A Current Understanding of Bile Acids in Chronic Liver Disease

Naba Farooqui [*], Anshuman Elhence [†], Shalimar [‡]

[*] Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA, [†] Department of Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India and [‡] Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, New Delhi, India

Chronic liver disease (CLD) is one of the leading causes of disability-adjusted life years in many countries. A recent understanding of nuclear bile acid receptor pathways has increased focus on the impact of crosstalk between the gut, bile acids, and liver on liver pathology. While conventionally used in cholestatic disorders and to dissolve gallstones, the discovery of bile acids' influence on the gut microbiome and human metabolism offers a unique potential for their utility in early and advanced liver diseases because of diverse etiologies. Based on these findings, preclinical studies using bile acid-based molecules have shown encouraging results at addressing liver inflammation and fibrosis. Emerging data also suggest that bile acid profiles change distinctively across various causes of liver disease. We summarize the current knowledge and evidence related to bile acids in health and disease and discuss culminated and ongoing therapeutic trials of bile acid derivatives in CLD. In the near future, further evidence in this area might help clinicians better detect and manage liver diseases. (J Clin Exp Hepatol 2022;12:155–173)

Liver cirrhosis is the 8th most common cause of death in low-middle-income countries as per the World Health Organization's official report.[1] The most common etiologies of cirrhosis are alcohol, chronic viral hepatitis, and the emerging menace of nonalcoholic fatty liver disease (NAFLD).[2–4] Decompensated cirrhosis, in particular, has high mortality with a median survival of 2 years. In patients with cirrhosis, bacterial translocation further aggravates fluid shifts and increases mortality risk due to sepsis.[5] Recent insights about the role of bile acid (BA) composition on insulin sensitivity and their dialog with the gut microbiome, apart from their well-known digestive functions, have drawn attention to their relevance in chronic liver disease (CLD). From numerous animal and human studies, we now know that through nuclear receptors, BAs play a role in regulating the qualitative and quantitative BA pool, gut microbiome, and glucose and lipid metabolism.[6–8]

An increase in studies characterizing the fecal and serum BA pool in cirrhosis has helped improve our understanding of the pathogenesis and enabled the development of diagnostic or prognostic markers of liver disease. As expected, many BA receptor–based therapeutics are currently in the pipeline. In this narrative review, we expand on the synthesis and metabolism of BAs, their effect on the gut microbiome, and the progression of CLD, in addition to discussing their emerging role in diagnostics and therapeutics.

BILE ACID SYNTHESIS AND METABOLISM IN HEALTH

Primary BAs are synthesized from cholesterol in hepatocytes predominantly via the neutral/classic pathway and in small amounts by the acidic/alternate pathway. The rate-limiting and regulatory step in BA synthesis via classic pathway is enzymatically catalyzed by cholesterol 7 α

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Address for correspondence. Shalimar, Additional Professor, Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi, 110019, India.
E-mail: drshalimar@yahoo.com

Abbreviations: ALP: Alkaline phosphatase; AD: Acute decompensation; AMACR: α-methylacyl-CoA racemase (AMACR); ASBT: Apical sodium dependent bile salt transporter; BA: Bile acid; BSEP: Bile salt export pump; BSH: Bile salt hydrolase; CA: Cholic acid; CLD: Chronic Liver Disease; CDCA: Chenodeoxycholic acid; CYP7A1: Cholesterol 7 α hydroxylase; CTP: Child-Turcotte-Pugh; DCA: Deoxycholic acid; DR5: Death receptor 5; ELF: Enhanced Liver Fibrosis; FXR: Farnesoid X receptor; FGF-19: Fibroblast growth factor-19; FGFR4: FGF receptor 4; GCA: Glycocholic acid; GDCA: Glycodeoxycholic acid; GLP-1: Glucagon-like peptide1; HVPG: Hepatic Venous Pressure Gradient; HBV: Hepatitis B virus; HCV: Hepatitis C virus; LCA: Lithocholic acid; LPS: Lipopolysaccharide; MELD: Model for End-Stage Liver Disease (MELD); MRI-PDFF: Magnetic resonance imaging derived proton density fat fraction; NAFLD: Non-alcoholic fatty liver disease; NAS: NAFLD activity score; NASH: Nonalcoholic steatohepatitis; NTCP: Sodium taurocholate cotransporting polypeptide; OCA: Obeticholic acid; OST: Organic solute transporter; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis; PFIC: Progressive familial intrahepatic cholestasis; PXR: Pregnane X receptor; SHP: Small heterodimer partner; TBA: Total bile acids; TGR5: Takeda G-protein coupled receptor 5; TRAIL: TNF-related apoptosis-inducing ligand; UDCA: Ursodeoxycholic acid; UPLC-MS: Ultra-performance liquid chromatography with tandem mass spectrometry; VDR: Vitamin D receptor

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hydroxylase (CYP7A1).[9] This step is sensitive to negative feedback regulation via excess BAs. The primary BAs include cholic acid (CA) and chenodeoxycholic acid (CDCA) and are further conjugated to amino acids glycine and taurine (3:1) to increase their solubility. The BAs secreted by the hepatocytes are stored in the gallbladder and released postprandially into the proximal small bowel, where they carry out their well-known digestive functions such as emulsification and formation of micelles aiding in the absorption of fat- and fat-soluble vitamins. They are transported into the distal small bowel from where they are actively absorbed into the portal circulation and transported to the hepatocytes for resecretion via the enterohepatic cycle (Figure 1). The majority of the BAs are salvaged actively from the distal small bowel, and a small proportion escapes into the colon, where they are deconjugated by bacterial bile salt hydrolase and dehydroxylated to secondary BAs: deoxycholic acid and lithocholic acid. Ursodeoxycholic acid (UDCA) is a secondary BA formed from the bacterial epimerization of CDCA. These secondary BAs are also absorbed and form a minor fraction of the total BA pool in the body.

Apart from their well-known digestive functions in the gut, BAs act on a myriad of nuclear receptors such as farnesoid X receptor (FXR), vitamin D receptor and pregnane X receptor, and surface receptors such as Takeda G protein–coupled receptor 5 (TGR5) (Figure 2) to bring about their signaling effects. FXR receptors are expressed widely in hepatocytes as well as enterocytes. In the hepatocytes, BAs inhibit CYP7A1 via induction of small heterodimer partner, while in the enterocytes, they induce the production of fibroblast growth factor-19 (FGF-19), which acts via FGF receptor 4 (FGFR4) to inhibit CYP7A1 and BA synthesis. BAs also act on surface receptors such as TGR5 on enteroendocrine cells, causing the release of glucagon-like peptide-1, which acts as an incretin and plays a role in adipose tissue browning.[9]

DYSREGULATED BILE ACID METABOLISM, CIRRHOSIS, AND DYSBIOSIS: A MULTIDIRECTIONAL RELATIONSHIP

BA pool is depleted in patients with cirrhosis because of decreased synthesis and secretion of BAs from hepatocytes and disproportionate partitioning of BAs. Furthermore, the accumulation of BAs in the blood and within hepatocytes compounds the inhibition of CYP7A1, contributing to the BA pool depletion.[10] The decreased levels of fecal BAs promote depletion of Firmicutes, particularly *Blautia* and *Ruminococcus* species, and expansion of proinflammatory pathogenic bacteria of the phylum Proteobacteria, particularly *Enterobacteriaceae* (Figure 3). This is because colonic microbial groups are responsible for deconjugation and 7-alpha dehydroxylation of BAs, and it is hypothesized that the presence of microbe toxic BAs (particularly deoxycholic acid [DCA]) in the intestine is one of the factors that keep undesirable microbial populations under control.[11–15] The dysbiosis is linked to loss of membrane disrupting activity of secondary BAs directly as well as indirectly via loss of secretion of receptor-mediated antimicrobial peptides via BA signaling.[11] The decrease in Firmicutes is possibly because BAs serve as primary fermentative

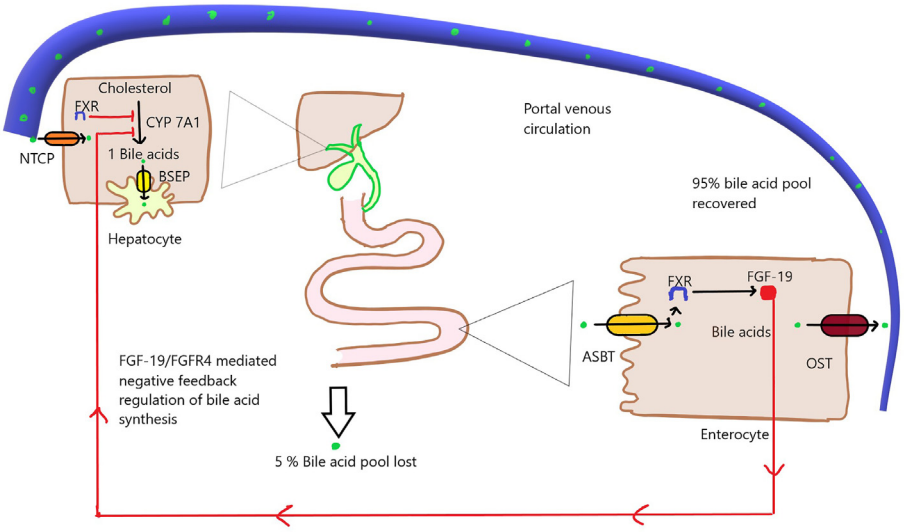

Figure 1 Enterohepatic cycle: Primary bile acids are synthesized predominantly via the classic pathway (80%) and secreted via bile salt export pump (BSEP) following conjugation with glycine and taurine. Following storage, in the gallbladder, they are released postprandially into the proximal small bowel. The secreted bile acids are actively absorbed via luminal apical sodium-dependent bile salt transporter (ASBT) in the distal small bowel from where they are transported to the portal circulation via organic solute transporter (OST). The reabsorbed bile acids are taken up by hepatocyte sinusoidal membrane protein sodium taurocholate cotransporting polypeptide (NTCP) and resecreted. The efficient enterohepatic cycling enables reabsorption of up to 95% of the bile acids and loss of around 5% of the pool which is resynthesized. CYP7A1: cytochrome P450 family 7 subfamily A member 1; FGF-19:fibroblast growth factor 19; FGFR4: fibroblast growth factor receptor 4; FXR: farnesoid X receptor.

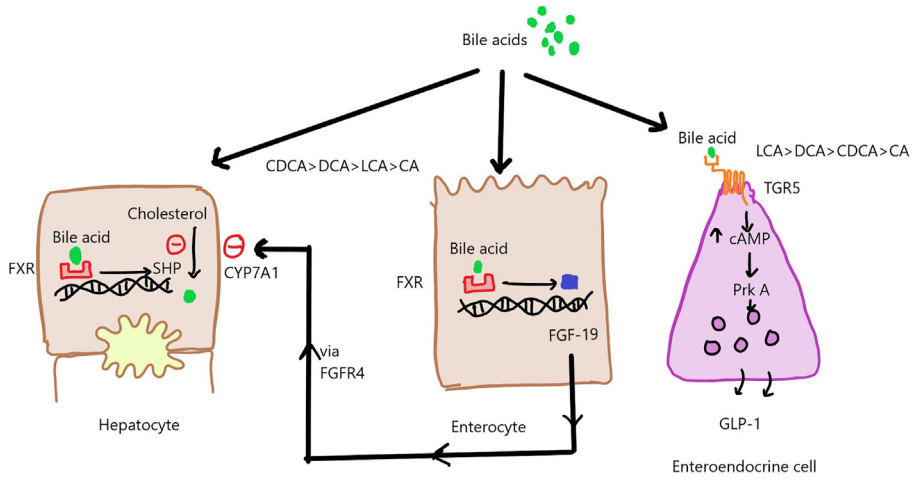

Figure 2 Bile acid signaling via nuclear and surface receptors. CA: cholic acid; cAMP: cyclic adenosine monophosphate; CDCA: chenodeoxycholic acid; CYP7A1: cytochrome P450 family 7 subfamily A member 1; DCA: deoxycholic acid; FGF19: fibroblast growth factor 19; FGFR4: fibroblast growth factor receptor 4; FXR: farnesoid X receptor; GLP-1: glucagon-like peptide-1; LCA: lithocholic acid; PRK A: phosphoribulokinase A; SHP: small heterodimer partner; TGR-5: takeda-G-protein-receptor-5.

electron acceptors for these bacteria.[11] The resulting inflammation contributes to increased translocation of bacterial products such as lipopolysaccharide and compounds the progression of cirrhosis. Apart from depleted BA pool, reduced intestinal motility decreased gastric and pancreatobiliary secretions, and portal hypertensive enteropathy can disturb the normal intestinal microbial community in patients with cirrhosis.

Apart from these changes in patients with cirrhosis, the proinflammatory cytokines inhibit the classic pathway's key enzyme CYP7A1. Hence, the alternate pathway forms the major source of BA synthesis in cirrhosis.[16] Patients with cirrhosis also have a depletion of 7α dehydroxylation bacteria, which leads to decreased secondary: primary BA ratio in cirrhosis.[16] Only the emergence of new microbial groups does not foster a pathologic milieu. Even the normally prevalent microbial groups often shift toward a more toxin-producing metabolic pathway because of survival benefits in a dysbiotic environment, thereby disturbing homeostasis.[17] Ridlon et al hypothesize that a decrease in DCA across different etiologies of cirrhosis could be beneficial as DCA could compound bacterial translocation and endotoxemia by irritating the gut mucosa, considering its membrane solubilizing potency.[18]

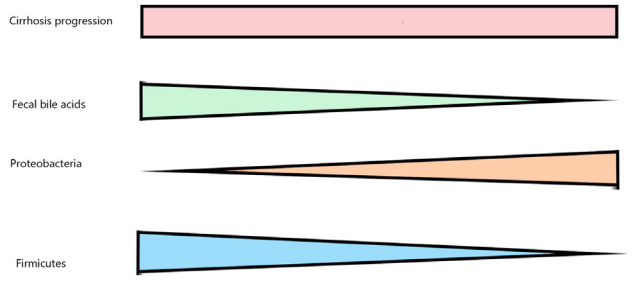

Figure 3 Effect of progression of cirrhosis on bile acids and microbiota.

GUT MICROBIOTA COMPOSITION AND BA LEVELS IN LIVER DISEASE

Primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) patients have decreased alpha/intraindividual diversity of microbes with higher proinflammatory genera levels than healthy controls, with some groups responding after 6 months of UDCA therapy.[19] Interestingly, Enterococcus was associated with an increase in TLCA (taurolithocholic acid) levels, a highly hydrophobic BA, in PSC patients.[20–23]

In chronic hepatitis B virus (HBV) cirrhosis, there is a decrease in the conversion of primary to secondary BAs and increase in FGF-19 (feedback inhibition of de novo BA synthesis), with advancing fibrosis.[24]

In alcoholic cirrhosis, an increase in primary BAs correlated with Enterobacteriaceae populations,[25] whereas in NAFLD, lower levels of Ruminococcaceae were associated with higher primary BAs in both obese and lean patients with NAFLD, whereas Veillonellaceae showed a positive association with primary BAs in lean patients only.[26]

Patients with autoimmune hepatitis (AIH) patients show lower alpha diversity compared with healthy controls with lower levels of commensal flora even after controlling for potential confounders, such as age, sex, and antibiotic use,[27] but no correlation with BA levels has been done.[28]

BA-MEDIATED TOXICITY AND INFLAMMATION

BAs can induce cell necrosis by solubilizing the plasma membrane or by signaling programmed cell death/

apoptosis. However, elevated BA concentrations, even in diseased states, rarely cause physical cellular damage.[29] BAs as signaling molecules can trigger cellular death pathways or release chemokines to recruit inflammatory cells.[30–32] In vitro studies in hepatocytes across species have shown that intracellular accumulation of BAs can cause oligomerization of the Fas receptor and activation of TNF-related apoptosis-inducing ligand receptor or death receptor 5.[33,34] This leads to activation of death-inducing signaling complex, which leads to caspase 8 activation. Caspase 8 brings about further cleavage of antiapoptotic proteins and activation of proapoptotic proteins. The downstream signaling brings about mitochondrial permeability transition, the release of cytochrome C, and mitochondrial dysfunction.

Glycodeoxycholic acid (GCDCA) is primarily known to induce apoptosis of cholangiocytes and hepatocytes.[13] In addition, reactive oxygen species generated due to excessive BAs (via phospholipase A2-induced membrane damage and interaction with nuclear receptors) overwhelm glutathione, which normally checks on repeated expected and stochastic cellular oxidative stressors. This increases the probability of cellular damage and necrosis. BAs can even increase Ca++ release from the endoplasmic reticulum (ER), triggering extracellular calcium entry into cells and activating caspases. A molecule called CHOP is involved in the ER stress pathway, and interestingly, CHOP knockout models have shown decreased liver fibrosis.[33] The cytokines released from BA-induced cellular damage activate the hepatocyte stellate cells, repeated cycles of which can lead to irreversible fibrosis. However, intrahepatocellular accumulation of BAs is key to inducing hepatocyte damage.[35] Exposure of hepatocytes to elevated BA concentrations as seen in obstructive cholestasis has shown to cause an increase in cytokines (interleukin [IL]-1β and IL-10), chemokines such as macrophage inflammatory protein cell adhesion molecules (ICAM-1 and VCAM-1), enzymes such as COX-2, and thereby influence immune cell levels and function.[31]

EVIDENCE ON THE DIAGNOSTIC AND PROGNOSTIC UTILITY OF BAS

BA derangements are common in the diseased liver and therefore may serve as markers of derangement and potential targets to help restore normal physiology. In healthy subjects, CA:CDCA typically ranges between 0.6 and 1, while in cirrhosis, it is reduced to 0.1 to 0.5 and further decreases as the severity of cirrhosis increases. The greatest decrease in CA occurs before overt symptoms appear, i.e., in early stages of cirrhosis.[36] The standard assay to measure BAs in serum, urine, and stool is ultraperformance liquid chromatography with tandem mass spectrometry. Other separation-based assays include gas chromatography (GC)-MS, high-performance liquid chromatography-MS, supercritical fluid chromatography, capillary electrophoresis, enzyme-linked immunosorbent assay, thin-layer chromatography, and nuclear magnetic resonance (NMR) spectroscopy–based assays. However, the more commonly used 3-α-hydroxysteroid dehydrogenase–based colorimetric assays are better suited for total BA measurements than for individual BAs, and LC/GC-MS remains the gold standard.[37]

Kim et al found the fasting serum BA concentration to be a more sensitive test of disordered hepatobiliary function than conventional liver function tests. They observed that some of their patients with cirrhosis had higher serum BA concentrations despite normal transaminases.[36]

Across various etiologies of CLD, Alamoudi et al found that total urinary BAs were maximally increased in patients with PBC and only marginally in HBV infection. The increase in total urinary CA and CDCA was highest in PSC and lowest in HBV infection. The total urinary primary/secondary BA ratio was high in most cases of CLD and low only in PBC.[38] In another study, including patients with alcoholic or nonalcoholic cirrhosis, serum conjugated primary BAs such as GCA, GCDCA, TCA, TCDCA, and TUDCA were higher in Child-Turcotte-Pugh (CTP) B and C than in CTP A, whereas secondary BAs showed a decreasing trend with advancing cirrhosis.[39] Higher total (but not individual) BAs were also associated with increased 6-month mortality. Prediction accuracies of BAs were slightly lower than those of the Model for End-Stage Liver Disease score; yet, BAs can still serve as prognostic indicators for cirrhotic patients.

Alcoholic Liver Disease

In alcoholic cirrhosis, the total fecal BA pool shrinks with a reduction in both primary and secondary BAs.[25,36] Taurine conjugates are higher than glycine conjugates. Although the absolute CDCA pool is significantly reduced in early alcoholic cirrhosis, it is largely unchanged in advanced stages. CA synthesis progressively decreases with cirrhosis; hence CA:CDCA ratio becomes a useful index of disease severity.[36,40] Kakiyama et al found that alcohol intake increases stool BAs even in healthy subjects, with fecal total bile acid (TBA) levels being lowest in abstinent alcoholic cirrhotics. Second, alcoholic cirrhosis patients have higher serum BAs than nonalcoholic cirrhosis, and serum BAs were higher in both alcoholic/nonalcoholic cirrhosis in comparison to healthy subjects irrespective of alcohol intake.[25]

Nonalcoholic Fatty Liver Disease

NAFLD with liver fibrosis has higher total plasma BAs (with a disproportionate increase in primary BAs), which increase with advancing fibrosis. Glycine-conjugated CA and CDCA, 7-keto DCA, and GUDCA specifically show

association with advancing fibrosis, whereas most secondary BAs show no significant difference.[41]

The fecal BA profile in NAFLD differs in obese and nonobese/lean patients (body mass index <25 kg/m^2). In Asian lean NAFLD patients, total stool BAs were higher, with an increase in both unconjugated (CA and CDCA) and conjugated BAs (GUDCA and GCDCA) and fecal TBAs among those with advanced fibrosis.[26] In obese NAFLD patients with liver fibrosis, fecal TBAs, total conjugated BAs, unconjugated BAs, and total fecal secondary BAs progressively decrease as fibrosis advances. The secondary to primary BA ratio and the unconjugated to conjugated BA ratios also show a decrease. There is no clinically relevant change in unconjugated primary BAs but conjugated primary BAs increase with advancing fibrosis.[26]

Viral Hepatitis

Serum TBAs are also significantly increased in cirrhosis because of HBV and hepatitis C virus (HCV) infection.[42,43] Yan et al demonstrated an association of cirrhosis and serum TBAs to total cholesterol ratio in noncholestatic chronic HBV infection.[44] Serum TBAs, particularly conjugated BAs, are elevated in HBV cirrhosis with a progressive increase as CTP grade advances.[45] Fecal TBAs, unconjugated BAs, total fecal secondary BAs, and unconjugated secondary BAs decrease with advancing fibrosis as do the ratios of total secondary to primary BAs, DCA to CDCA, and unconjugated to conjugated BAs. In HCV cirrhosis, fasting serum TBAs are higher in severe cirrhosis than nonsevere cirrhosis, whereas biliary secondary BAs decrease as the cirrhosis advances.[40] However, serum BAs show an increase in advanced cirrhosis, even with normal bilirubin.[46]

Hepatocellular Carcinoma

In early cirrhosis, the concentrations of serum TBAs and primary conjugated BAs are significantly higher in patients with underlying hepatocellular carcinoma (HCC) and can help suspect HCC before overt manifestations. However, TBAs do not show an association with mortality in this case.[39]

Primary Sclerosing Cholangitis and Primary Biliary Cholangitis

Compared with healthy controls, fecal glycine-conjugated primary BAs and serum total primary BAs are higher in PBC, whereas serum total secondary BAs are lower.[47] Both serum and fecal secondary to primary BA ratios and hydrophobic to hydrophilic BA ratios are decreased in PBC, whereas serum and fecal conjugated to unconjugated BA ratios are increased. The data for serum BAs in PSC are confounded by UDCA therapy. But PSC patients have similar DCA levels, higher TBA levels, and decreased glycine:taurine BA ratios of CDCA and DCA, compared with controls. In a study by Mousa et al, serum TBAs were predominantly elevated only in patients on UDCA treatment. In the subset of patients who were not on UDCA, their concentration was higher than in healthy subjects (but within the normal range), increasing the conjugated fraction.[48] An increase in serum levels of conjugated BAs (TCA and TCDCA) has been seen in experimental AIH models, but there is no evidence in humans yet.[49]

Acute Liver Failure

Horvatits et al investigated the role of fasting serum BAs as markers for acute decompensation (AD) of noncholestatic cirrhosis in hospitalized patients (excluding patients with PBC or PSC or those on UDCA). They found that in AD, TBAs, taurine, and glycine conjugates of primary BAs as well as UDCA, DCA, and unconjugated CDCA levels increase with an increase in CTP grade. Among the individual CTP components, BA levels correlated with total bilirubin and INR. In their sample of 39 AD patients, TCDCA had the strongest association with AD, whereas among the 11 ACLF patients, it was TCA and GCA. Interestingly, in a subsequent year-long follow-up in patients without AD/ACLF on initial admission, TBAs showed an independent association with new-onset ACLF and AD and increased CDCA and TBA levels more than $\geq$36.9 μmol/L predicted AD/ACLF with a 78% sensitivity.[50] In this study, TBAs also positively correlated with the hepatic venous pressure gradient.

Pediatric Cholestatic Disorders

Serum BAs are elevated in childhood cholestatic disorders, such as Dubin-Johnson syndrome, progressive familial intrahepatic cholestasis (PFIC 1–3), and Alagille syndrome (AGS) with higher serum BA concentration in PFIC (PFIC 2 > PFIC 1) compared with AGS.[51–53] There is no difference between total biliary bile salts between PFIC1 and PFIC2 (bile salt export pump deficiency). Pawlikowska et al found biliary bile salts and CDCA/CA ratio lower in PFIC, whereas the taurine:glycine (T:G) ratio and hydrophobicity index were not any different. Postsurgical diversion, AGS patients have a higher biliary CDCA/CA ratio compared with PFIC. A normalization of BAs postdiversion was associated with a longer duration of survival with native liver.[54] Serum TBAs are typically low to normal in cholestasis because of BA synthesis disorders.[55] Duodenal biliary TBA (dTBA), dTBA/sTBA ratio, and dTBA/serum gamma-glutamyl transferase (GGT) ratio were lower in infants with biliary atresia than healthy infants.[56] BAs have not been shown to correlate with the severity of pruritis in childhood cholestatic disorders.[57] Serum primary BAs (especially GCDCA and TCDCA) are higher in cholestasis due to biliary atresia than due to other causes and may help in noninvasively distinguishing etiology of childhood cholestasis.[58]

BILE ACID THERAPEUTICS

Several therapies based on BAs and their signaling have been tested in various etiologies of CLD in various clinical trials and summarized in the following sections.

Ursodeoxycholic Acid

UDCA is a naturally occurring hydrophilic BA produced by epimerization of CDCA by colonic bacteria. On UDCA administration, there is a reduction in FXR-activating BAs in the pool, indirectly negating the FXR-mediated inhibition of BA synthesis. UDCA is approved as the first-line therapy for PBC. In therapeutic doses (>13 mg/kg), it improves liver transplant–free survival and is associated with fewer deaths per year in PBC with and without evidence of cirrhosis, with a more substantial effect in younger patients and those with higher baseline alkaline phosphatase (ALP).[59]

Studies have shown an improvement in Mayo risk score with UDCA in PSC, indicating improved survival.[60–62] UDCA has also consistently been shown to improve liver biochemistry, but that is now considered to be a contentious endpoint in PSC.[63–66] A systematic review and a meta-analysis of randomized controlled trials evaluating UDCA in PSC showed no significant reduction in risk of death, liver transplant, ascites, encephalopathy, histological grade, and progression to cholangiocarcinoma.[67,68] Despite questionable benefits, worsening symptoms and liver biochemistry were observed within 3 months of UDCA withdrawal in PSC patients.[69] However, the American College of Gastroenterology (ACG) categorically recommends against doses more than 28 mg/kg because of higher incidence of adverse events.[70,71]

Multiple studies show biochemical and improvement in pruritis with UDCA therapy in cholestatic disorders, such as PFIC and AGS, with more significant benefit with doses >20 mg/kg.[72–74] In PFIC, UDCA therapy even demonstrated reversal of liver fibrosis.[72] In pediatric cholestasis secondary to long-term total parenteral nutrition, UDCA has shown improvement in liver biochemistry and resolution of splenomegaly, whereas in extrahepatic biliary atresia, it only offers symptomatic benefit.[74–77]

norUDCA

norUDCA is a shortened derivative of UDCA relatively resistant to amidation. This helps in increasing its cholehepatic shunting. In 2017, a Phase II double-blind randomized controlled trial (DBRCT) of norUDCA showed a significant improvement in ALP levels in both sexes irrespective of pretreatment with UDCA within 12 weeks of therapy (NCT01755507). This was dose dependent, with the maximum dose of 1500 mg displaying a safety profile comparable to placebo. The 1000 mg and 1500 mg treatment groups also showed a decrease in spleen sizes. While pruritus, an expected adverse effect was not higher compared with placebo, the 1500 mg norUDCA group reported an increased number of headaches. However, this trial excluded patients with CTP B and C and those with serum bilirubin >3 mg/dL. Patients are currently being recruited for a 2-year long Phase 3 trial to evaluate 1500 mg norUDCA in PSC (NCT03872921).

Oral CA supplementation improves bile flow and liver function tests in inborn errors of BA synthetic pathway, such as 3β-hydroxysteroid dehydrogenase(3βHSDH) deficiency and α-methylacyl-CoA racemase (AMACR) deficiency. This is partly due to feedback inhibition of the BA synthetic pathway. In genetic defects of BA conjugation, conjugated primary BA supplementation may be potentially helpful.[78]

FXR-BASED THERAPEUTICS

Obeticholic acid (OCA) or 6-alpha ethyl CDCA is a potent and selective synthetic steroidal FXR agonist. It is known to promote bile flow or choleresis, which prevents the accumulation of hydrophobic BAs in the liver.[79] FLINT trial–tested OCA in noncirrhotic nonalcoholic steatohepatitis (NASH) (NCT01265498). OCA in a dose of 25 mg showed significant improvement in NAFLD activity score (NAS), without worsening of fibrosis at the end of 72 weeks.[80] There was also a significant improvement in fibrosis in the OCA group. However, the treatment group did not show resolution of NASH or reversal of diagnosis. Pruritus was seen in nearly 20%, with 2–3% developing very severe episodes of pruritus requiring withholding and/or discontinuation of the medication. In addition, OCA was associated with an increase in low-density lipoprotein (LDL) cholesterol levels. The 18-month interim analysis from the REGENERATE trial showed a significant improvement in fibrosis with no worsening of NASH in both 10 mg and 25 mg arms compared with placebo; however, there was no significant difference in NASH resolution without worsening of fibrosis.[81] An extended follow-up of the same study showed significant improvement in NASH, with no worsening of fibrosis in the OCA group.[82] Currently, the approval of OCA for noncirrhotic NASH hangs by a thin thread because of a significant proportion of patients experiencing pruritus (50%) and increased LDL cholesterol (17%) in the REGENERATE trial. The data on hypercholesterolemia are particularly concerning because most NASH patients have other risk factors for coronary artery disease. Currently, the REVERSE trial (Phase 3 NCT03439254) is ongoing to study the efficacy of 10 mg and 10–25 mg OCA in patients with compensated cirrhosis due to NASH.[83] A recent review has discussed the role

Table 1 Summary of Studies Evaluating Therapeutics in NAFLD and NASH.

Author	Drug and dose	Inclusion criteria, stage of fibrosis	Type of study	Duration of therapy	Outcome
Ongoing	Elobixibat (IBAT inhibitor) 5 mg once daily	NAFLD, NASH	Randomized double-blind, placebo-controlled Phase 2 study	16 weeks	Change in serum LDL cholesterol
Nadinskaia et al. WJG 2021[101]	N = 174; 15 mg/kg/d UDCA 121 (69.5%) men and 53 (30.5%) women Men significantly younger than women	Ultrasound-diagnosed NAFLD; FLI >60	Open-label, multicenter, international uncontrolled trial	6 months	Δ decrease in ALT, AST, and GGT during 0–3 m > 3–6 m. ↔ in NFS, FIB-4. Significant ↑ in HDL, ↓ in LDL-C, TC, TG Sex differences in response observed
Traussnigg et al, Wien Klin Wochenschr, 2021[102]	5 mg PX-104 (nonsteroidal FXR agonist) once daily	Nondiabetic NAFLD (n = 12)	Open-label Phase 2a study	4 weeks	↑ IS ↓ ALT and GGT ↔ ALP or serum lipids. ↔Hepatic steatosis: MRI-PDFF, 1H-MRS, and CAP ↔ Serum BAs Cardiac arrhythmia in two patients led to the termination of the study.
Newsome et al. Journal of hepatology 2020[103]	N = 197 were randomized to receive Volixibat 5 mg (n = 49), Volixibat 10 mg (n = 50), Volixibat 20 mg (n = 49), or placebo (n = 49) once daily	Adults, ≥5% steatosis, and NASH without cirrhosis	Phase 2 randomized, double-blind, Phase II, placebo-controlled study	48 weeks	Volixibat did not meet interim endpoints (24 weeks), i.e., ≥5% reduction in MRI-PDFF and ≥20% reduction in serum ALT. The study was terminated owing to a lack of efficacy
Pockros et al. Liver international, 2019[104]	5 mg, 10 mg, 25 mg OCA once daily	Biopsy-confirmed NASH without hepatic decompensation	Randomized, double-blind, placebo-controlled, Phase 2 study	16 weeks	↓ LDL-C with OCA + statin
Palmer et al. BMC pharmacology and toxicology 2018[105]	20, 40, or 80 mg Volixibat (n = 63); placebo (n = 21) once daily	Overweight and obese adults	Phase 1 study	12 days	Volixibat (≥20 mg/day): maximal fecal BA excretion in obese and overweight adults
Harrison et al Hepatology 2020[106]	Subcutaneous NGM282 1 mg (n = 24) NGM282 3 mg (n = 19) once daily	Paired biopsies, NASH as per NASH CRN criteria; F1-F3; liver fat ≥8%; ↑ ALT	Open-label, multicenter trial	12 weeks	50% and 68% in the 1 mg and 3 mg treatment arms, respectively, achieved significant histological improvement. 12% and 10% in the 1 mg and 3 mg groups, respectively, achieved NASH resolution without fibrosis worsening at 12 weeks
Younossi et al. Lancet 2019[81]	N = 931; placebo (n = 311) OCA 10 mg (n = 312); or OCA 25 mg (n = 308)	Stage F2-F3 fibrosis	Phase 3 multicenter, randomized, placebo-controlled trial	Interim analysis at 18 months (total study period = 4 years)	OCA 25 mg significantly improved fibrosis without worsening of NASH by 1.9 times (95% CI 1·4–2·8) c/w placebo. Greater proportion of patients receiving 25 mg OCA showed improvement in liver histology and in serum ALT and AST
Harrison et al. Lancet 2018[91]	Subcutaneous NGM282 (FGF-19 analog) 3 mg (n = 27), NGM282 6 mg (n = 28), or placebo (n = 27) once daily	Biopsy-confirmed NASH; Stage 1–3 fibrosis; liver fat ≥8%; ↑ ALT	Multicenter international randomized, double-blind, placebo-controlled, Phase 2 trial	12 weeks	NGM282: ↓ ALT/AST, ↓ liver fat ↓ TGs seen only in 6 mg group and ↑ total and LDL cholesterol in both groups

(*Continued on next page*)

Table 1 (*Continued*)

Author	Drug and dose	Inclusion criteria, stage of fibrosis	Type of study	Duration of therapy	Outcome
Neuschwander-Tetri et al (FLINT trial) Lancet 2015[80]NCT01265498	OCA 25 mg (n = 141) or Placebo (n = 142) once daily	Histologically proven NASH or borderline NASH	Phase 3 Randomized double-blind, placebo-controlled trial	72 weeks	OCA: improved liver histology (≥2-point NAS without worsening of fibrosis). Mean change in NAS: OCA > placebo. OCA: ↓ ALT and AST. ↑ ALP, ↓ GGT which reversed after stopping OCA. NASH resolution (OCA = placebo). OCA (ADE): ↑ LDL-C, Pruritis: 23% vs. 6% in placebo
Siddiqui et al Journal of Hepatology. 2020[107]	Serum and biopsy samples of 196 patients who were enrolled in the FLINT trial; OCA group (n = 99), placebo group (n = 97) once daily	Histologically proven NASH or borderline NASH		72 weeks	OCA: ↑ increase in lipoprotein levels, which improves after drug discontinuation
Mueller et al. Journal of hepatology 2015[108]	20 mg/kg/day UDCA (n = 19) in two daily doses; Controls (n = 18)	Morbidly obese (BMI >35 kg/m^2) NAFLD scheduled for laparoscopic Roux-en-Y gastric bypass	Randomized open-label study	3 weeks	UDCA: ↓ AST, GGT, free FA, total and LDL-C, and ↑ TGs
Mudaliar et al. Gastroenterology 2013[109] NCT00501592	Placebo (n = 23), 25 mg OCA (n = 20), or 50 mg OCA (n = 21) once daily	Patients with type 2 diabetes mellitus and NAFLD	Phase 2 Multicenter, randomized, double-blind, placebo-controlled study	6 weeks	OCA: ↑ IS by 28% as c/w placebo OCA: ↓ GGT, ALT, and dose-related weight loss. ↑ LDL-C and FGF-19, a/w ↓ 7α-hydroxy-4-cholesten-3-one and endogenous BAs
Ratziu et al J Hepatol. 2011[110]	N = 126; high-dose UDCA (HD-UDCA; 28–35 mg/kg per day)	Biopsy-proven NASH and elevated ALT	Phase 2 randomized, double-blind, placebo-controlled multicenter trial	12 months	HD-UDCA: ↓ ALT
Leuschner UF, Hepatology. 2010[111]	N = 185; UDCA 23–28 mg/kg (n = 94) or placebo (n = 91) daily in three divided doses	NASH (per NAS and modified brunt score)	Randomized, double-blind, placebo-controlled study	18 months	No difference in histology c/w placebo
Lindor et al. Hepatology 2004[112]	N = 166; 13–15 mg/kg/day of oral UDCA (n = 80) or placebo (n = 86) four divided doses daily	Patients with biopsy-proven NASH	Prospective, randomized, double-blind, placebo-controlled trial	24 months	UDCA (13–15 mg/kg/d): not effective in patients with NASH
Laurin et al. Hepatology 1996[113]	13–15 mg/kg/day of oral UDCA in divided doses with meals (n = 24); 2 g/day Clofibrate in two divided doses (n = 16 with NASH + hypertriglyceridemia)	Biopsy-proven NASH	Open-label study	12 months	UDCA: ↓ ALP, ALT, GGT, and hepatic steatosis

IS, insulin sensitivity**; IBAT**, ileal bile acid transporter; **LDL-C**, low-density lipoprotein cholesterol; **OCA**, obeticholic acid; **NAFLD**, nonalcoholic fatty liver disease; **NASH**, nonalcoholic steatohepatitis; **LDL**, low-density lipoprotein; **FXR**, Farnesoid X receptor; **ALT**, alanine aminotransferase; **AST**, aspartate aminotransferase; **GGT**, γ-glutamyltransferase; **ALP**, alkaline phosphatase; **MRI-PDFF**, magnetic resonance imaging–proton density fat fraction; **^{1}H-MRS**, proton magnetic resonance spectroscopy; **CAP**, controlled attenuation Parameter; **BA**, bile acid; **NASH CRN**, nonalcoholic steatohepatitis clinical research network; **FGF-19**, fibroblast growth factor 19; **PSC**, primary sclerosing cholangitis; **NAS**, NAFLD activity score; **TG**, triglycerides; **BMI**, body mass index; **UDCA**, ursodeoxycholic acid; **ADE**, adverse drug events; **FA**, fatty acids; **CI**, confidence interval; **NFS**, NAFLD fibrosis score; **FLI**, Fatty Liver Index; **FIB-4**, Fibrosis-4 Index; **TC**, total cholesterol.

Table 2 Summary of Studies in Primary Cholestatic Disorders in Adults.

Author	Drug	Inclusion criteria, stage of fibrosis	Type of study, drug dose	Duration of therapy	Outcome
Kjærgaard, Kristoffer et al. Journal of Hepatology 2021[114]	N = 8; daily oral OCA dose 5 mg for 1 month and 10 mg for 2 months or matching placebo	PBC	Single-center, double-blind placebo-controlled, crossover study	3 months each with placebo or treatment	c/w placebo, OCA ↑ hepatic blood perfusion (11%). No significant difference in pruritis between the two treatments.
Kowdley et al. Journal of hepatology 2020[115] AESOP (Assessment of Efficacy and Safety of OCA in PSC)	N = 76; Placebo (n = 25), OCA 1.5–3.0 mg (n = 25), and OCA 5–10 mg (n = 26) once daily	PSC based on cholangiography Concomitant UDCA therapy in ≤50%	Randomized, double-blind, placebo-controlled phase 2 trial	24 weeks	Significant ↓ in serum ALP in the OCA 5–10 mg group compared with placebo. Pruritus was worse in the 5–10 mg OCA group.
Trauner et al. Hepatology 2019[87]	N = 52; Cilofexor 100 mg (n = 22), 30 mg (n = 20), or placebo (n = 10) orally once daily 46% patients on UDCA	PSC patients without cirrhosis	Multicenter, randomized, double-blind, placebo-controlled, Phase 2 trial	12 weeks	Significant ↓ in ALP, ALT, and AST, secondary BAs, HDL-C in 100 mg group, and ↓ GGT in both groups. Dose dependent ↓ in relative and absolute ALP concentration. Common ADEs: pruritus, abdominal pain, and nasopharyngitis.
Hegade et al. Lancet 2017[116]	N = 22; IBAT inhibitor GSK2330672 or placebo (n = 11 each), 45 mg twice daily on days 1–3, followed by 90 mg twice daily on days 4–14	PBC on UDCA for >8 weeks, serum ALP ≤10 times ULN	Phase 2a randomized, double-blinded, two-period crossover trial	14 days	GSK2330672 reduced pruritus severity. Diarrhea, the most common adverse event
Fickert et al Journal of hepatology 2017[117]	N = 159; norUDCA 500 mg (n = 39), 1000 mg (n = 41), 1500 mg (n = 39) or placebo (n = 40) once daily	PSC without concomitant UDCA therapy and with elevated serum ALP	Multicenter randomized, double-blind, placebo-controlled Phase 2 study	12 weeks. Follow-up after 4 weeks after finishing study	↓ in ALP (nearly to baseline), in all treatment arms. Notable ADEs: abdominal pain, fatigue, nasopharyngitis, headache, and pruritus.
Nevens et al. The New England journal of medicine 2016[83] (POISE trial)	N = 216; OCA: 10 mg (n = 73); 5 mg with adjustment to 10 mg if applicable (n = 70), or placebo (n = 73)	PBC with inadequate response to UDCA/ intolerant to UDCA with 93% taking on UDCA	Randomized, double-blind, placebo-controlled parallel group Phase 3 trial	12 months	Significant ↓ in ALP and total bilirubin in both treatment groups (irrespective of concomitant UDCA intake) Pruritus significantly higher in both treatment groups.
Trauner et al The lancet. Gastroenterology & hepatology 2019[118]	N = 193; placebo (n = 48); OCA 5–10 mg (n = 55); OCA 10 mg (n = 53)	Open-label extension (OLE) of OCA in PBC patients included in POISE trial	3-year interim analysis of 5-year open label extension of OCA in PBC patients included in POISE trial	3 years	ALP significantly ↓ at 12, 24, 36, and 48 m (including 12 months of POISE trial) and total bilirubin ↓ at 12 m and 48 m only. Mean ↓ in liver enzymes (AST, ALT, GGT, and ALP) were persistent as in POISE trial and significant at each yearly time point. ALP, GGT, ALT, and AST showed significant ↓ starting as early as 3 months. CRP was significantly ↓ at 12 and 48 m. Similar results were observed across treatment groups ADEs: pruritus, fatigue, and nasopharyngitis Hepatic ADEs: esophageal varices and ascites.
Bowlus et al. Clinical Gastroenterology and Hepatology 2020[119]	N = 17; OCA 5 mg or OCA 5–10 mg plus concomitant UDCA	PBC patients with inadequate response to or intolerance to UDCA	Analysis of paired biopsies from the POISE study	Biopsy at baseline and at 3 years	Improvements or stabilization of ductular injury, fibrosis, and collagen morphometry. Significant ↓ in ALP, ALT, AST, GGT, CK-18, CRP, APRI, and ↑ FGF-19.

(*Continued on next page*)

Table 2 (*Continued*)

Author	Drug	Inclusion criteria, stage of fibrosis	Type of study, drug dose	Duration of therapy	Outcome
Harms et al. JHEP reports 2020[120]	N = 187; Once daily OCA 5 mg, OCA 5–10 mg, or placebo At baseline: low risk (n = 47); moderate risk (n = 79); high risk (n = 89) based on GLOBE score and APRI.	PBC patients with inadequate response to or intolerance to UDCA	Post hoc analysis of data from the POISE study	12 months	Improvement or absence of worsening of baseline GLOBE score more likely in treatment groups. Significantly ↑ proportion of patients on OCA with ≥1 risk stage improvement irrespective of age-specific/nonspecific GLOBE scores used for defining risk stage.
Corpechot et al. Journal of Hepatology 2020[121]	N = 780; UDCA 10–15 mg/kg/day (n = 190) orally in two divided doses started within 2 weeks of liver transplantation vs no preventive UDCA (n = 590)	PBC patients with liver transplant	Multicenter retrospective cohort study	14 ± 7.4 yrs	Preventive UDCA therapy after liver transplant for recurrent PBC significantly a/w ↓ disease recurrence, graft failure, and 5-, 10-, 15-, 20-, and 25-year mortality.
Hirschfield et al. Gastroenterology 2015[85]	N = 165; OCA 10 mg (n = 38), 25 mg (n = 48), 50 mg (n = 41), placebo (n = 38) once daily N = 78 for OLE at 3–60 mg once daily (mean 20 mg)	PBC patients on stable dose of UDCA for 6 months	Randomized double-blind placebo-controlled trial	3 months	Daily doses of 10–50 mg OCA, significantly ↓ ALP, GGT, AST, and ALT. Significant ↓ in C4 and total endogenous BAs among all OCA groups and ↑ in FGF19 in 10 mg and 25 mg groups. Significant ↓ in total cholesterol and HDL-C across all dose groups and no significant change in LDL-C and TGs Significantly higher incidence of pruritus in OCA 25 mg and 50 mg groups.
Dilger K et al J Hepatol. 2012[122]	N = 22; UDCA (15 mg/kg/day) once daily (n = 11); HC (n = 11)	Female patients, biopsy-proven PBC stage I, II, or II–III	Controlled trial	3 weeks	PBC patients show higher rates of taurine conjugation in bile. ↓ after UDCA treatment
Lindor et al. Hepatology 2009[71]	High-dose UDCA (28–30 mg/kg/day in divided doses) vs placebo UDCA	PSC not on UDCA treatment in 3 months prior	Randomized, double-blind placebo-controlled trial	5 years	Death, liver transplant, minimal listing criteria for liver transplant, development of cirrhosis, esophageal and/or gastric varices, and cholangiocarcinoma more common in treatment group despite improved liver biochemistries. Study terminated after 6 years because of adverse effects.
Cullen et al. Journal of hepatology 2008[123]	N = 31; UDCA 10 mg/kg (n = 11); 20 mg/kg (n = 11); 30 mg/kg (n = 9)	PSC; Most patients with UC also	Randomized trial	24 months	Serum ALT and AST significantly ↓ with 20 mg/kg of UDCA, ALP, GGT with all three doses. Survival at all time points calculated (1–4 years) significantly ↑ only with 30 mg/kg/day. No significant histological improvement with any dose. No difference in side effect profile between the three doses of UDCA.
Olsson et al. Gastroenterology 2005[124]	N = 198; 17–23 mg/kg/day of UDCA (n = 97) or placebo (n = 101)	PSC based on cholangiography with conventional radiological criteria not on UDCA treatment	Randomized placebo-controlled study	5 years	No significant difference in liver enzymes and time to LT or death or percentage of patients with that endpoint in either group. ALP and ALT only tended to ↓ in UDCA-treated patients during the first 6 months.
Harnois et al. The American journal of gastroenterology 2001[60]	N = 128; high-dose UDCA (25–30 mg/kg/day, n = 23) in four divided doses, low-dose UDCA (13–15 mg/kg/day, n = 53), placebo (n = 52)	PSC without previous treatment with UDCA	Prospective study	12 months	Significant improvement in serum ALP, AST, albumin, and bilirubin with both low- and high-dose UDCA. Improved Mayo risk score (therefore 4-year survival) only with high-dose UDCA.

Table 2 (*Continued*)

Author	Drug	Inclusion criteria, stage of fibrosis	Type of study, drug dose	Duration of therapy	Outcome
Angulo et al. Journal of hepatology 1999[125]	N = 137; low dose 5–7 mg/kg/day (n = 47) standard dose 13–15 mg/kg/day (n = 45) high dose 23–25 mg/kg/day (n = 45) in four divided doses	Clinical and histological evidence of PBC not previously treated with UDCA	Randomized, double-blind trial	12 months	Improvement in ALP, AST, Mayo risk score: standard- and high-dose groups significantly > low-dose group; standard dose ≈ high-dose groups.
Lindor et al The New England journal of medicine 1997[126]	N = 102; UDCA 13–15 mg/kg/day in four divided doses (n = 51), placebo (n = 51)	PSC for at least 6 months with biopsy within 3 months	Multicenter, randomized, double-blind, placebo-controlled trial	24 months	Significant improvement in serum ALP, AST, bilirubin, and albumin levels at 1 and 2 years No difference in time to treatment failure, that is, death or histologic progression by two stages or decompensation or to liver transplantation irrespective of early histologic disease or presence of colitis. No significant changes in liver histology/symptoms/serum lipids after 2 years.
De Maria et al. Hepato-gastroenterology 1996[127]	N = 59; UDCA 300 mg twice daily (n = 20) colchicine 0.6 mg orally twice daily (n = 19); and untreated control group (n = 20)	PSC based on clinical, biochemical, and radiology	Randomized controlled study	24 months	No difference between groups in liver enzymes, liver function, liver size, and hepatic copper content.
van de Meeberg et al J Hepatol. 1996[128]	N = 27; UDCA (10 mg/kg/day) in a single dose at bedtime (n = 13) or in three divided doses with meals (n = 14)	Early stage (Stage I-II) disease. PSC (n = 19), PBC (n = 8)	RCT	3 months	Single- or multiple-dose UDCA have similar effects on liver biochemistry.

UDCA, ursodeoxycholic acid; **GGT**, γ-glutamyltransferase; **ALP**, alkaline phosphatase; **HC**, healthy controls; **PBC**, primary biliary cholangitis; **PSC**, primary sclerosing cholangitis; **IBD**, inflammatory bowel disease; **FGF19**, fibroblast growth factor-19; HDL-C, high-density lipoprotein cholesterol; **AST**, aspartate aminotransferase; **ALT**, alanine aminotransferase; **OCA**, obeticholic acid; **IBAT**, ileal bile acid transporter; **BA**, bile acid; **ERC**, endoscopic retrograde cholangiography; **ELF**, enhanced liver fibrosis; **MRCP**, magnetic resonance cholangiopancreatography; **TIMP-1**, tissue inhibitor of metalloproteinases-1; **CRP**, C-reactive protein; **GLOBE**, not an acronym; **CK-18**, cytokeratin 18; APRI, AST to platelet ratio index; **ITT**, intention-to-treat; **GLDH**, glutamate dehydrogenase; **LT**, liver transplantation; **C4**, 7 α-hydroxy-4-cholesten-3-one.

Table 3 A Summary of Studies Evaluating Bile Acids in Pediatric Cholestatic Disorders.

Author	Sample size and therapy	Inclusion criteria, stage of fibrosis	Type of study, drug dose	Duration of therapy	Outcome
Baumann et al, Clinics and research in hepatology and gastroenterology, 2021[129]	N = 24; 10–200 μg/kg oral odevixibat daily	PFIC (n = 13), Alagille syndrome (n = 6), biliary atresia (n = 3), other causes of intrahepatic cholestasis (n = 2)	Open-label, multicenter Phase 2 study	4 weeks	↓ serum BA compared with baseline levels (reductions up to 98%). Improved pruritus (three scales) and sleep. No serious ADEs
van Wessel et al. Hepatology, 2021[130]	N = 130, surgical biliary diversion	Compound heterozygous- or homozygous-predicted pathogenic ATP8B1 variants (PFIC)	Multicenter, combined retrospective, and prospective study	–	Postsurgical diversion serum BA concentrations <65 μmol/L show a trend of association ($P = 0.05$) with improved NLS
Deneau et al. The Journal of pediatrics 2019[131]	N = 263; GGT normalization after 12 months (n = 122); non-norm (n = 141)	PSC with baseline serum GGT levels >50 IU/L	Retrospectively reviewed patient records	12 months	↓ in AST, ALT, ALP; 5-year survival with native liver better in the GGT normalization group
Black et al. Hepatology communications 2019[132]	N = 22; Null (n = 7), ALT, and GGT persistently ≤29 IU/L; Flare (n = 8), ALT, and/or GGT >100 IU/L; indeterminant (n = 7), ALT, and/or GGT >29 IU/L and <100 IU/L; during UDCA dose reduction and withdrawal	PSC	Treatment withdrawal and reintroduction study	24 weeks; multiple phases	All flares responded to UDCA reinstitution
Shneider et al. Hepatology communications, 2018[133]	N = 37; Maralixibat 70, 140, or 280 μg/kg/day (n = 25) or placebo (n = 12) once daily	Children with Alagille syndrome	Randomized double-blind, placebo-controlled Phase 2b trial	13 weeks	Significant ↓ in ItchRO with 70 and 140 μg/kg/day but not 280 μg/kg/day (Maralixibat). A 1-point ↓ in pruritus in the drug-treated group. No serious ADEs
Dinler et al. The Turkish journal of pediatrics 1999[134]	N = 24; Follow-up biopsy N = 17; UDCA 15–20 mg/kg/day	Intrahepatic cholestasis (neonatal hepatitis 7, Byler disease 7, idiopathic intrahepatic cholestasis 10)	Uncontrolled trial	12 months	Complete resolution of pruritus in 16.7% with some improvement in all. Significant ↓ in AST, ALT, ALP, bilirubin, GGT
Dinler et al. Pediatrics international 1999[135]	Nine children aged between 1.5- and 9-years UDCA orally at doses of 15–20 mg/kg per day	PFIC1 (Byler disease)	Uncontrolled trial	12 months	Pruritus resolved completely in 22.2%, ↓ in 22.2% and unchanged in 55.6%. Significant ↓ AST. ↔ GGT, cholesterol, and serum TBAs. Cholestatic changes on histology resolved in 22.2%, ↓ in 33.3%, and ↔ in 22.2%. No ADEs
Narkewicz et al. Journal of pediatric gastroenterology and nutrition 1998[77]	N = 13; CF (n = 6), Alagille syndrome (n = 4), PFIC (n = 2), and nonsyndromic intrahepatic bile duct paucity (n = 1). UDCA 15–20 mg/kg per day for 12 months, off for 6 months, and on again for 12 months	Intrahepatic cholestatic liver disease for at least 6 months in a child >5 years old not previously on UDCA therapy	Open-label, crossover study	2.5 years	Majority (75%) had biochemical or symptomatic relapse on UDCA discontinuation, requiring retreatment with UDCA. No sustained improvements in the biochemical indices at 24 months

Table 3 (*Continued*)

Author	Sample size and therapy	Inclusion criteria, stage of fibrosis	Type of study, drug dose	Duration of therapy	Outcome
Jacquemin et al. Hepatology, 1997[72]	N = 39; UDCA orally (20–30 mg/kg/day), Group 1 (n = 26) nomal GGT, and group 2 (n = 13) high GGT. The dose of UDCA corresponded to a total daily dose of 28 ± 7 mg/kg of body weight in group 1 and 26 ± 7 mg/kg of body weight	Children with PFIC	Uncontrolled trial	Group 1: 26 ± 16 months Group 2: 49 ± 11 months	↓ in AST, ALT, serum TBAs in both groups and GGT only in group 2 Hepatomegaly and pruritus significantly ↓ in both groups. After therapy cessation: ↑ transaminases. No serious ADEs
Kardorff et al. Klinische Padiatrie, 1996[73]	N = 20; (biliary atresia n = 10, Alagille's syndrome n = 4, intrahepatic biliary hypoplasia n = 3, Byler disease/PFIC1 n = 3). UDCA 13 (7–26) mg/kg/d in two divided doses	Inherited disorders of cholestasis treated for at least 6 months	Retrospective analysis	–	Significant ↓ GGT and GLDH, AST, and ALT ↔ Bilirubin and albumin. First 12 months: pruritus improved (20% only)

BA, bile acid; **ADE**, adverse drug event; **ItchRO**, itch reported outcome; **UDCA**, ursodeoxycholic acid; **ALT**, alanine aminotransferase; **AST**, aspartate aminotransferase; **GGT**, γ-glutamyltransferase; **GLDH**, glutamate dehydrogenase; **PFIC**, progressive familial intrahepatic cholestasis; **NLS**, native liver survival; **TBA**, total bile acids.

of various drugs, including FXR agonists, in patients with NAFLD.[84]

OCA has also demonstrated improvement in total bilirubin and ALP in PBC patients who showed poor response to UDCA and is already FDA approved for the indication at a lower dose.[85,86] It is not recommended for patients with decompensated cirrhosis and is currently marketed for PBC with a black box warning for the same.

Cilofexor, a nonsteroidal non-BA FXR agonist, showed antifibrotic effects in a rat model of NASH (80). This was followed by a 12-week long Phase 2 DBRCT (NCT02943460). Total and primary serum BAs were lower in the 100 mg but not 30 mg cilofexor treatment group, albeit not significantly with secondary BAs showing a significant reduction. Liver enzymes decreased in a dose-dependent manner independent of UDCA use. There was no change in liver stiffness measured by FibroScan and enhanced liver fibrosis score, but serum TIMP-1, a fibrogenic cytokine, showed a decreasing trend in the 100 mg Cilofexor cohort.[87] In 2020, a Phase 2 DBRCT of Cilofexor was done in patients with noncirrhotic NASH (NCT02854605). Both 100 mg and 30 mg treatment groups significantly reduced serum primary BAs, whereas only the 30 mg group had a relative and absolute reduction in total and secondary BAs. The 100 mg group showed a meaningful reduction in steatosis (measured using MRI-PDFF), but this must be interpreted with caution as the placebo group had significantly higher baseline levels of steatosis.[88]

Tropifexor (LJN542) is a synthetic nonsteroidal FXR modulator that was manufactured to address the limitations of the adverse effect profile of OCA. In preclinical studies, it was found to be 20 times more potent than OCA and has shown improvement in the quality of the gut microbiome and decreased hepatic steatosis in experimental models of cholestasis and NASH. Phase 1 studies recently concluded in humans have demonstrated its safety (NCT04408937).[89]

Nidufexor (LMB763) is another nonsteroidal FXR agonist with FXR-dependent gene modulatory activity in vivo.[90] Nidufexor is currently being evaluated for NASH in Phase 2 clinical trials (NCT03804879).

FXR agonists are also being developed and evaluated in chronic hepatitis B infection, as they are hypothesized to interfere with viral replication (NCT03272009).

Aldafermin (NGM282), an FGF-19 analog (FXR downstream signaling molecule), has recently been tested in Phase 2 trials for PSC and NASH patients without cirrhosis, in doses of 0.3 mg, 1 mg, 3 mg, and 6 mg compared with placebo (NCT02443116).[91–94] In patients with NASH, at the end of 12 weeks, serum glycine-conjugated primary BAs significantly decreased from baseline. Greater reductions in GCA and GCDCA were observed with higher dose (1, 3, and 6 mg) groups. Aldafermin suppresses CYP7A1 and reduces the more

Table 4 A Summary of Studies Evaluating Bile Acids in Alcoholic Liver Disease and Hepatocellular Carcinoma.

Author	Study population	Parameter studied	Type of study	Duration of study	Outcome
Brandl et al. Journal of hepatology 2018[136]	Alcoholic hepatitis (n = 132), alcohol use disorder (n = 9), and controls (n = 9)	Serum BAs	Multicenter prospective cohort study	–	Alcoholic hepatitis vs controls: Significant ↑ total and conjugated BAs, serum FGF19 ↓ De novo bile acid synthesis
Kakiyama et al Am J Physiol Gastrointest Liver Physiol. 2014[25]	N = 103; HC (n = 19), alcohol intake w/o liver disease (n = 6), cirrhotic patients (n = 78): currently drinking (n = 10), no history of alcohol consumption (n = 30), and alcoholic cirrhosis and abstinent for >6 m (n = 38)	Fecal BAs	Prospective cohort study	–	MELD score significantly negatively correlated with total fecal BAs, total secondary BAs, and the secondary-to-primary BA ratio. Active alcohol intake influences BA composition independent of cirrhosis
Thomas et al. Cancers 2021[137]	N = 32,535; incident HCC N = 216 among participants who provided a prediagnostic serum sample	Serum BAs	Prospective population-based cohort study Nested case–control study	10 years	↑ serum primary BAs and T:G ratio were strongly associated with HCC risk of ↑ 2°:1° primary BAs inversely associated with HCC risk
Petrick et al. International journal of cancer 2020[138]	HBV N = 185; matched controls N = 61 from REVEAL-HBV cohort; HCV cases N = 96; matched controls N = 96 from REVEAL-HCV cohort	15 serum BAs measured using LC-MS	Case–control study	Mean duration of follow-up HBV: 13 years HCV: 15 years	↑ glycine- and taurine-conjugated primary BAs a/w 2–8 fold ↑ risk of HBV- and HCV-related HCC. ↑ DCA inversely a/w HBV-related HCC risk No ↑ risk of liver cancer with ↑ secondary BAs observed

MELD, model for end-stage liver disease; BA, bile acid; FGF19, fibroblast growth factor 19; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, Hepatitis C virus; T:G, taurine:glycine.

hydrophobic glycine-conjugated BAs than hydrophilic taurine-conjugated BAs. In PSC, it showed a preferential reduction of secondary BAs.

TGR-5 receptor agonists have shown increased insulin sensitivity in mouse models of NAFLD.[95,96]

INT-767 is an FXR and TGR-5 agonist that improves high-fat diet–induced effects and promotes more insulin-sensitive adipocytes. In a rabbit model with high-fat diet–induced metabolic syndrome, INT-767 increased brown adipogenesis and prevented the development of NASH.[97]

All-trans retinoic acid (ATRA) is a permissive activator of FXR. Mice studies of ATRA have shown a marked reduction of fibrosis.[98] In the clinical study of ATRA in combination with UDCA, only alanine aminotransferase (ALT) and BA intermediates showed a reduction.[99]

Apical sodium-dependent bile salt transporter and BA sequestrants have recently attracted attention owing to an experimental molecule, IMB17–15 demonstrating decreased hepatic fat content in hamsters fed a high-fat diet. Ge MX et al recently showed that IMB17-15 improves insulin sensitivity through activation of AMPK and PPAR-α pathway.[100]

A summary of studies evaluating BA-based therapeutics in CLD can be found in Tables 1–4.

In conclusion, Bile acid pathophysiology is a promising avenue for improving diagnosis and assessing the severity of CLD, especially secondary to NAFLD. Specific BA profile signatures can serve as early hints for certain types of CLD and help modulate metabolism to address the root cause of metabolic liver disease. However, the accuracy of BAs as noninvasive markers of advanced liver disease still needs more evaluation. We also need literature on BA profiles in autoimmune hepatitis. While approval of obeticholic acid came three decades after the experience with UDCA, the next decade should see a steep increase in BA-based drugs as can be estimated from the barrage of novel molecules currently being tested. Although BA-based drugs are now commonly used for cholestatic disorders and a fair number of studies are ongoing in the context of NAFLD and NASH, their therapeutic utility needs to be characterized in viral hepatitis. The cost of therapy is also an area that must be addressed. For example, OCA use in PBC currently costs a patient 1800–4500 per month. This becomes especially relevant in a country such as India, where the average monthly income of many would make it difficult to adhere to therapy for long.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

N.F. contributed to writing the article, concept, and critical revision. A.E. contributed to critical revision and figures. S. contributed to concept and critical revision.

CONFLICTS OF INTEREST

The authors have none to declare.

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