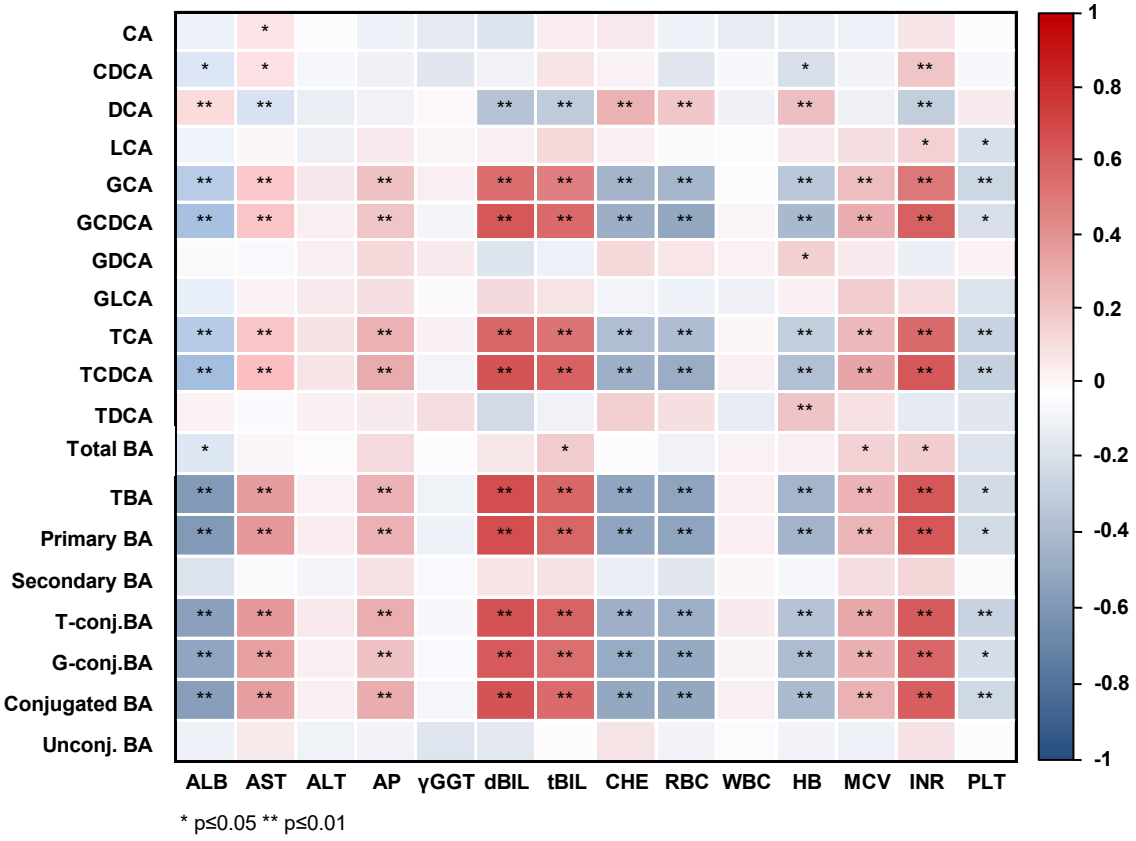


Figure 13. Cohort 1: Heatmap of Spearman correlation coefficients between bile acid variables and laboratory parameters in patients with liver cirrhosis.

3.6.4 Bile Acid Profiles Display Significant Changes in Liver Cirrhosis

Bile acid profiles of cohort 1 are provided in the following figures and tables and show the qualitative changes in liver cirrhosis. They display that primary bile acids, mostly conjugated forms of CDCA and CA, were elevated in systemic circulation in patients with liver

cirrhosis. Among the cirrhotic patients, those with Childs B&C cirrhosis exhibited higher absolute amounts of taurine and glycine conjugated CA and CDCA when compared to patients with Childs A cirrhosis (**Figure 14A**).

Comparing the relative levels between healthy controls, Childs A and Childs B&C, taurine-conjugates exhibited higher-fold increases than glycine-conjugates. The ratio of taurine- to glycine-conjugates was higher in cirrhotic patients indicating increased taurine and/or reduced glycine conjugation. When comparing healthy controls to liver cirrhosis, the percentage of taurine-conjugated CDCA (TCDCA) was 2.8% versus 23.1% (ratio = 8.25) and taurine-conjugated CA (TCA) was 1.8% versus 8.1% (ratio = 4.5), respectively. On the other hand, glycine-conjugated CDCA (GCDCA) was 21.9% versus 40.2% (ratio = 1.84) and glycine-conjugated CA (GCA) was 11.5% versus 17.2% (ratio = 1.5) (**Figure 14B**). In contrast, relative concentrations of all unconjugated bile acid derivates were lower percentage- wise, with slight absolute variations (**Figure 14**). In fact, unconjugated CA, CDCA and UDCA did not show significant differences between healthy controls and cirrhotic patients (**Table 11**). The ratio of unconjugated to conjugated BAs decreased significantly with the progression of liver cirrhosis, from 0.8 to 0.3 in Child A and 0.2 in Child B+C ($p=0.007$ and $p<0.001$) respectively (**Table 12**).

Among secondary bile acids, DCA and LCA exhibited a declining trend in percentage frequency with increasing severity of liver cirrhosis, while CDCA-conjugates in particular were more prevalent, accounting for 67% of TBA in Child B+C compared to only 24.7% in healthy controls. The proportion of total DCA decreased from 15.6% in healthy controls to 3.1% in Child B+C cirrhosis, total LCA from 3.1% to 0.4% (**Figure 16A**). This was also reflected in the ratio of secondary to primary BAs, which was significantly lower in Child B+C cirrhosis (0.6±0.7 in healthy controls vs. 0.1±0.4 in Child B+C; $p=0.004$) (**Table 12**).

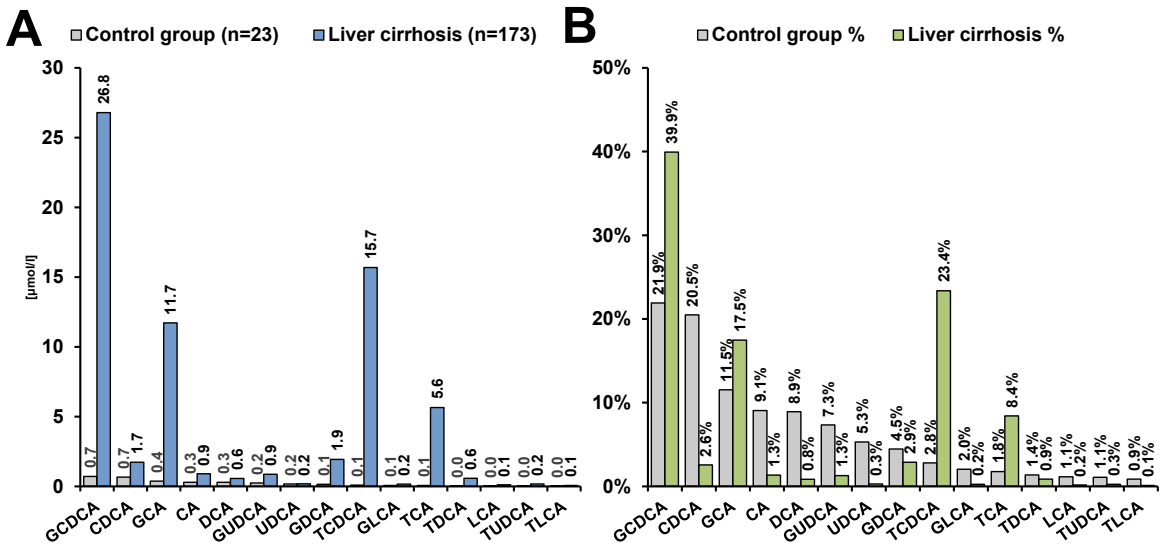


Figure 14. Cohort 1: Individual bile acid profile of the control group and liver cirrhosis given as (A) absolute concentrations and as (B) percentage of total bile acids.

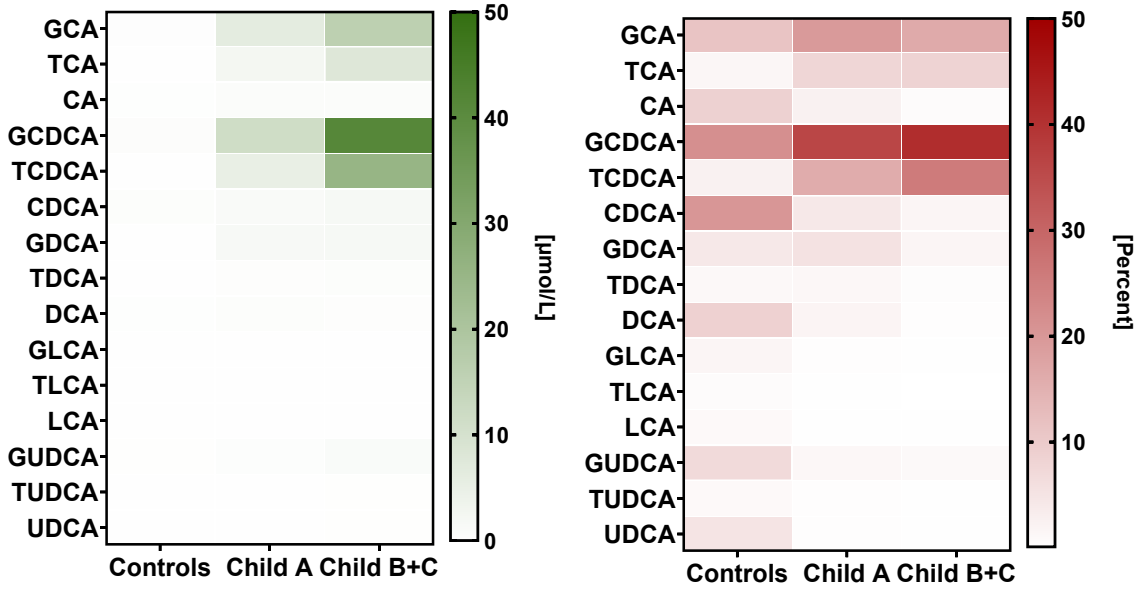


Figure 15. Cohort 1: Heatmaps of BA profile across Controls, Child A and Child B+C given as (A) absolute concentrations and (B) as percentage of total bile acids.

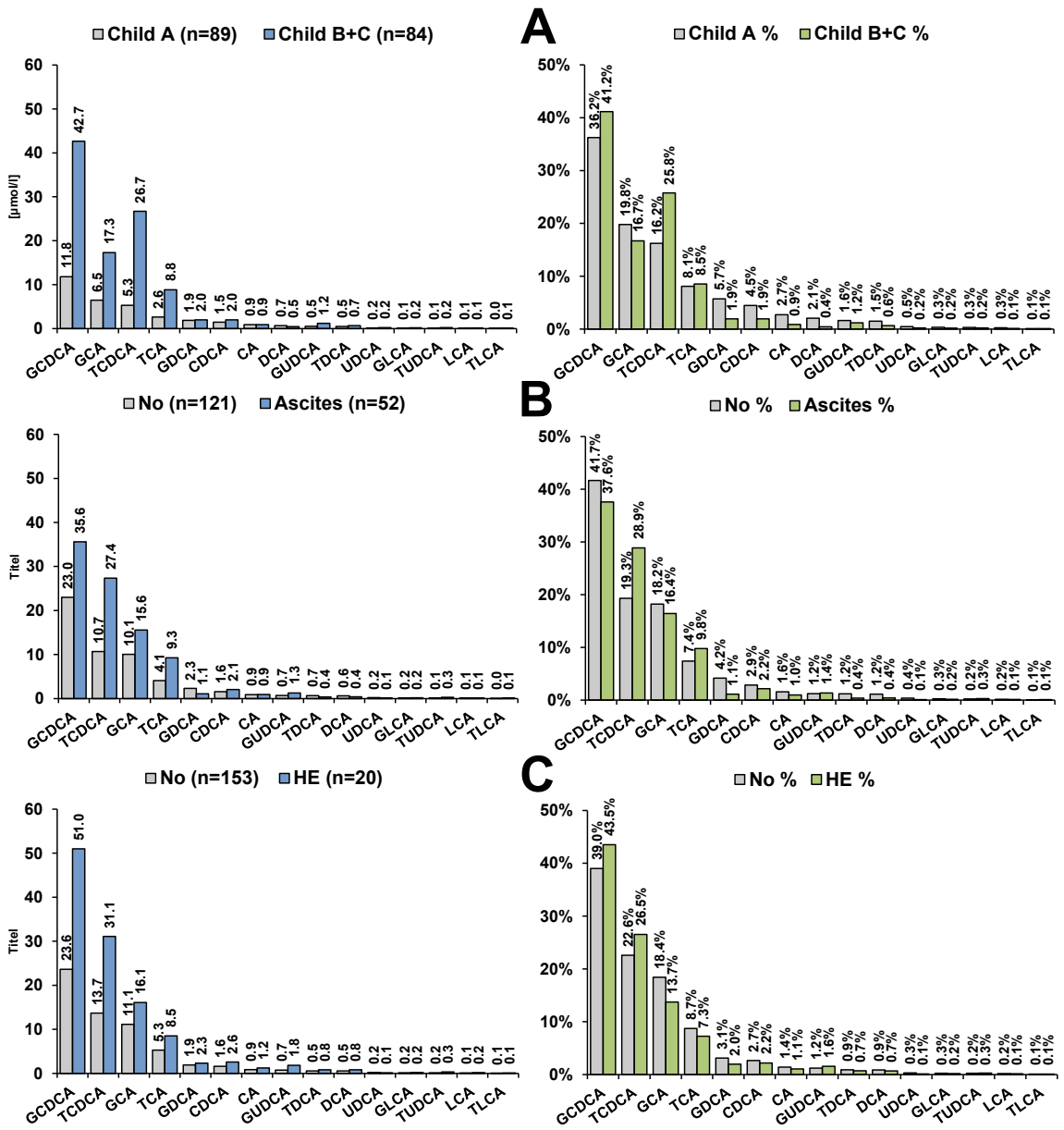


Figure 16. Cohort 1: Individual bile acid profile in Child-Pugh-Stages and in absence or presence of clinical complications in absolute (left) and relative (right) terms. (A) Child-Pugh-Stages; (B) Ascites; (C) Hepatic Encephalopathy.

Table 11. Cohort 1: Individual bile acid profile of Controls, Child A and Child B+C

Bile Acid Variable	Controls n=23	Child A n=89	Child B+C n=80	p-value
Total Bile Acids (µmol/l)	3.2±2.6	32.6±33	103.6±85	$p<0.001^{\&,\$,\S}$
Primary and Secondary BAs				
Total CA (µmol/l)	0.7±0.6	10±11.9	27±23.5	$p<0.001^{\&,\$,\S}$

GCA (µmol/l)	0.4±0.4	6.5±8.1	17.3±16.7	$p<0.001^{\&,\$,\S}$
TCA (µmol/l)	0.1±0.1	2.6±3.8	8.8±9	$p<0.001^{\&,\$,\S}$
CA (µmol/l)	0.3±0.6	0.9±2.9	0.9±3	n.s.
Total CDCA (µmol/l)	1.5±2	18.6±19.9	71.4±67.4	$p<0.001^{\&,\$,\S}$
GCDCA (µmol/l)	0.7±0.6	11.8±12.7	42.7±44.8	$p<0.001^{\&,\$,\S}$
TCDCA (µmol/l)	0.1±0.1	5.3±7.5	26.7±28.7	$p<0.001^{\&,\$,\S}$
CDCA (µmol/l)	0.7±1.7	1.5±3.4	2±5	n.s.
Total DCA (µmol/l)	0.5±0.5	3±3.5	3.2±4.7	$p<0.001^{\$}$ $p<0.001^{\S}$
GDCA (µmol/l)	0.1±0.2	1.9±2.6	2±3.3	$p<0.001^{\$}$ $p<0.001^{\S}$
TDCA (µmol/l)	0±0.1	0.5±0.7	0.7±1.2	$p<0.001^{\$}$ $p<0.001^{\S}$
DCA (µmol/l)	0.3±0.4	0.7±0.8	0.5±0.9	$p<0.001^{\$}$
Total LCA (µmol/l)	0.1±0.1	0.2±0.3	0.4±0.6	$p=0.007^{\&}$ $p=0.008^{\$}$ $p<0.001^{\S}$
GLCA (µmol/l)	0.1±0.1	0.1±0.2	0.2±0.3	$p=0.021^{\&}$ $p<0.001^{\S}$
TLCA (µmol/l)	0±0	0±0.1	0.1±0.2	n.s.
LCA (µmol/l)	0±0.1	0.1±0.1	0.1±0.2	$p=0.003^{\$}$ $p<0.001^{\S}$
Total UDCA (µmol/l)	0.4±0.7	0.8±1.1	1.7±2.2	$p<0.001^{\&,\S}$
GUDCA (µmol/l)	0.2±0.5	0.5±0.8	1.2±1.8	$p=0.001^{\&}$ $p=0.041^{\$}$ $p<0.001^{\S}$
TUDCA (µmol/l)	0±0.1	0.1±0.2	0.2±0.3	$p=0.001^{\&}$ $p=0.006^{\$}$ $p<0.001^{\S}$
UDCA (µmol/l)	0.2±0.3	0.2±0.3	0.2±0.9	n.s.

Note. Data are mean ± standard deviation. $^{\&}$Difference between Child-Pugh A and Child-Pugh B+C; $^{\$}$Difference between Child-Pugh A and Controls; §Difference between Child-Pugh B+C and Controls; Differences calculated via t-test for independencies; n.s.= not significant.

Table 12. Cohort 1: Differences of calculated bile acid parameters between Controls, Child A and Child B+C

Bile Acid Variable	**Controls n=23**	**Child A n=89**	**Child B+C n=80**	**p-value**
Primary Bile Acids (µmol/l)	2.2±2.5	28.6±30.8	98.4±83.4	$p<0.001^{\&,\$,\S}$
Secondary Bile Acids (µmol/l)	0.6±0.4	3.3±3.6	3.6±5.1	$p<0.001^{\$}$ $p<0.001^{\S}$
Secondary to primary (ratio)	0.6±0.7	0.3±0.4	0.1±0.4	$p=0.003^{\&}$ $p=0.004^{\S}$
Taurine conjugated (µmol/l)	0.3±0.3	8.6±11.5	36.5±35.9	$p<0.001^{\&,\$,\S}$
Glycine conjugated (µmol/l)	1.5±0.9	20.8±21.9	63.4±58.1	$p<0.001^{\&,\$,\S}$
Taurine to glycine (ratio)	0.2±0.2	0.4±0.4	3±21.6	n.s.
Unconjugated (µmol/l)	1.5±2.2	3.3±6.4	3.7±8.3	n.s.
Conjugated (µmol/l)	1.7±1.7	19±20.7	59.3±46.8	$p<0.001^{\&,\$,\S}$
Unconjugated to conjugated (ratio)	0.8±0.8	0.3±0.4	0.2±0.5	$p=0.007^{\$}$ $p<0.001^{\S}$
Hydrophilic (µmol/l)	1.2±0.9	10.8±12.3	28.7±24	$p<0.001^{\&,\$,\S}$

Hydrophobic (µmol/l)	2.1±2.1	21.8±21.7	73.8±67.9	$p<0.001^{\&,\$,\S}$
Hydrophilic to hydrophobic (ratio)	0.8±0.6	0.5±0.5	0.6±0.9	n.s.

Note. Data are mean ± standard deviation. $^{\&}$Difference between Child-Pugh A and Child-Pugh B+C; $^{\$}$Difference between Child-Pugh A and Controls; §Difference between Child-Pugh B+C and Controls; Differences calculated via t-test for independencies; n.s.= not significant. Primary BAs, total CA + total CDA; Secondary BAs, total DCA + total LCA; Taurine conjugated, TCA + TCDCA + TDCA +TLCA; Glycine conjugated, GCD + GCDCA + GDCA + GLCA; Unconjugated, CA + CDCA + DCA + LCA; Conjugated, taurine conjugated + glycine conjugated; Hydrophilic, total CA + total UDCA; Hydrophobic, total CDCA + total DCA + total LCA.

Table 13. Cohort 2: Individual bile acid profile of Controls, Child A and Child B+C

	Controls	**Child A**	**Child B+C**	**p-value**
Bile Acid Variable	**n=10**	**n=79**	**n=21**	
Total Bile Acids (µmol/l)	2.8±1.6	33.3±32.2	118.4±102.8	$p<0.001^{\&,\$,\S}$
Primary and Secondary BAs				
Total CA (µmol/l)	0.6±0.4	10.3±12.6	29.7±23.3	$p<0.001^{\&,\$,\S}$
GCA (µmol/l)	0.4±0.3	7±8.7	21.6±19.4	$p<0.001^{\&,\$,\S}$
TCA (µmol/l)	0±0	2.6±4.4	7±7	$p=0.003^{\&}$ $p<0.001^{\$,\S}$
CA (µmol/l)	0.2±0.2	0.7±3	1±1.8	$p=0.025^{\S}$
Total CDCA (µmol/l)	1.4±0.9	17.3±18.1	82.2±86.9	$p<0.001^{\&,\$,\S}$
GCDCA (µmol/l)	1±0.4	11.8±12.9	55.2±63.5	$p=0,001^{\&}$ $p<0.001^{\$,\S}$
TCDCA (µmol/l)	0.1±0.1	4.4±6.6	23.9±28.8	$p=0,001^{\&}$ $p<0.001^{\$,\S}$
CDCA (µmol/l)	0.3±0.5	1±2.9	3.1±8.1	n.s.
Total DCA (µmol/l)	0.6±0.5	3.4±3.7	4.2±6.2	$p<0.001^{\$}$ $p=0.005^{\S}$
GDCA (µmol/l)	0.2±0.2	2.2±2.7	2.8±4.4	$p<0.001^{\$}$ $p=0.004^{\S}$
TDCA (µmol/l)	0±0	0.6±0.7	1±1.6	$p<0.001^{\$}$ $p=0.005^{\S}$
DCA (µmol/l)	0.4±0.4	0.7±0.8	0.4±0.6	n.s.
Total LCA (µmol/l)	0±0	0.3±0.5	0.4±0.5	$p<0.001^{\S}$
GLCA (µmol/l)	0±0	0.1±0.3	0.2±0.3	$p<0.001^{\S}$
TLCA (µmol/l)	0±0	0±0.1	0.1±0.1	$p=0.042^{\&}$ $p=0.009^{\S}$
LCA (µmol/l)	0±0	0.1±0.2	0.1±0.2	$p<0.001^{\&}$ $p=0.003^{\S}$
Total UDCA (µmol/l)	0.1±0.1	2±6.5	1.9±2.5	$p<0.001^{\S}$
GUDCA (µmol/l)	0.1±0.1	1.5±5.6	1.4±2	$p=0.001^{\S}$
TUDCA (µmol/l)	0±0	0.2±0.7	0.3±0.4	$p<0.001^{\S}$
UDCA (µmol/l)	0±0.1	0.3±0.9	0.2±0.3	n.s.

Note. Data are mean ± standard deviation. $^{\&}$Difference between Child-Pugh A and Child-Pugh B+C; $^{\$}$Difference between Child-Pugh A and Controls; §Difference between Child-Pugh B+C and Controls; Differences calculated via t-test for independencies; n.s.= not significant.

Table 14. Cohort 2: Differences of calculated bile acid parameters between Controls, Child A and Child B+C

Bile Acid Variable	Controls n=10	Child A n=79	Child B+C n=21	p-value
Primary Bile Acids (µmol/l)	2±1.2	27.6±29.7	111.8±99.5	$p<0.001^{\&,\$,\S}$
Secondary Bile Acids (µmol/l)	0.8±0.6	5.7±8.5	6.5±7.2	$p<0.001^{\S}$
Secondary to primary (ratio)	0.4±0.3	0.6±1.6	0.1±0.1	$p=0.006^{\&}$ $p<0.001^{\S}$
Taurine conjugated (µmol/l)	0.1±0.2	7.8±11.4	32.3±34.5	$p<0.001^{\&,\$,\S}$
Glycine conjugated (µmol/l)	1.8±0.8	22.7±23.3	81.2±77.1	$p<0.001^{\&,\$,\S}$
Taurine to glycine (ratio)	0.1±0.1	0.3±0.4	0.5±0.6	$p=0.034^{\S}$
Unconjugated (µmol/l)	0.9±1	2.8±6.2	4.9±10	n.s.
Conjugated (µmol/l)	1.9±0.9	30.5±31	113.5±101.3	$p<0.001^{\&,\$,\S}$
Unconjugated to conjugated (ratio)	0.5±0.5	0.2±0.6	0.1±0.4	$p=0.027^{\S}$
Hydrophilic (µmol/l)	0.8±0.5	12.4±14.2	31.5±24	$p<0.001^{\&,\$,\S}$
Hydrophobic (µmol/l)	2±1.1	20.9±19.8	86.8±87.8	$p<0.001^{\&,\$,\S}$
Hydrophilic to hydrophobic (ratio)	0.4±0.1	0.7±0.8	0.5±0.4	n.s.

Note. Data are mean ± standard deviation. $^{\&}$Difference between Child-Pugh A and Child-Pugh B+C; $^{\$}$Difference between Child-Pugh A and Controls; §Difference between Child-Pugh B+C and Controls; Differences calculated via t-test for independencies; n.s.= not significant. Primary BAs, total CA + total CDCA; Secondary BAs, total DCA + total LCA; Taurine conjugated, TCA + TCDCA + TDCA +TLCA; Glycine conjugated, GCD + GCDCA + GDCA + GLCA; Unconjugated, CA + CDCA + DCA + LCA; Conjugated, taurine conjugated + glycine conjugated; Hydrophilic, total CA + total UDCA; Hydrophobic, total CDCA + total DCA + total LCA.

4 Discussion

This thesis aimed to characterize changes in BA composition in liver cirrhosis, by firstly analyzing retrospective BA data of cirrhosis patients and correlate them to the disease severity and clinical features and secondly by measuring BA concentrations before and after TIPS.

In our retrospective analysis of two independent datasets, we demonstrate that total serum bile acid (TBA) levels were higher with increasing disease severity, as previously described by other authors (134-136). Correlation analyses demonstrated significant associations between TBA concentrations and the MELD-Score, the CP-Score, as well as the FIB4-Score. The MELD-Score is effective in assessing survival in cirrhotic patients, strongly predicting 6-month survival. Although BAs show slightly lower predictive accuracy than the MELD-Score, they could still serve as valuable prognostic indicators in cirrhotic patients (134). The etiology of liver cirrhosis might influence the strength of the correlations, as we observed variations in BA levels between different etiologies. For instance, alcohol-induced liver cirrhosis was associated with the highest mean TBA levels in our analyses. This may be due to an alcohol induced change in BA transporter expression found in rats, with an upregulation of hepatic BSEP and MRP2, as well as ileal ASBT and OSTß, consequently increasing the total BA reabsorption. Furthermore, ethanol up-regulates the expression of CYP7A1, the rate-limiting enzyme for bile acid synthesis. Overall, genes involved in the synthesis and efflux transport seem to be up-regulated and genes regulating influx transport in the liver are down-regulated in alcoholic cirrhosis (137). However, it is important to consider the potential influence of liver cirrhosis severity on the higher TBA levels we saw. In one of the two cohorts the alcoholic patient group had a higher prevalence of Child B and Child C stages, which are indicative of more advanced liver dysfunction, introducing the possibility of bias in our results. In the second cohort, however, the severity distribution was balanced with comparable results to the other cohort. Taken together, this suggests that the etiology of liver cirrhosis influences the magnitude of TBA elevation, independent of the liver cirrhosis severity.

Overall, the correlations indicate that serum concentrations of TBA increase with the scale of decompensation and may be of diagnostic and predictive value in liver cirrhosis. However, some studies have found no superiority for assessment of TBA concentrations in

comparison to other liver tests in the diagnosis and prognosis of cirrhosis (138). Instead, the individual bile acid profile may offer a more promising approach. For instance, a study by X. Wang et al. demonstrated that dynamic alterations in serum bile acids can serve as staging and monitoring biomarkers for liver function in patients with hepatitis B-associated liver cirrhosis (139). Individual bile acid profiles could also be useful as staging biomarkers in alcoholic liver cirrhosis, as they exhibit highly characteristic changes that distinguish it from other etiologies (140).

Comparing BA profiles between healthy individuals and cirrhosis patients, we observed a significant increase in the conjugated forms of primary bile acids. With progressive liver cirrhosis, primary bile acids further increased, while secondary bile acids (DCA and LCA) showed a decreasing trend in relative terms. Therefore, especially GCDCA, TCDCA, GCA and TCA could be of great worth in diagnosis and evaluation of liver cirrhosis progression (134). In a study analyzing fecal samples from cirrhotic patients, a decreased conversion of primary to secondary fecal bile acids and lower levels of total secondary bile acids were observed in advanced cirrhosis and in patients treated with rifaximin, an antibiotic substance that is used to treat and prevent hepatic encephalopathy. This may explain the decreasing trend of secondary BAs in serum and emphasizes the importance of the gut-liver axis (45). LCA is known as the strongest endogenous ligand of TGR5. The activation of TGR5 triggers the secretion of glucagon-like peptide 1 (GLP-1), which then supports glucose-dependent postprandial insulin secretion from pancreatic ß-cells (33, 50, 52). GLP-1-agonists, originally established for the treatment of type 2 diabetes, have shown promising results in reversing hepatic steatosis, positioning them as a great hope in the treatment of MASLD (141). This prompts the question: Could the relative reduction of LCA in cirrhosis, as we have observed, lead to reduced GLP-1 activity, thereby counteracting the beneficial effects seen with these new “liver miracle drugs”? Is the deficiency of certain BAs such as LCA both the result and trigger of chronic liver diseases and would this mean, for example, that GLP-1 agonists could also be effective in cases of alcohol-associated liver disease, where we observed the greatest BA alteration, including LCA depletion as seen in MASLD? GLP-1-agonists might compensate for the lack of LCA, potentially alleviating above-mentioned microbiome changes associated with cirrhosis and providing a promising treatment avenue beyond MASLD.

The unconjugated/conjugated BA ratio decreased with liver cirrhosis and disease progression. One hypothesis for this observation is that conjugated CDCA is the main ligand for FXR, and as such, the increase in CDCA could induce FGF 19 formation in the gut, subsequently reducing the synthesis of unconjugated bile acids by inhibition of CYP7A1 in the liver (21). Concerning the relationship between BAs and clinical complications, higher TBA levels were found in the presence of complications like ascites and hepatic encephalopathy. The measurement of TBA levels could function as an extra marker to assess the risk of complications in cirrhotic patients. However, the determination of appropriate cut-off values is necessary for practical use. In a study that invasively investigated the association between portal hypertension and TBA levels, significant correlations were observed (135), consistent also with non-invasive studies (136) and our findings. Serum BAs may indirectly represent the degree of portal hypertension. Overall, it is important to acknowledge that the increase in BAs in the systemic circulation may only be significant in relative terms when compared to the absolute bile acid pool, primarily confined to the enterohepatic circulation without reaching the systemic circulation. The impact of this systemic increase remains to be further elucidated. Therefore, a better sense of the dynamics of BAs in cirrhotic patients is of great importance.

TIPS is acknowledged as an efficient treatment for complications linked to portal hypertension in liver cirrhosis. Despite its clinical utility, the consequences of TIPS on hepatic metabolism and systemic metabolite levels remain poorly understood. To investigate changes in BA metabolism following TIPS, we quantified BA levels at different anatomical locations and timepoints. Initial observations showed high variability in BA values pre- and post-TIPS, possibly due to the non-standardized blood sampling regarding food intake. A literature review was conducted to determine the correct time of measurement. Several studies demonstrate a marked elevation of postprandial serum bile acid (SBA) levels in individuals with hepatobiliary disease, whereas healthy individuals show minimal postprandial increases (142-145). Despite conflicting reports on postprandial SBA levels in healthy subjects (146-148), the healthy liver effectively extracts and excretes BAs, maintaining low systemic levels with minor fluctuations (149). In cirrhosis, large fluctuations throughout the day have been observed including postprandial episodes (146, 149). Kaplowitz et al. and Angelico et al. demonstrated that postprandial SBA levels are more sensitive than fasting levels for detecting liver dysfunction, with increased levels

indicating impaired hepatic excretory function (142, 144). However, despite the higher sensitivity of postprandial SBA, multiple common routine liver tests combined outperform the sensitivity of postprandial SBA determination alone in diagnosing liver diseases (144). Contrarily, Mannes et al. found no superior sensitivity of postprandial BAs compared to fasting BAs (147). Their study revealed that the radioimmunoassay (RIA) method had higher sensitivity for both fasting (93%) and postprandial (93%) levels, whereas enzymatic methods showed lower sensitivity in fasting BA levels. The gas-chromatographic method (GLC) demonstrated even higher sensitivity (98% fasting, 95% postprandial). This indicates that determination of fasting concentrations is sufficient for diagnostic purposes when using an RIA or GLC method. Additionally, since the postprandial rise in bile acids is influenced by numerous factors such as gastric emptying, intestinal motility, and gallbladder contraction, which are known to be impaired in liver cirrhosis, the anticipated increase in SBA levels following a meal could be inconsistent. This suggests that fasting bile acids are more reproducible in the assessment of liver disease (150). Festi et al. also mentioned the impact of methodology as a reason why postprandial SBA determination was suggested to be more sensitive. In their study involving 322 patients and 93 controls, they found that fasting SBA determination was more sensitive than postprandial determination (151). To enhance the timing of postprandial sampling, Fausa and Kelbaek et al. found peaks in BA concentrations occurring between 90-120 minutes, with some individuals showing increases as early as one hour after a meal and individual variations due to disease type and meal composition (146, 152). The composition of the ingested meal was also found to be crucial, with meals high in fat leading to more pronounced increases in postprandial SBA levels, likely due to the stimulation of gallbladder contraction by fats. The consistency of the meal may also be a contributing factor, as it affects the rate of gastric emptying and intestinal absorption (148, 153). Overall, while the optimal timing for BA measurement remains unclear, fasting BA levels appear to provide a more consistent diagnostic approach.

In the peripheral vein, total BA levels decreased within 12-72 hours post-TIPS (median time difference was 19-21 hours). These findings align with those of Dantas et al., who reported a decrease in BA levels immediately after TIPS implantation, prior to post-anesthesia care unit admission (time difference 1-3 hours). However, BA levels returned to baseline prior to patient discharge (after 14-19 hours). Notably, the risk of developing post-TIPS hepatic encephalopathy (HE) correlated with early post-TIPS changes in BA levels, with more

severe HE outcomes linked to pronounced decreases in BA levels. This observation suggests a protective role of certain bile acids against HE development (126) and indicates that reductions in post-TIPS BA levels may increase the risk for later HE episodes. Pertinent investigations on BA metabolism after surgical placement of a portosystemic shunt contrarily showed an elevation in SBA levels after insertion, as evidenced by BA determination conducted between 2 weeks and 6 months post-procedure (154-157). The underlying reasons can be theoretically attributed to multiple factors. The partial impairment of the enterohepatic circulation results in a diversion of portal blood flow into the systemic circulation consequently diminishing hepatic blood flow, possibly resulting in reduced hepatic extraction of BAs. Also, a higher BA synthesis rate and frequency of enterohepatic circulation may contribute to this phenomenon (155-158). Interestingly, when serum BA concentrations were measured shortly after surgery (within 1-4 days), no significant differences were observed compared to pre-shunt levels. Thus, blood overflow is a determinant of SBA concentrations, BAs should theoretically increase immediately postoperatively. However, factors such as reduced intestinal absorption, intravenous feeding and absence of stimuli for gallbladder contraction after surgery may explain for the lack of an immediate post-operative increase (157). Also, the BA composition changes notably after surgical placement of a portocaval shunt. In non-shunted patients with liver cirrhosis, the CA/CDCA ratio in serum is lower than in bile. In individuals with shunts, there is a tendency for the serum and biliary BA patterns to become analogous, resulting in a subsequent elevation of the CA/CDCA ratio. Consequently, the peripheral BA composition may be influenced by the composition of portal blood in shunted patients. Furthermore, DCA, a bile acid known to decrease in liver cirrhosis, was further decreased after portosystemic shunt insertion (154).

The reduction in SBA concentrations following TIPS, despite the theoretical increase in bile-acid-rich blood shunted away from hepatic extraction due to the iatrogenic shunt, may be partly explained by a compensatory increase in hepatic arterial blood flow. The liver, which is a dual perfused organ, receiving 20-30% of its blood supply by the hepatic artery and 70% from the portal vein under normal circumstances, can adjust through the hepatic arterial buffer response (HABR) to maintain liver perfusion when portal venous perfusion is decreased. Post-TIPS measurements indicate preserved HABR in cirrhotic patients, even those with advanced cirrhosis, as evidenced by a lower resistance index in the hepatic artery

(as measured by doppler duplex ultrasonography) (159). Additionally, studies using endoluminal sensors to measure hepatic arterial blood flow during TIPS have documented increases in average and maximum arterial peak velocity within seconds after TIPS, suggesting a reflex-like mechanism that enhances hepatic arterial flow (160). CT perfusion studies further support this by showing an increase in the hepatic arterial fraction (HAF) post-TIPS, which also correlates with the changes in hepatic venous pressure gradient (HVPG), demonstrating that the proportion of HAF increases as the HVPG increases. Despite this compensatory mechanism, the overall effective hepatic blood supply is still diminished post-TIPS, because the increased arterial flow does not fully compensate for the reduced portal vein flow (161). TIPS creation may also restore portal venous flow, since TIPS is effectively used in portal vein thrombosis and helps to resolve the thrombus and recanalize the portal venous system (162, 163).

In our cohort of seven patients the hepatic extraction fraction of bile acids was markedly low in some patients, at only 0.14 (14%) and correlated negatively with the Child-Pugh-score and HVPG. This is in stark contrast to the extraction rates reported for healthy individuals, which range from 70-90% for cholic and glycocholic acid. In patients with chronic liver disease, the extraction rates are significantly reduced to between 7-69%, as noted previously (164). Our method of measuring the hepatic extraction fraction is the most direct, enhancing the robustness and reliability of our findings compared to those derived from the less direct methodology in the previously cited study. Such findings underscore the diminished hepatocellular function in liver cirrhosis, impairing the maintenance of physiological enterohepatic circulation and resulting in a significant reduction in the hepatic extraction of BAs. Overall, it must be noted that the individual proportion of blood flow through the hepatic artery is unknown, so indeed the hepatic extraction fraction as calculated here only gives an estimate of liver function (158). The possible error introduced by variations in the flow ratio between portal venous and hepatic arterial blood flow should not be overlooked (42).

We observed that peripheral BA concentrations were consistently higher than those in the liver and portal vein, likely due to individual variations in vascular territories and the effects of portosystemic shunting in cirrhosis. Under physiological conditions, peripheral venous concentrations of BAs do not surpass those in liver venous blood (158). However, in cirrhosis, portosystemic shunting facilitates the diversion of blood still containing high levels

of BAs away from the liver. This allows BAs to enter the venous system via other vascular pathways and add to the concentrations of peripheral venous blood.

A limitation of the present study is the small number of cases. A larger sample size would be necessary to prove further significance in differences post-TIPS, especially regarding individual bile acid profile differences. Another limitation is the high interindividual variability of BA levels. Again, a larger sample size would be necessary to stratify groups according to cirrhosis severity and etiology to better distinguish between differences caused by the stage or etiology of liver cirrhosis. Also, we had some outliers in BA data, primarily confined to our methodology, reducing the strength of our data.

In conclusion, this thesis demonstrates that BA levels in liver cirrhosis correlate with disease severity and clinical scores such as the MELD score, suggesting that total BA measurements could serve as prognostic indicators in cirrhosis. Nonetheless, BA levels alone may not outperform regular liver function tests, but individual bile acid profiles may provide more diagnostic insight. Cirrhosis progression was linked to specific changes in BA composition, including an expansion in conjugated primary BAs and a decline in secondary bile acids. Serum BA levels decreased the day after TIPS, despite increased portosystemic shunting. BA determination before and after TIPS could serve as a measure to help identify patients at a higher risk of complications, especially hepatic encephalopathy. Additionally, this study demonstrated reduced hepatic extraction of BAs in cirrhosis and elevated peripheral BA levels relative to liver vein BA levels. The effects of TIPS on BA dynamics warrant further research but underline the complexity of BA regulation in cirrhosis.

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