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Yu LY, Zhang XF. Beyond efficacy: Insights from the norursodeoxycholic acid phase III trial in metabolic dysfunction-associated steatotic liver disease. *World J Hepatol* 2026; 18(8): 117543 [DOI: 10.4254/wjh.117543]

OPINION REVIEW

Tu HS, Chen ML, Hong J, He L. Chrono-metabolic regulation of hepatic fibrosis *via* NR1D1-mediated hepatic stellate cell activation. *World J Hepatol* 2026; 18(8): 118194 [DOI: 10.4254/wjh.118194]

REVIEW

Drazilova S, Jarcuska P, Koky T, Harhovsky M, Komarova S, Vasil J, Janicko M. Second-line treatment of primary biliary cholangitis: To whom, which molecule, and when. *World J Hepatol* 2026; 18(8): 121491 [DOI: 10.4254/wjh.121491]

Reshetnyak VI, Reshetnyak AA, Maev IV. Peribiliary and colonic crypt glands in primary sclerosing cholangitis and inflammatory bowel disease pathogenesis: New research perspectives. *World J Hepatol* 2026; 18(8): 123377 [DOI: 10.4254/wjh.123377]

MINIREVIEWS

Khandwala K, Sajjad M, Hussain Z, Zeeshan M, Hilal K. Hepatic hydatid cyst disease in low- and middle-income countries: Spectrum of imaging findings and complications. *World J Hepatol* 2026; 18(8): 123946 [DOI: 10.4254/wjh.123946]

Blaske B, Ward M, Thandassery RB. Metabolic dysfunction-associated steatotic liver disease and the consequence of extra-hepatic malignancy. *World J Hepatol* 2026; 18(8): 124224 [DOI: 10.4254/wjh.124224]

ORIGINAL ARTICLE

Retrospective Study

Rohland O, Schwarzer L, Schwenk L, Pollmann N, Ali Deeb A, Settmacher U, Rauchfuss F, Dondorf F, Amrein K, Kocijan R, Ardelt M. Examination of bone mineral density in patients with varied liver diseases - a cross-sectional study. *World J Hepatol* 2026; 18(8): 120697 [DOI: 10.4254/wjh.120697]

Yardeni D, Gershbaum Tobin J, Itkowitz B, Lipnizkiy I, Abu Juma'a A, Abu-Freha N, Keren-Naus A, Turgeman M, Nevo-Shor A, Gish RG, Etzion O. Acute hepatitis B in the vaccination era: Predictors of functional cure and chronicity. *World J Hepatol* 2026; 18(8): 124108 [DOI: 10.4254/wjh.124108]

Khongviwatsathien S, Tanwandee T. Age-male-albumin-bilirubin-platelet score stratifies hepatocellular carcinoma risk after sustained virological response in hepatitis C-related compensated advanced chronic liver disease. *World J Hepatol* 2026; 18(8): 124474 [DOI: 10.4254/wjh.124474]

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Clinical Trials Study

Huang YF, Wu XQ, Yang YD, Ke BL, Luo JN, Chang BY, Zhou CR, Huang XL, Pan XM, Lai XM, Zhang LJ, Wu C, Wu B. Effect of hepatic venous pressure gradient in different hepatic venous branches on assessing portal hypertension in cirrhotic patients. *World J Hepatol* 2026; 18(8): 123580 [DOI: 10.4254/wjh.123580]

Observational Study

Ruiz-Margáin A, Fierro-Angulo OM, González-Regueiro JA, Hernández-Jiménez SC, García-Ulloa AC, Landa-Anell V, Bril F, Roman-Calleja BM, Romo-Arellano R, Pereira-García A, Chalasani N, Macías-Rodríguez RU. Prevalence and risk factors for metabolic dysfunction-associated steatotic liver disease and fibrosis in recently diagnosed type 2 diabetes. *World J Hepatol* 2026; 18(8): 123278 [DOI: 10.4254/wjh.123278]

Koky T, Drazilova S, Janicko M, Harhovsky M, Hubkova B, Tothova I, Rabajdova M, Marekova M, Jarcuska P. Small dense low-density lipoprotein in primary biliary cholangitis: Independent association with metabolic syndrome. *World J Hepatol* 2026; 18(8): 124436 [DOI: 10.4254/wjh.124436]

Basic Study

Huang L, Wu CG, Zhu TF, Li QL, Yu QS, Zhang Q, Shen Y. Suppression of the STAT3/MYC/MCL-1 pathway by the fixed Jianpi Huoxue Formula relieves early biochemical abnormalities in liver fibrosis. *World J Hepatol* 2026; 18(8): 122262 [DOI: 10.4254/wjh.122262]

CORRESPONDENCE

Tian R, Wu YX, Guo JY, Chen XZ, Tang JF, Zhou CF. Letter to the Editor: Natural product intervention in metabolic dysfunction-associated steatotic liver disease: From allyl isothiocyanate to signaling networks. *World J Hepatol* 2026; 18(8): 117382 [DOI: 10.4254/wjh.117382]

Forlano R, Castellaneta NM, Suppressa P. Letter to the Editor: Filling the gaps in non-invasive assessment of liver fibrosis in metabolic dysfunction-associated steatotic liver disease. *World J Hepatol* 2026; 18(8): 117777 [DOI: 10.4254/wjh.117777]

Makhlouf NA. Letter to the Editor: Efficacy and safety of the generic forms of sofosbuvir/ledipasvir in chronic hepatitis C virus adolescents in Upper Egypt. *World J Hepatol* 2026; 18(8): 118661 [DOI: 10.4254/wjh.118661]

Zhang JW. Letter to the Editor: Induction immunotherapy in hyperimmunized liver transplantation: Rebalancing immunological risk and graft protection. *World J Hepatol* 2026; 18(8): 118687 [DOI: 10.4254/wjh.118687]

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EDITORIAL

Hepatic ischemia reperfusion injury: Understanding the little components of the larger picture

Eyad Abdulrazzak, Eric R Kallwitz

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Abstract

Hepatic ischemia reperfusion injury is especially relevant in the field of liver surgery and transplantation. In the most severe cases, significant liver dysfunction and liver failure can occur, resulting in death or re-transplantation. The mechanisms are complex; however, the most basic concept is that the restoration of blood flow to an ischemic organ can lead to an inflammatory response. The mechanisms that trigger this immune response are not completely understood; however, multiple factors were found to contribute. These include the release of inflammatory cytokines/chemokines, hepatocellular apoptosis, and oxidative stress. Multiple studies, mainly animal models, have examined the potential for various therapies, including pharmacotherapy, to ameliorate ischemia-reperfusion injury. The study by Kelleni *et al* recently published in the recent issue of the *World Journal of Hepatology* examines the potential hepatoprotective effect of aprepitant, an antiemetic agent commonly used for nausea related to chemotherapy. In an animal model of ischemia-reperfusion injury, the authors found improved inflammatory markers and survival in rats treated with aprepitant. There have been multiple animal models suggesting various pharmacotherapies to reduce the severity of ischemia-reperfusion injury. The use of aprepitant is unique as it is currently available for use in humans, although further data is needed, side effects need to be monitored, and significant drug interactions could occur.

Key Words: Surgical complications; Liver allograft; Hepatocellular injury; Ischemia prevention; Organ preservation

Core Tip: Despite advancements in surgical techniques and preservation, hepatic ischemia-reperfusion injury (H-IRI) remains a critical hurdle in liver transplantation, contributing to early allograft dysfunction. While machine perfusion represents a major macro-level advancement, pharmacological strategies targeting specific molecular pathways remain an important avenue for mitigating H-IRI. This editorial briefly outlines the complexity of H-IRI mechanisms, discusses emerging pharmacological and mechanical interventions, and highlights recent findings on the neurokinin-1 receptor antagonist aprepitant as a potential therapeutic agent.

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This editorial refers to "*Targeting sirtuin 1/nuclear factor erythroid 2-related factor 2/tumor necrosis factor-a pathway to modulate hepatic ischemia reperfusion-induced injury*" by Kelleni *et al*, 2025; https://doi.org/10.4254/wjh.v17.i12.110733.

INTRODUCTION

Hepatic ischemia-reperfusion injury (H-IRI) is a common and clinically significant complication of liver surgery, especially liver transplantation. Mechanistically, H-IRI is driven by mitochondrial dysfunction, oxidative stress, and profound energy depletion, which together trigger hepatocyte death through apoptotic and necrotic pathways[1]. As the name suggests, H-IRI develops after restoration of perfusion to the liver after an ischemic period caused during surgery. Upon reperfusion, the release of damage-associated molecular patterns activates Kupffer cells and recruits neutrophils and monocyte-derived macrophages, which together amplify inflammation and propagate tissue injury[1]. This type of injury is especially notable during liver transplant surgery. Collectively, these interconnected processes manifest as clinically detectable graft injury in the post-transplant setting. In a cohort of 506 liver transplant recipients, histologic H-IRI was present in 87.4% of grafts, and moderate-to-severe injury occurred in 13.8%[2]. Importantly, increasing H-IRI severity was associated with higher rates of early allograft dysfunction (EAD) and reduced 6-month graft survival[2]. H-IRI is also linked to the development of post-transplant biliary complications, which carry significant morbidity and mortality[3].

A large body of experimental research has examined the molecular pathways that both cause and mitigate H-IRI, leading to the opportunity for pharmacologic intervention[1]. Although many pharmacologic agents have demonstrated benefit in animal models, no drug has yet been universally adopted into clinical guidelines for routine prevention of H-IRI in liver transplantation. Machine perfusion technologies have markedly advanced the field by improving organ preservation and enabling more reliable use of higher-risk grafts. However, as the transplant community increasingly relies on marginal or extended-criteria donor livers, which exhibit heightened susceptibility to H-IRI, there remains a compelling need to explore targeted strategies that further mitigate H-IRI and expand the safe utilization of these grafts.

INVESTIGATIONAL PHARMACOLOGIC THERAPIES FOR H-IRI

Preclinical studies have demonstrated that a variety of pharmacological agents can attenuate H-IRI by targeting factors such as mitochondrial stability, oxidative stress, and/or innate immune activation[1]. Examples include select anesthetics, hypoxia-inducible factor activators, immunosuppressants, and antioxidants such as N-acetylcysteine[4-6]. A recent preclinical study of the *World Journal of Hepatology* by Kelleni *et al*[7] highlights the therapeutic promise of the anti-emetic aprepitant, a neurokinin-1 receptor antagonist with broad anti-inflammatory, antioxidant, and anti-apoptotic properties. In a rat model of warm H-IRI, aprepitant pretreatment produced dose-dependent improvements across several mechanistic domains of injury. It significantly reduced hepatocellular damage (lower aminotransferase levels), mitigated oxidative stress by lowering malondialdehyde concentrations, and attenuated inflammatory signaling through suppression of tumour necrosis factor alpha and interleukin-6. Aprepitant also reduced caspase-3 activation and enhanced expression of sirtuin-1 and nuclear factor erythroid 2-related factor 2, suggesting coordinated modulation of mitochondrial homeostasis, redox balance, and apoptosis. These pleiotropic effects, together with improvements in histologic injury and overall survival, support further exploration in future studies. However, key questions remain, including optimal dosing, timing (donor, perfusion, or recipient phases), feasibility of administration, and possible drug-drug interactions. In addition, preclinical H-IRI models do not fully capture the immunologic complexity, donor comorbidities, and perioperative variables inherent to human liver transplantation, reinforcing the need for staged translational validation before clinical application.

Beyond animal models, clinical evaluation of pharmacologic strategies to mitigate H-IRI remains limited. A recent randomized clinical trial in the liver transplantation setting tested a multistep, nine-agent regimen that included oral antioxidants, an *ex-situ* epoprostenol flush, and perioperative anti-inflammatory and cytoprotective agents[6]. Despite the mechanistic rationale, the intervention did not improve peak aminotransferase levels or the rate of EAD, nor did it reduce ischemic cholangiopathy or graft loss over 12 months of follow-up[6]. These findings highlight the difficulty of translating complex, downstream pharmacologic approaches into clinical benefit and underscore the growing rationale for prioritizing upstream graft-conditioning strategies within modern preservation techniques.

THE ROLE OF MACHINE PERFUSION

Machine perfusion has reshaped liver preservation by reducing the magnitude and duration of ischemia and thereby limiting the downstream cascade of H-IRI. Unlike static cold storage, which imposes prolonged hypoxia and promotes mitochondrial dysfunction, perfusion strategies maintain oxygen delivery, support aerobic metabolism, and blunt the oxidative burst that occurs at reperfusion[1]. These mechanistic advantages are particularly relevant for donation after cardiac death (DCD) grafts, which are inherently more susceptible to ischemic injury. An increasingly adopted perfusion approach, normothermic regional perfusion, restores *in situ* oxygenated circulation after circulatory arrest[1]. Normothermic machine perfusion (NMP) maintains the liver *ex situ* at physiologic temperatures, preserving aerobic metabolism and allowing real-time functional viability testing before transplantation[8]. In contrast, hypothermic oxygenated perfusion offers a simpler, lower-cost alternative focused on early mitochondrial reoxygenation at low temperatures, limiting succinate accumulation and attenuating reactive oxygen species formation during reperfusion[1].

Clinical data increasingly support the benefits of these technologies. In a recent clinical cohort, NMP significantly reduced EAD, particularly among DCD grafts[9]. In this study, EAD decreased from 50% with static cold storage to 17.5% with NMP[9]. Additional clinical benefits included reduced transfusion requirements, shorter hospital and intensive care unit stays, and lower one-year graft failure risk (by nearly 80% overall, and 87% in DCD grafts)[9]. Likewise, a recent randomized clinical trial evaluating hypothermic oxygenated perfusion demonstrated reductions in EAD, biliary complications, and re-transplantation rates in DCD transplantation[10]. As machine perfusion becomes increasingly integrated into clinical practice, it also presents a unique translational opportunity: The delivery of targeted pharmacologic agents directly to a metabolically active graft within a controlled perfusion environment. Whether such perfusion-based therapeutic strategies provide additive or synergistic protection against H-IRI remains an important question for future investigation.

CONCLUSION

H-IRI is an important factor in surgical outcomes, particularly in transplant programs that increasingly rely on ECD and DCD grafts. Mechanistic insights from preclinical work continue to highlight promising targets, yet clinical translation remains limited. At the same time, advances in machine perfusion have improved early graft performance and expanded the safe utilization of marginal organs. Looking ahead, integration of these two approaches may offer the greatest opportunity for progress. Pharmacologic agents delivered systemically or within the controlled environment of perfusion could provide additional protection, although questions regarding optimal timing, cellular targets, and feasibility remain. Studies such as the one by Kellini *et al*[7] provide a potential framework for future clinical trials in settings such as liver transplantation.

REFERENCES

1 **Zhao J**, Hou L, Dery KJ, Yuan X, Kim KH, Kupiec-Weglinski JW, Hall DR, Thornley CJ, Hobeika MJ, Eltzschig HK, Ju C. Hepatic ischemia-reperfusion injury: Underlying mechanisms and concepts in liver surgery and liver transplantation. *Hepatology* 2025 [*RCA*] [PMID: 41217423 DOI: 10.1097/HEP.0000000000001609] [FullText] [Full Text(PDF)]

2 **Ito T**, Naini BV, Markovic D, Aziz A, Younan S, Lu M, Hirao H, Kadono K, Kojima H, DiNorcia J 3rd, Agopian VG, Yersiz H, Farmer DG, Busuttil RW, Kupiec-Weglinski JW, Kaldas FM. Ischemia-reperfusion injury and its relationship with early allograft dysfunction in liver transplant patients. *Am J Transplant* 2021; **21**: 614-625 [*RCA*] [PMID: 32713098 DOI: 10.1111/ajt.16219] [FullText]

3 **Cursio R**, Gugenheim J. Ischemia-Reperfusion Injury and Ischemic-Type Biliary Lesions following Liver Transplantation. *J Transplant* 2012; **2012**: 164329 [*RCA*] [PMID: 22530107 DOI: 10.1155/2012/164329] [FullText] [Full Text(PDF)]

4 **Liu X**, Pan Z, Su D, Yang Z, Zheng B, Wang X, Tian J. Remifentanil Ameliorates Liver Ischemia-Reperfusion Injury Through Inhibition of Interleukin-18 Signaling. *Transplantation* 2015; **99**: 2109-2117 [*RCA*] [PMID: 25919765 DOI: 10.1097/TP.0000000000000737] [FullText]

5 **Nakamura K**, Kageyama S, Ito T, Hirao H, Kadono K, Aziz A, Dery KJ, Everly MJ, Taura K, Uemoto S, Farmer DG, Kaldas FM, Busuttil RW, Kupiec-Weglinski JW. Antibiotic pretreatment alleviates liver transplant damage in mice and humans. *J Clin Invest* 2019; **129**: 3420-3434 [*RCA*] [PMID: 31329160 DOI: 10.1172/JCI127550] [FullText]

6 **Meurisse N**, Mertens M, Fieuws S, Gilbo N, Jochmans I, Pirenne J, Monbaliu D. Effect of a Combined Drug Approach on the Severity of Ischemia-Reperfusion Injury During Liver Transplant: A Randomized Clinical Trial. *JAMA Netw Open* 2023; **6**: e230819 [*RCA*] [PMID: 36853611 DOI: 10.1001/jamanetworkopen.2023.0819] [FullText]

7 **Kelleni MT**, Abdelzaher WY, Adly M, Attya ME, Fawzy MA, Ibrahim MA. Targeting sirtuin 1/nuclear factor erythroid 2-related factor 2/

tumor necrosis factor-α pathway to modulate hepatic ischemia reperfusion-induced injury. *World J Hepatol* 2025; **17**: 110733 [*RCA*] [PMID: 41479522 DOI: 10.4254/wjh.v17.i12.110733] [FullText] [Full Text(PDF)]

8 **Dutkowski P**, Guarrera JV, de Jonge J, Martins PN, Porte RJ, Clavien PA. Evolving Trends in Machine Perfusion for Liver Transplantation. *Gastroenterology* 2019; **156**: 1542-1547 [*RCA*] [PMID: 30660724 DOI: 10.1053/j.gastro.2018.12.037] [FullText]

9 **Nguyen MC**, Zhang C, Chang YH, Li X, Ohara SY, Kumm KR, Cosentino CP, Aqel BA, Lizaola-Mayo BC, Frasco PE, Nunez-Nateras R, Hewitt WR, Harbell JW, Katariya NN, Singer AL, Moss AA, Reddy KS, Jadlowiec C, Mathur AK. Improved Outcomes and Resource Use With Normothermic Machine Perfusion in Liver Transplantation. *JAMA Surg* 2025; **160**: 322-330 [*RCA*] [PMID: 39878966 DOI: 10.1001/jamasurg.2024.6520] [FullText] [Full Text(PDF)]

10 **Schlegel A**, Mueller M, Muller X, Eden J, Panconesi R, von Felten S, Steigmiller K, Sousa Da Silva RX, de Rougemont O, Mabrut JY, Lesurtel M, Cerisuelo MC, Heaton ND, Allard MA, Adam R, Monbaliu D, Jochmans I, Haring MPD, Porte RJ, Parente A, Muiesan P, Kron P, Attia M, Kollmann D, Berlakovich G, Rogiers X, Petterson K, Kranich AL, Amberg S, Müllhaupt B, Clavien PA, Dutkowski P. A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation. *J Hepatol* 2023; **78**: 783-793 [*RCA*] [PMID: 36681160 DOI: 10.1016/j.jhep.2022.12.030] [FullText]

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EDITORIAL

Beyond efficacy: Insights from the norursodeoxycholic acid phase III trial in metabolic dysfunction-associated steatotic liver disease

Ling-Ying Yu, Xian-Feng Zhang

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Abstract

The therapeutic landscape for metabolic dysfunction-associated steatotic liver disease (MASLD) is undergoing rapid transformation with recent drug approvals. This editorial contextualizes a recent phase III trial of norursodeoxycholic acid within this dynamic progress. Panuganti *et al* recently published a study in *World Journal of Hepatology*, while demonstrating significant alanine aminotransferase normalization and FibroScan-based liver stiffness improvement, the trial's short duration (24 weeks), reliance on non-invasive endpoints, and absence of histological confirmation underscore persistent challenges in MASLD drug development.

Key Words: Clinical endpoints; Drug development; Liver fibrosis; Metabolic dysfunction-associated steatotic liver disease; Norursodeoxycholic acid; Pharmacotherapy; Clinical trial

Core Tip: The phase III trial of norursodeoxycholic acid (norUDCA) in metabolic dysfunction-associated steatotic liver disease reflects broader methodological challenges in the field: Short duration, reliance on non-invasive surrogate endpoints, and high placebo response rates. While norUDCA's mechanism and safety profile differ from newly approved agents, its future role may be further evaluated.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has cemented its position as the most prevalent chronic liver condition worldwide, with a global prevalence estimated at 30%-35% and projections pointing to a relentless rise in tandem with obesity and type 2 diabetes mellitus pandemics[1-3]. Although recent consensus redefined NAFLD as metabolic dysfunction-associated steatotic liver disease (MASLD) to better reflect metabolic drivers, population-level prevalence estimates remain comparable due to overlapping diagnostic criteria. Its progressive form, metabolic dysfunction-associated steatohepatitis (MASH), is a leading cause of cirrhosis, hepatocellular carcinoma, and liver-related mortality, while also contributing significantly to cardiovascular disease burden[4-6]. For decades, the management of MASLD rested almost exclusively on the pillars of lifestyle modification-weight loss, dietary changes, and increased physical activity[7]. While effective, the long-term sustainability of these interventions is often poor, leaving a vast therapeutic void for a disease affecting billions.

The year 2024 marked a historic turning point with the United States Food and Drug Administration approval of resmetirom, the first drug specifically indicated for the treatment of MASH with moderate-to-advanced liver fibrosis[8,9]. This milestone was quickly followed by the accelerated approval of semaglutide for MASH in 2025[10,11]. These approvals have shattered the long-standing therapeutic nihilism in MASLD, validating key biological targets and catalyzing an explosive phase of drug development. The pipeline now includes agents targeting a diverse array of pathways: Nuclear receptors [farnesoid X receptor (FXR), peroxisome proliferator-activated receptor (PPAR)], metabolic hormones [glucagon-like peptide-1 (GLP-1), fibroblast growth factor 21 (FGF21)], fibrogenic processes, and the gut-liver axis[12-15].

It is within this newly energized and competitive landscape that the Phase III trial of norursodeoxycholic acid (norUDCA) must be evaluated. NorUDCA is not a newcomer; its distinct pharmacology and early clinical signals have been recognized for years[16]. In a recent issue of the *World Journal of Hepatology*, Panuganti *et al*[17] reported a prospective study. This recent large-scale, randomized, placebo-controlled study provides preliminary evidence for its efficacy and safety in a MASLD population. This editorial offers a dual perspective: A granular critique of the norUDCA phase III trial and a strategic analysis of where this agent might fit within an increasingly complex, multimodal MASLD treatment paradigm.

This editorial argues that the norUDCA trial functions less as a therapeutic breakthrough and more as a "stress test" for current clinical trial paradigms-particularly regarding endpoint selection, duration, placebo response, and global generalizability in an era where histology-based fibrosis regression is now the regulatory gold standard.

EXPANDING PHARMACOPEIA: A SNAPSHOT OF CURRENT MASLD DRUG DEVELOPMENT

To appreciate the significance of any single agent, one must understand the spectrum of approaches being pursued. The current MASLD drug development ecosystem can be categorized by primary mechanism of action:

Thyroid hormone receptor-beta agonists

Resmetirom's approval is seminal. It acts selectively on hepatic thyroid hormone receptor-beta (TRβ), boosting mitochondrial function and lipid metabolism, leading to reductions in hepatic fat, inflammation, and, crucially, fibrosis [8]. Its success has validated hepatic metabolic reprogramming as a potent anti-MASH strategy.

GLP-1 receptor agonists

Semaglutide's MASH resolution is likely driven predominantly by profound weight loss (approximately 13% in ESSENCE), though direct anti-inflammatory hepatic effects cannot be excluded[10]. Their cardiorenal benefits make them particularly attractive for the comorbid MASLD patient[18].

FXR agonists

FXR activation regulates bile acid homeostasis and exerts anti-inflammatory and anti-fibrotic effects directly in the liver [19]. While obeticholic acid (first-gen) showed antifibrotic efficacy at the cost of pruritus and low-density lipoprotein (LDL) elevation[14], next-generation FXR modulators (*e.g.*, tropifexor, cilofexor) aim for improved tolerability through

partial agonism or gut-restricted action.

PPAR agonists

This class, including pan-PPAR agonists like lanifibranor, targets multiple facets of MASLD: PPAR-α for lipid metabolism, PPAR-δ for fatty acid oxidation, and PPAR-γ for insulin sensitization and anti-inflammation[12]. They offer a broad-spectrum approach but require careful monitoring for side effects like weight gain.

FGF21 analogues

Pegbelfermin and other FGF21 mimetics mimic a metabolic hormone that improves insulin sensitivity, lipid metabolism, and energy expenditure. They have shown promise in reducing hepatic fat and biomarkers of cell stress in clinical trials [15].

Anti-fibrotic & anti-inflammatory agents

Directly targeting the fibrosis cascade (*e.g.*, through LOXL2 inhibition) or key inflammatory pathways represents a complementary strategy, often envisioned for use in combination with metabolic agents[20].

Some of these drugs have undergone high-quality clinical trials, demonstrating their therapeutic efficacy against MASH and their ability to improve fibrosis (Table 1). This rich pipeline signifies a shift from seeking a single "magic bullet" to building a multi-pronged, often combinatorial, therapeutic toolkit tailored to disease stage and patient phenotype[21].

NORUDCA: MECHANISM AND THE PHASE III TRIAL IN FOCUS

NorUDCA, a 23-carbon homologue of the familiar ursodeoxycholic acid (UDCA), possesses a unique pharmacological profile. Unlike UDCA-which primarily stabilizes hepatocyte membranes-and FXR agonists-which systemically modulate bile acid synthesis-norUDCA acts locally *via* cholehepatic shunting to exert direct antifibrotic and cytoprotective effects without significantly altering systemic bile acid pools. Its shortened side chain confers resistance to amidation, allowing it to undergo "cholehepatic shunting". This means it is efficiently absorbed from the bile ducts back into hepatocytes, leading to high and sustained intrahepatic concentrations-a pharmacokinetic advantage over UDCA[16]. Mechanistically, it primarily downregulates key profibrogenic pathways, including transforming growth factor-beta signaling and collagen I expression, thereby directly countering the fibosis[22]. Also, it promotes a healthier bile acid profile by indirectly influencing FXR signaling and regulating bile acid transporter expression. This shifts the bile acid pool toward a more hydrophilic and less toxic composition, which supports metabolic homeostasis[4]. It further mitigates inflammation and provides direct protection to hepatocytes, shielding them from various injurious stimuli[22].

The phase III trial evaluated norUDCA (1500 mg/day) *vs* placebo over 24 weeks in 165 Indian patients with MASLD [17]. Its design and outcomes offer a concrete case study in modern MASLD trial execution. The co-primary endpoints were alanine transaminase (ALT) normalization and improvement in liver stiffness measured by FibroScan at week 12. Key efficacy results showed a significantly higher proportion of patients in the norUDCA group achieved ALT normalization at week 12 compared to the placebo group (89.8% *vs* 76.4%; $P = 0.022$). The regression in liver stiffness at week 12 was observed in 57.4% of the norUDCA group *vs* 40.0% in the placebo group ($P = 0.035$), with this antifibrotic signal strengthening to 83.3% *vs* 65.5% by week 24 ($P = 0.009$). The drug was well-tolerated with no serious adverse events reported; adverse events were mostly mild-to-moderate and gastrointestinal.

CROSS-CUTTING ANALYSIS: INTERROGATING THE NORUDCA TRIAL THROUGH THE LENS OF FIELD-WIDE CHALLENGES

The norUDCA trial, while a positive study, vividly illustrates several universal challenges in MASLD drug development.

Conundrum of trial duration and meaningful endpoints

The 24-week duration of the norUDCA trial is both a pragmatic strength and a scientific limitation-a tension common in the field[23]. While it efficiently demonstrates ALT normolization and transient elastography improvements, MASLD is a chronic, indolent disease. True "disease-modification" likely requires treatment durations of 72 weeks or more, as seen in the registration trials for resmetirom and semaglutide[8,10]. The choice of FibroScan as a primary endpoint, while ethically and practically justified over repeated liver biopsies, also sparks debate about its sensitivity for early fibrosis and its correlation with hard clinical outcomes[24]. This reflects a field-wide struggle to balance feasibility with regulatory and clinical rigor[25].

Pervasive and potent placebo effect

The norUDCA results demonstrate the powerful placebo effect in MASLD trials. The placebo group achieved a remarkable 76.4% ALT normalization rate at week 12. This phenomenon, observed across countless studies, is attributed to the "trial effect": Intensive lifestyle counseling and heightened medical attention leading to behavioral changes[26]. The trial protocol reportedly included standard dietary advice, but adherence was not monitored-potentially contributing

Table 1 Comparative overview of metabolic dysfunction-associated steatotic liver disease/metabolic dysfunction-associated steatohepatitis pharmacotherapies

Drug class/agent (example)	Sample size (total randomized)	Trial duration	Baseline fibrosis stage[1]	Primary regulatory endpoint(s) achieved	Placebo-subtracted effect size[2]	Key safety trade-offs
TRβ agonist resmetirom	966 (MAESTRO-NASH, phase III)	52 weeks	F1B-F3	√ MASH resolution with no worsening of fibrosis; √ fibrosis improvement with no worsening of MASH (≥ 1 stage)	MASH resolution with no worsening of fibrosis: 16.4%-20.7%; fibrosis improvement with no worsening of MASH: 10.2%-11.8%; liver fat: -26.7% to 37.9% (MRI-PDFF)	GI intolerance (diarrhea and nausea); generally well tolerated
GLP-1 RA semaglutide	1197 (ESSENCE, phase III)	72 weeks	F2-F3	√ MASH resolution with no worsening of liver fibrosis; √ fibrosis improvement with no worsening of MASH	MASH resolution with no worsening of liver fibrosis: 28.7%; fibrosis improvement with no worsening of MASH: 14.4%; Weight loss: -8.5%	GI intolerance (nausea/vomiting); contraindicated in medullary thyroid cancer risk; low hypoglycemia risk
FXR agonist obeticholic acid	1968 (REGENERATE, phase III)	18 months	F1-F3	× MASH resolution with no worsening of liver fibrosis; √ fibrosis improvement with no worsening of MASH	MASH resolution with no worsening of fibrosis: 3%-4% (NS); fibrosis improvement with no worsening of MASH: 6%-11%	Pruritus (28%-51%, often grade 1- grade 2); LDL-C ↑ (1.4-2.7 mg/dL); HDL ↓
Pan-PPAR agonist lanifibranor	247 (NATIVE, phase IIb)	24 weeks	F1-F3	√ A decrease of SAF-A score and no worsening of fibrosis	A decrease of SAF-A score and no worsening of fibrosis: 15%-22%; resolution of MASH without worsening of fibrosis: 17%-27%; fibrosis improvement with no worsening of MASH: 5%-17%	Weight gain (2.4-2.7 kg); mild edema; GI intolerance (diarrhea and nausea)

[1]Baseline fibrosis stage: Based on central histology (nonalcoholic steato hepatitis clinical research network system) unless otherwise noted.
[2]Effect sizes: Approximate placebo-adjusted differences.
NS: Not significant; MAESTRO-NASH: Metabolic associated steatotic liver disease and metabolic associated steatohepatitis research network; MASH: Metabolic dysfunction-associated steatohepatitis; GI: Gastrointestinal; MRI-PDFF: Magnetic resonance imaging - proton density fat fraction; LDL-C: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein; REGENERATE: Randomized global phase 3 study to evaluate the impact on NASH with fibrosis of obeticholic acid treatment; NATIVE: Name of a phase 2b/3 clinical trial evaluating the efficacy and safety of lanifibranor in adult patients with nonalcoholic steatohepatitis and liver fibrosis; SAF-A: Steatosis, activity, fibrosis - activity score; TRβ: Thyroid hormone receptor β; GLP-1 RA: Glucagon-like peptide-1 receptor agonist; FXR: Farnesoid X receptor; PPAR: Peroxisome proliferator-activated receptor.

to the high placebo response. Also, the trial's lack of a pre-randomization run-in period likely allowed this effect to inflate the placebo response, thereby potentially underestimating the true treatment effect size[23].

Decoupling biochemical from histological/structural response

An intriguing finding is the temporal discordance between ALT normalization (peak effect at week 12) and FibroScan improvement (progressive through week 24). This underscores a key principle: Improvement in liver enzymes is not a surrogate for antifibrotic efficacy[25]. The field is gradually moving towards accepting composite endpoints that include both resolution of steatohepatitis and improvement in fibrosis stage. In norUDCA trial, FibroScan improvement was implicitly treated as a surrogate for fibrosis regression, though its correlation with histologic change remains imperfect-especially over 12-24 weeks.

Limitations of ethnic and regional generalizability

South Asian populations display a unique metabolic phenotype, characterized by higher visceral adiposity and insulin resistance, which leads to a greater prevalence of MASLD even at lower body mass indices. Given norUDCA's primary effect on bile acid metabolism, its efficacy may differ in populations with distinct genetic and physiological backgrounds-such as variations in bile acid pool composition or transporter expression (*e.g.*, apical sodium-dependent bile acid transporter polymorphisms). Therefore, future clinical trials should move beyond simple replication in Western cohorts and instead incorporate region-stratified analyses or adaptive designs to better evaluate potential population-specific responses.

Safety in a metabolically vulnerable population

The safety profile of norUDCA appears favorable. However, the occurrence of metabolic adverse events (dyslipidemia,

new-onset diabetes) in both arms highlights a crucial point: MASLD trial populations are, by definition, metabolically unstable. Disentangling drug-related metabolic effects from the natural progression of underlying cardiometabolic risk factors is exceptionally difficult, a challenge also faced by other drug classes like FXR agonists[14]. Given MASLD's association with cardiovascular risk, 24-week safety data are inadequate to rule out long-term metabolic or bile acid-related adverse effects.

POSITIONING NORUDCA IN THE CURRENT AND FUTURE TREATMENT PARADIGM

Given its efficacy, safety data and its combined anti-inflammatory and direct anti-fibrotic action, norUDCA could be beneficial across MASLD stages[27]. Its bile acid-centric mechanism may offer particular utility in patients with overlapping MASLD and cholestatic features[22]. Its favorable safety and tolerability-notably the absence of pruritus or LDL elevation-contrasts with the known limitations of FXR agonists. Furthermore, its mechanism of action is independent of weight loss, offering a potential alternative for patients intolerant of or unresponsive to GLP-1 receptor agonists.

However, significant evidence gaps must be addressed for norUDCA to achieve broader clinical relevance. Critically, the absence of robust histologic data showing ≥ 1-stage fibrosis improvement after ≥ 72 weeks of treatment remains a major limitation; such proof of durable histological benefit is essential for competitiveness. Furthermore, there are no head-to-head or additive trials comparing norUDCA with approved agents like resmetirom or semaglutide, and a lack of long-term (> 1 year) efficacy and safety data places it at a disadvantage in a market where newer therapies are supported by multi-year trial results.

Combination therapy represents a promising frontier in MASLD treatment[4,28,29], with norUDCA positioned as a compelling candidate for rational pairing. Its unique, bile acid-focused mechanism supports two particularly viable strategies: First, with a GLP-1 receptor agonist such as semaglutide, to concurrently address systemic metabolic drivers-*via* improved glycemic control, weight loss, and reduced inflammation-while providing direct hepatic anti-fibrotic action, an approach especially compelling for patients with obesity, insulin resistance, and established fibrosis; and second, with a thyroid hormone receptor-beta agonist like resmetirom, creating a dual-pronged regimen that enhances hepatic metabolic function while modulating the bile acid pool. From a safety perspective, these combinations carry a low risk of pharmacokinetic interactions, as none are significant CYP450 substrates, though additive gastrointestinal side effects may require careful clinical management. The primary development challenge lies in the substantial evidence required for regulatory approval: Agencies will demand clear demonstration of an added histological benefit over monotherapy, necessitating large, long-term trials with strategic patient selection, which inherently increases both the cost and complexity of bringing such combinations to market.

SYNTHESIS AND FUTURE DIRECTIONS: LESSONS FROM NORUDCA FOR THE FIELD

The norUDCA trial represents more than a report on a single therapeutic agent; it serves as a microcosm of the contemporary endeavor to treat MASLD. The discussion of the trial reinforce several imperatives for future trial design, with the following three as top priorities: Extend pivotal trials to ≥ 72 weeks with histologic endpoints. Standardize and validate non-invasive tests across diverse populations. Launch early-phase combination trials with mechanistic synergy.

To mitigate the placebo effect, incorporating run-in periods is feasible strategy[23,26]. Also, Future research should seek to identify biomarkers that predict response to specific mechanisms, moving from a one-size-fits-all to a tailored approach. Baseline serum bile acid profiles or expression of bile acid transporters (*e.g.*, apical sodium-dependent bile acid transporter) could serve as predictive biomarkers for norUDCA response[30].

CONCLUSION

The phase III trial of norUDCA demonstrates its capacity to safely improve key hepatic parameters, thereby expanding the available therapeutic arsenal for MASLD. However, its arrival underscores the evolving and increasingly rigorous standards of the field. In this context, the trial serves as a salient case study, exposing key systemic constraints in contemporary MASLD drug development: The limitations of trials with relatively short duration, a reliance on non-invasive surrogate endpoints, and the confounding impact of high placebo response rates. As effective therapies like resmetirom and semaglutide establish new benchmarks with multi-year histological data, the imperative for more definitive evidence grows. Consequently, the future path for novel agents must involve longer, histology-anchored trials enriched by validated biomarkers. This shift necessitates more exacting scrutiny from regulators, clinicians, and scientific journals alike to ensure that new additions to the therapeutic toolbox meet the heightened evidentiary threshold now demanded in the post-approval era.

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REFERENCES

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [*RCA*] [PMID: 26707365 DOI: 10.1002/hep.28431] [Full Text]

2 **Miao L**, Targher G, Byrne CD, Cao YY, Zheng MH. Current status and future trends of the global burden of MASLD. *Trends Endocrinol Metab* 2024; **35**: 697-707 [*RCA*] [PMID: 38429161 DOI: 10.1016/j.tem.2024.02.007] [FullText]

3 **Kalligeros M**, Vassilopoulos A, Vassilopoulos S, Victor DW, Mylonakis E, Noureddin M. Prevalence of Steatotic Liver Disease (MASLD, MetALD, and ALD) in the United States: NHANES 2017-2020. *Clin Gastroenterol Hepatol* 2024; **22**: 1330-1332.e4 [*RCA*] [PMID: 37949334 DOI: 10.1016/j.cgh.2023.11.003] [FullText]

4 **Loomba R**, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* 2021; **184**: 2537-2564 [*RCA*] [PMID: 33989548 DOI: 10.1016/j.cell.2021.04.015] [FullText] [Full Text(PDF)]

5 **Åberg F**, Helenius-Hietala J, Puukka P, Färkkilä M, Jula A. Interaction between alcohol consumption and metabolic syndrome in predicting severe liver disease in the general population. *Hepatology* 2018; **67**: 2141-2149 [*RCA*] [PMID: 29164643 DOI: 10.1002/hep.29631] [FullText]

6 **Lee HH**, Lee HA, Kim EJ, Kim HY, Kim HC, Ahn SH, Lee H, Kim SU. Metabolic dysfunction-associated steatotic liver disease and risk of cardiovascular disease. *Gut* 2024; **73**: 533-540 [*RCA*] [PMID: 37907259 DOI: 10.1136/gutjnl-2023-331003] [FullText]

7 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J Hepatol* 2024; **81**: 492-542 [*RCA*] [PMID: 38851997 DOI: 10.1016/j.jhep.2024.04.031] [FullText]

8 **Harrison SA**, Bedossa P, Guy CD, Schattenberg JM, Loomba R, Taub R, Labriola D, Moussa SE, Neff GW, Rinella ME, Anstee QM, Abdelmalek MF, Younossi Z, Baum SJ, Francque S, Charlton MR, Newsome PN, Lanthier N, Schiefke I, Mangia A, Pericàs JM, Patil R, Sanyal AJ, Noureddin M, Bansal MB, Alkhouri N, Castera L, Rudraraju M, Ratziu V; MAESTRO-NASH Investigators. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. *N Engl J Med* 2024; **390**: 497-509 [*RCA*] [PMID: 38324483 DOI: 10.1056/NEJMoa2309000] [FullText]

9 **Keam SJ**. Resmetirom: First Approval. *Drugs* 2024; **84**: 729-735 [*RCA*] [PMID: 38771485 DOI: 10.1007/s40265-024-02045-0] [FullText]

10 **Sanyal AJ**, Newsome PN, Kliers I, Østergaard LH, Long MT, Kjær MS, Cali AMG, Bugianesi E, Rinella ME, Roden M, Ratziu V; ESSENCE Study Group. Phase 3 Trial of Semaglutide in Metabolic Dysfunction-Associated Steatohepatitis. *N Engl J Med* 2025; **392**: 2089-2099 [*RCA*] [PMID: 40305708 DOI: 10.1056/NEJMoa2413258] [FullText]

11 **Bansal MB**, Patton H, Morgan TR, Carr RM, Dranoff JA, Allen AM. Semaglutide therapy for metabolic dysfunction-associated steatohepatitis: November 2025 updates to AASLD Practice Guidance. *Hepatology* 2026; **83**: 1326-1340 [*RCA*] [PMID: 41201884 DOI: 10.1097/HEP.0000000000001608] [FullText]

12 **Francque SM**, Bedossa P, Ratziu V, Anstee QM, Bugianesi E, Sanyal AJ, Loomba R, Harrison SA, Balabanska R, Mateva L, Lanthier N, Alkhouri N, Moreno C, Schattenberg JM, Stefanova-Petrova D, Vonghia L, Rouzier R, Guillaume M, Hodge A, Romero-Gómez M, Huot-Marchand P, Baudin M, Richard MP, Abitbol JL, Broqua P, Junien JL, Abdelmalek MF; NATIVE Study Group. A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. *N Engl J Med* 2021; **385**: 1547-1558 [*RCA*] [PMID: 34670042 DOI: 10.1056/NEJMoa2036205] [FullText]

13 **Iwaki M**, Kobayashi T, Nogami A, Ogawa Y, Imajo K, Sakai E, Nakada Y, Koyama S, Kurihashi T, Oza N, Kohira T, Okada M, Yamaguchi Y, Iwane S, Kageyama F, Sasada Y, Matsushita M, Tadauchi A, Murohisa G, Nagasawa M, Sato S, Maeda K, Furuta K, Shigefuku R, Seko Y, Tobita H, Kawata K, Kawanaka M, Sugihara T, Tamaki N, Iwasa M, Kawaguchi T, Itoh Y, Kawaguchi A, Takahashi H, Nakajima A, Yoneda M. Pemafibrate for treating MASLD complicated by hypertriglyceridaemia: a multicentre, open-label, randomised controlled trial study protocol. *BMJ Open* 2024; **14**: e088862 [*RCA*] [PMID: 39581726 DOI: 10.1136/bmjopen-2024-088862] [FullText]

14 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [*RCA*] [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4] [FullText]

15 **Sanyal A**, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, Lawitz EJ, Halegoua-DeMarzio D, Kundu S, Noviello S, Luo Y, Christian R. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet* 2019; **392**: 2705-2717 [*RCA*] [PMID: 30554783 DOI: 10.1016/S0140-6736(18)31785-9] [FullText]

16 **Halilbasic E**, Fiorotto R, Fickert P, Marschall HU, Moustafa T, Spirli C, Fuchsbichler A, Gumhold J, Silbert D, Zatloukal K, Langner C, Maitra U, Denk H, Hofmann AF, Strazzabosco M, Trauner M. Side chain structure determines unique physiologic and therapeutic properties of norursodeoxycholic acid in Mdr2-/- mice. *Hepatology* 2009; **49**: 1972-1981 [*RCA*] [PMID: 19475687 DOI: 10.1002/hep.22891] [FullText] [Full Text(PDF)]

17 **Panuganti VK**, Alluri CV, Mohammad J, Dundigalla MR, Madala PK, Kssvv S, Shaik A. Phase III, multicenter, randomized, double-blind, placebo-controlled study of norursodeoxycholic acid in metabolic dysfunction-associated steatotic liver disease patients. *World J Hepatol* 2025; **17**: 113658 [*RCA*] [PMID: 41479524 DOI: 10.4254/wjh.v17.i12.113658] [FullText] [Full Text(PDF)]

18 **Newsome PN**, Buchholtz K, Cusi K, Linder M, Okanoue T, Ratziu V, Sanyal AJ, Sejling AS, Harrison SA; NN9931-4296 Investigators. A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis. *N Engl J Med* 2021; **384**: 1113-1124 [*RCA*] [PMID: 33185364 DOI: 10.1056/NEJMoa2028395] [FullText]

19 **Trauner M**, Fuchs CD, Halilbasic E, Paumgartner G. New therapeutic concepts in bile acid transport and signaling for management of cholestasis. *Hepatology* 2017; **65**: 1393-1404 [*RCA*] [PMID: 27997980 DOI: 10.1002/hep.28991] [FullText]

20 **Harrison SA**, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, Lawitz EJ, Rockey DC, Schall RA, Jia C, McColgan BJ,

McHutchison JG, Subramanian GM, Myers RP, Younossi Z, Ratziu V, Muir AJ, Afdhal NH, Goodman Z, Bosch J, Sanyal AJ; GS-US-321-0105 and GS-US-321-0106 Investigators. Simtuzumab Is Ineffective for Patients With Bridging Fibrosis or Compensated Cirrhosis Caused by Nonalcoholic Steatohepatitis. *Gastroenterology* 2018; **155**: 1140-1153 [*RCA*] [PMID: 29990488 DOI: 10.1053/j.gastro.2018.07.006] [Full Text]

21 **Wei S**, Wang L, Evans PC, Xu S. NAFLD and NASH: etiology, targets and emerging therapies. *Drug Discov Today* 2024; **29**: 103910 [*RCA*] [PMID: 38301798 DOI: 10.1016/j.drudis.2024.103910] [FullText]

22 **Fickert P**, Pollheimer MJ, Silbert D, Moustafa T, Halilbasic E, Krones E, Durchschein F, Thüringer A, Zollner G, Denk H, Trauner M. Differential effects of norUDCA and UDCA in obstructive cholestasis in mice. *J Hepatol* 2013; **58**: 1201-1208 [*RCA*] [PMID: 23369794 DOI: 10.1016/j.jhep.2013.01.026] [FullText]

23 **Rinella ME**, Tacke F, Sanyal AJ, Anstee QM; participants of the AASLD/EASL Workshop. Report on the AASLD/EASL joint workshop on clinical trial endpoints in NAFLD. *J Hepatol* 2019; **71**: 823-833 [*RCA*] [PMID: 31300231 DOI: 10.1016/j.jhep.2019.04.019] [FullText]

24 **Eddowes PJ**, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Guha IN, Cobbold JF, Deeks JJ, Paradis V, Bedossa P, Newsome PN. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **156**: 1717-1730 [*RCA*] [PMID: 30689971 DOI: 10.1053/j.gastro.2019.01.042] [FullText]

25 **Sanyal AJ**, Friedman SL, McCullough AJ, Dimick-Santos L; American Association for the Study of Liver Diseases; United States Food and Drug Administration. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. *Hepatology* 2015; **61**: 1392-1405 [*RCA*] [PMID: 25557690 DOI: 10.1002/hep.27678] [FullText] [Full Text(PDF)]

26 **Han MAT**, Altayar O, Hamdeh S, Takyar V, Rotman Y, Etzion O, Lefebvre E, Safadi R, Ratziu V, Prokop LJ, Murad MH, Noureddin M. Rates of and Factors Associated With Placebo Response in Trials of Pharmacotherapies for Nonalcoholic Steatohepatitis: Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2019; **17**: 616-629.e26 [*RCA*] [PMID: 29913275 DOI: 10.1016/j.cgh.2018.06.011] [FullText]

27 **Traussnigg S**, Schattenberg JM, Demir M, Wiegand J, Geier A, Teuber G, Hofmann WP, Kremer AE, Spreda F, Kluwe J, Petersen J, Boettler T, Rainer F, Halilbasic E, Greinwald R, Pröls M, Manns MP, Fickert P, Trauner M; Austrian/German NAFLD-norUDCA study group. Norursodeoxycholic acid versus placebo in the treatment of non-alcoholic fatty liver disease: a double-blind, randomised, placebo-controlled, phase 2 dose-finding trial. *Lancet Gastroenterol Hepatol* 2019; **4**: 781-793 [*RCA*] [PMID: 31345778 DOI: 10.1016/S2468-1253(19)30184-0] [FullText]

28 **Ratziu V**, Charlton M. Rational combination therapy for NASH: Insights from clinical trials and error. *J Hepatol* 2023; **78**: 1073-1079 [*RCA*] [PMID: 36603662 DOI: 10.1016/j.jhep.2022.12.025] [FullText]

29 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [*RCA*] [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9] [FullText] [Full Text(PDF)]

30 **Zerem E**, Kunosic S, Kurtcehajic A, Zerem D, Zerem O. Bile acids in metabolic dysfunction-associated steatotic liver disease. *World J Hepatol* 2025; **17**: 108606 [*RCA*] [PMID: 40901599 DOI: 10.4254/wjh.v17.i8.108606] [FullText] [Full Text(PDF)]

FOOTNOTES

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OPINION REVIEW

Chrono-metabolic regulation of hepatic fibrosis *via* NR1D1-mediated hepatic stellate cell activation

Hou-Shu Tu, Meng-Lin Chen, Jing Hong, Ling He

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Novelty: Grade B, Grade C, Grade C
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Abstract

Hepatic fibrosis (HF) is a progressive condition driven by persistent activation of hepatic stellate cells (HSCs), metabolic dysregulation, and inflammatory signaling. Emerging evidence identifies the nuclear receptor NR1D1 as a central node linking circadian rhythms, HIF-1α signaling, and ammonia-mediated HSC activation, providing a mechanistic framework for chrono-metabolic therapeutic strategies. Preclinical studies demonstrate that pharmacological or natural compound-mediated modulation of NR1D1, including *Hedyotis diffusa*, ferulic acid, and dihydroartemisinin, restores circadian and metabolic homeostasis, attenuates HSC activation, and reduces extracellular matrix deposition. Integrative chrono-informed interventions leveraging NR1D1 offer potential advantages over conventional single-target therapies by synchronizing drug action with endogenous circadian oscillations, optimizing dosing, and enabling multi-pathway modulation. This review highlights the NR1D1-centered chrono-metabolic axis as a promising therapeutic target for HF, emphasizing translational potential and the need for preclinical and early-phase clinical validation.

Key Words: Hepatic fibrosis; NR1D1; Hepatic stellate cells; Circadian rhythm; Chronotherapy; Ammonia metabolism; HIF-1α signaling; Natural compounds

Core Tip: This review summarizes the NR1D1-centered chrono-metabolic regulation of hepatic fibrosis, detailing how circadian rhythms, HIF-1α signaling, and ammonia-mediated hepatic stellate cell activation converge. It emphasizes the therapeutic potential of chrono-informed interventions using pharmacological agents and natural compounds, offering a translational roadmap for more precise and effective antifibrotic strategies.

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INTRODUCTION

Hepatic fibrosis (HF) is a dynamic, highly integrated wound-healing response triggered by chronic liver injury. It is characterized by excessive deposition of the extracellular matrix (ECM), disruption of tissue architecture, and progressive evolution toward cirrhosis and hepatocellular carcinoma[1-3]. Globally, liver fibrosis accounts for over one million deaths annually, with major etiologies including chronic viral hepatitis, metabolic dysfunction-associated steatohepatitis/nonalcoholic steatohepatitis (MASH/NASH), and alcohol-related liver disease[4-6]. Despite extensive research, no therapies have yet been approved to directly reverse fibrosis, highlighting a critical unmet clinical need[7-9].

Hepatic stellate cells (HSCs) are central drivers of liver fibrogenesis. In their quiescent state, HSCs primarily store vitamin A-containing lipid droplets. Upon liver injury, they transdifferentiate into activated myofibroblast-like cells, secreting collagen and other ECM proteins that promote fibrosis progression[7,10,11]. HSC activation is orchestrated by multiple factors, including paracrine signals from injured hepatocytes and immune cells, systemic metabolic disturbances, epigenetic modifications, and microenvironmental cues such as oxidative and endoplasmic reticulum stress [12-15]. Hyperammonemia exerts particularly deleterious effects, inducing HSC activation, cytoplasmic vacuolization, endoplasmic reticulum enlargement, and upregulation of pro-fibrotic genes. Pharmacological ammonia-lowering strategies, such as L-ornithine phenylacetate, reduce HSC activation and portal hypertension in preclinical models, emphasizing ammonia metabolism as a potential therapeutic target[12,16]. Beyond HSCs, ammonia also directly induces hepatocyte apoptosis and autophagy *via* the Bax/Bcl-2 and mTOR/ATG5/LC3BII pathways, creating a pro-fibrotic microenvironment that further exacerbates liver injury[17].

Circadian CLOCK components have emerged as key modulators of HF. Genes including *NR1D1*, *BMAL1*, *CLOCK*, and *REV-ERBα* regulate HSC activation, cellular metabolism, transforming growth factor-beta (TGF-β) signaling, and inflammatory responses[18-21]. Dysregulation of these CLOCK genes exacerbates fibrosis, whereas pharmacological modulation, such as with melatonin or REV-ERB agonists, attenuates HSC activation and ECM deposition, highlighting the potential of chrono-informed therapeutic strategies[18,21-24]. Recent evidence also suggests bidirectional communication between the liver and central circadian rhythms, whereby liver pathology can disrupt systemic circadian homeostasis, establishing a feedback loop that aggravates disease progression[25].

Non-coding RNAs (ncRNAs), including long ncRNAs and microRNAs, further regulate HSC activation through TGF-β signaling, epigenetic modifications, and competitive endogenous RNA (ceRNA) networks. For instance, MALAT1 and lnc-LFAR1 promote HSC activation *via* TGF-β signaling, whereas HIF1A-AS1 and lincRNA-p21 inhibit activation through DNA methylation and ceRNA mechanisms[26-28]. Dysregulation of these ncRNAs contributes to both initiation and progression of fibrosis, representing potential molecular targets for therapy.

Complementing these mechanistic insights, natural products and traditional Chinese medicines have shown significant antifibrotic potential. *Hedyotis diffusa* alleviates CCl4-induced fibrosis by restoring NR1D1 expression, modulating HIF-1α and urea cycle pathways, and regulating gut microbiota-bile acid homeostasis[21,29,30]. Other bioactive compounds, such as ferulic acid, dihydroartemisinin, and eriocitrin, attenuate HSC activation and ECM deposition through TGF-β/Smad signaling, lipophagy regulation, and PPARα-mediated inflammasome modulation[31-35].

Collectively, liver fibrosis arises from a complex interplay among HSC activation, circadian and metabolic dysregulation, inflammatory signaling, and epigenetic control. These interconnections highlight the therapeutic potential of integrated chrono-metabolic interventions and provide a framework for translating mechanistic insights into effective clinical strategies[1,3,22,29,36].

CURRENT ADVANCES

Circadian regulation in HF

Circadian CLOCK machinery plays a critical role in maintaining liver homeostasis and regulating fibrogenesis. HSCs possess functional circadian oscillators, exhibiting rhythmic expression of fibrogenic genes such as *NR1D1/Rev-erbα* and *BMAL1*[1,10,18,20,24,37-39]. Disruption of these CLOCK genes enhances HSC activation, ECM deposition, and fibrosis progression[12,40]. Mechanistically, circadian dysregulation affects TGF-β signaling, metabolic pathways, and inflammatory responses in both hepatocytes and HSCs[19,21]. Preclinical studies indicate that restoring circadian function—genetically or pharmacologically (*e.g.*, REV-ERB agonists or melatonin)—suppresses HSC activation and ECM accumu-

lation, highlighting the therapeutic potential of chrono-metabolic regulation. Taken together, these findings suggest that circadian CLOCK components provide temporal control over HSC activation and fibrogenesis, offering a mechanistic foundation for chrono-informed interventions. Table 1 summarizes key circadian regulators and their roles in HSC activation and HF.

HIF-1α signaling and ammonia-mediated HSC activation

Metabolic stress signals, particularly hyperammonemia, are central drivers of HSC activation. Elevated ammonia promotes HSC proliferation, reactive oxygen species generation, endoplasmic reticulum stress, and upregulation of α-SMA and PDGF-Rβ[12,41]. NR1D1 functions upstream to inhibit HIF-1α signaling, mitigating ammonia-induced HSC activation, stabilizing mitochondrial function, and reducing local inflammatory responses[16,20,29,30,42-44]. This NR1D1–HIF-1α–ammonia axis integrates circadian and metabolic regulation, linking chrono-metabolic dysfunction to fibrogenesis and highlighting ammonia metabolism as a pharmacological target. Pharmacological or genetic restoration of NR1D1 effectively suppresses HSC activation and ameliorates fibrosis progression.

ncRNAs, including MALAT1 and lnc-LFAR1, interact with HIF-1α signaling, modulating ammonia-induced HSC activation through ceRNA networks[26,27]. These examples underscore the multifaceted regulation of HSC activation at both transcriptional and post-transcriptional levels. Taken together, these data support the convergence of circadian modulation and ammonia metabolism in controlling HSC activation, providing a strong rationale for integrated chrono-metabolic interventions. Table 2 presents experimental evidence linking NR1D1 to HIF-1α inhibition and ammonia-mediated HSC activation.

Bioactive compounds and experimental validation

Natural compounds demonstrate potent anti-fibrotic effects by modulating the NR1D1–HIF-1α–ammonia axis. *Hedyotis diffusa* extracts restore NR1D1 expression, normalize urea cycle function, reduce ammonia accumulation, and attenuate HSC activation in preclinical models[21,22,29]. Other bioactive molecules, including ferulic acid, dihydroartemisinin, and eriocitrin, exert protective effects through TGF-β/Smad signaling, lipophagy regulation, and PPARα-mediated inflammasome modulation[31-33,45-52]. Nanoparticle-based strategies, such as hyaluronic acid–bilirubin conjugates, selectively target activated HSCs and reduce oxidative stress, further mitigating fibrosis[34].

Network pharmacology analyses indicate that these compounds act on multiple nodes within the circadian-metabolic-fibrogenic network, supporting a multi-target, chrono-metabolic intervention approach that may be superior to single-target therapies. Taken together, these findings provide strong experimental validation that chrono-metabolic modulation *via* natural compounds or pharmacological agents is feasible and effective in preclinical models of HF. Table 3 lists recent preclinical studies of *Hedyotis diffusa* and other bioactive compounds targeting the NR1D1–HIF-1α–ammonia axis.

MAJOR CONTROVERSIES AND UNRESOLVED ISSUES

Despite substantial progress in elucidating the NR1D1–HIF-1α–ammonia axis in HF, several critical controversies and unresolved questions remain, which limit the translational application of chrono-metabolic interventions[1,19]. Here, we highlight three major areas of uncertainty.

Disease-specific applicability of the NR1D1–HIF-1α–ammonia axis

Most mechanistic studies have been conducted in CCl4-induced fibrosis or NASH/MASH models, raising uncertainty regarding the relevance of this axis in other etiologies, including alcoholic liver disease (ALD), drug-induced fibrosis, congenital HF, or nonalcoholic fatty liver disease (NAFLD)-related fibrosis[12,41,53-55]. Differences in liver microenvironment, HSC heterogeneity, and systemic metabolic context may modulate the activity of this pathway and its therapeutic responsiveness. Circadian disruption has been shown to prime HSCs for accelerated activation in NAFLD models, suggesting broader applicability but also mechanistic differences across disease contexts. Expanding experimental validation across multiple fibrosis models is therefore necessary to confirm the generalizability of NR1D1–HIF-1α-ammonia signaling[20,54].

Single-target vs chrono-metabolic integrated interventions

While NR1D1 modulation alone has demonstrated anti-fibrotic efficacy in preclinical models[20,29], HF is a multifactorial disease driven by circadian disruption, metabolic stress, ammonia accumulation, and chronic inflammation[14,30,56]. Bioactive compounds such as dihydroartemisinin and notoginsenoside R1 have been shown to regulate HSC lipophagy *via* NR1D1-mediated mechanisms and modulate PPAR-γ/TGF-β signaling, respectively, illustrating the potential advantage of multi-target chrono-metabolic strategies over single-node interventions[32,55,56]. It remains unresolved whether targeting a single node is sufficient or if combinatorial strategies—such as NR1D1 activation combined with ammonia-lowering therapy, bile acid modulation, or circadian-aligned pharmacotherapy—are necessary for robust and durable therapeutic outcomes[21,32].

Clinical translation challenges

Implementation of chrono-informed therapies faces practical obstacles. Optimizing dose, timing, and patient stratification is crucial, as individual differences in circadian rhythms, fibrosis stage, and etiology-specific metabolic alterations may profoundly influence treatment efficacy[16,30,57]. Existing preclinical models often fail to fully capture human disease

Table 1 Key circadian regulators in hepatic stellate cell activation and liver fibrosis

Circadian gene/regulator	Model/cell type	Intervention/condition	Main findings	Mechanistic insight	Ref.
NR1D1/Rev-erbα	Mouse liver, HSCs	Circadian disruption/CCl4	Dysregulated NR1D1/Rev-erbα; enhanced HSC activation	Loss of NR1D1 promotes TGF-β signaling and ECM deposition	[1,10,20,24]
BMAL1	HSCs, LX2 cells	TGF-β1 induction	Downregulated *BMAL1*; increased glycolysis and HSC activation	*BMAL1* inhibits phenotypic transformation *via IDH1*/α-KG-mediated glycolysis	[23,38,40]
CLOCK, *Per1*-3	HSCs	CCl4-induced fibrosis	Reduced *CLOCK* gene expression	Disruption of CC contributes to HSC proliferation and collagen synthesis	[18,21,37]
REV-ERBα	HSCs, mice	Melatonin/SR9009	Upregulation prevented HSC activation	Modulates circadian clock and PPARα signaling	[18,39]

This table summarizes the primary circadian clock components involved in hepatic stellate cell activation and hepatic fibrosis, including experimental models, interventions, main findings, mechanistic insights, and supporting references. HSC: Hepatic stellate cell; CC: Circadian clock; TGF-β: Transforming growth factor-beta; ECM: Extracellular matrix.

Table 2 Experimental evidence linking NR1D1 to HIF-1α inhibition and ammonia-mediated hepatic stellate cell activation

Pathway/target	Model/cell type	Intervention	Main findings	Mechanistic insight	Ref.
NR1D1–HIF-1α	LX2, primary HSCs	CCl4-induced fibrosis/NR1D1 overexpression	NR1D1 restoration suppressed HIF-1α, reduced α-SMA and collagen	NR1D1 inhibits HIF-1α signaling, reduces ammonia-mediated HSC activation	[20,29]
Ammonia	LX2, human HSCs	NH4Cl/hyperammonemia	Increased proliferation, ROS, ER stress, α-SMA, PDGF-Rβ	Ammonia promotes HSC activation; toxicity reversed by NR1D1	[12,41]
HIF-1α	HSCs	Hypoxia/metabolic stress	Upregulated fibrogenic genes	HIF-1α drives ammonia-induced fibrogenesis	[19,29]

This table summarizes experimental studies investigating the NR1D1–HIF-1α–ammonia axis, including models, interventions, main findings, mechanistic insights, and supporting references. HSC: Hepatic stellate cell; ROS: Reactive oxygen species; ER: Endoplasmic reticulum; α-SMA: Alpha-smooth muscle actin; PDGF-Rβ: Platelet-derived growth factor receptor beta.

heterogeneity, limiting predictive validity for clinical translation[4,21]. Developing more representative models and incorporating patient-derived systems will be critical to bridge this translational gap and facilitate clinical application.

Taken together, these considerations underscore the need for integrated experimental, preclinical, and clinical strategies to validate the NR1D1–HIF-1α–ammonia axis across diverse fibrosis contexts. Table 4 summarizes key controversies in NR1D1–HIF-1α–ammonia research, detailing issues, supporting evidence, and knowledge gaps.

AUTHOR PERSPECTIVES

NR1D1 has emerged as a pivotal regulatory node in HF, integrating circadian rhythm, HIF-1α signaling, and ammonia metabolism. Experimental evidence demonstrates that NR1D1 activation suppresses HIF-1α–driven transcriptional programs, reduces ammonia accumulation, and limits HSC activation, thereby mitigating fibrogenesis[20-22,29,40]. These multifaceted effects position NR1D1 as a unique chrono-metabolic target, capable of coordinating temporal regulation of both hepatic metabolic and fibrotic pathways.

From a therapeutic perspective, chrono-informed interventions targeting NR1D1 offer distinct advantages. Aligning drug administration with the endogenous circadian oscillation of NR1D1 may optimize efficacy and minimize off-target effects, while simultaneously modulating systemic ammonia levels and HSC activation cycles[18,19,21]. Preclinical studies indicate that natural compounds, such as *Hedyotis diffusa* extract, ferulic acid, and dihydroartemisinin, can restore NR1D1 expression, normalize downstream HIF-1α and urea cycle pathways, and attenuate HSC activation[21,29,32].

Figure 1 illustrates the proposed mechanistic framework: NR1D1 acts upstream to repress HIF-1α signaling, thereby limiting ammonia-induced HSC activation. Potential pharmacological and botanical intervention points are indicated, including additional modulatory nodes such as bile acid signaling, ncRNAs (*e.g.*, MALAT1 and lnc-LFAR1), and PPARα-mediated inflammasome regulation[26,27,34]. Together, these insights reinforce NR1D1 as a core node for integrated temporal and metabolic control, bridging molecular mechanisms to potential clinical interventions and suggesting that chrono-metabolic strategies may surpass conventional single-target approaches[3,34].

Table 3 Recent preclinical studies of *Hedyotis diffusa* and other bioactive compounds targeting the NR1D1-HIF1-ammonia axis

Compound/extract	Model	Intervention	Main findings	Mechanistic insight	Ref.
Hedyotis diffusa	CCl4/HF mice, LX2	*Hedyotis diffusa* extract/injection	Reduced α-SMA, collagen; restored NR1D1	Modulates NR1D1–HIF1–ammonia axis; normalizes urea cycle	[21,29]
Dihydroartemisinin	CCl4 mice, HSCs	Dihydroartemisinin treatment	Restored lipid droplets in HSCs; inhibited activation	NR1D1-mediated Rab7 ubiquitination regulates lipophagy	[32,51,52]
Ferulic acid	LX2 cells, SD rats	TGF-β1/CCl4	Inhibited α-SMA, collagen, p-Smad 2/3	Blocks TGF-β/Smad signaling	[31,47]
Eriocitrin	TAA mice, LX2 cells	Eriocitrin treatment	Reduced inflammasome activation and collagen deposition	PPARα-mediated NLRP1/NLRC4 pathway	[33]
HDW extract	CCl4 mice	HDW treatment	Reduced HSC activation; improved liver function	Modulates gut microbiota, FXR/SHP/CYP7A1 pathway; chrono-metabolic effects	[21]
FA11	CCl4 mice	FA11 treatment	Reduced α-SMA, collagen	Inhibits TGF-β1-induced HSC activation	[46]
Ghrelin	CCl4 mice	Ghrelin treatment	Decreased HSC proliferation, ECM deposition	Modulates HIF-1α and ROS pathways	[45]
Physalin D	HSCs	PD treatment	Reduced HSC activation	Blocks TGF-β/Smad and YAP signaling	[49]

This table summarizes interventions, experimental models, main findings, mechanistic insights, and references for natural and pharmacological compounds affecting the *NR1D1*–HIF-1α–ammonia pathway. HSC: Hepatic stellate cell; ECM: Extracellular matrix; ROS: Reactive oxygen species; TGF-β: Transforming growth factor-beta.

Table 4 Key controversies in NR1D1–HIF-1α–ammonia axis research

Controversy	Evidence/context	Knowledge gap	Ref.
Disease-specific applicability	Most mechanistic studies performed in CCl4-induced fibrosis or NASH/MASH models; relevance to ALD, drug-induced fibrosis, congenital fibrosis, or NAFLD unclear	Uncertain if *NR1D1*–HIF-1α–ammonia axis functions similarly across diverse etiologies; need multi-model validation	[12,41,53-55]
Single-target *vs* integrated chrono-metabolic therapy	NR1D1 modulation alone shows anti-fibrotic effects; bioactive compounds (*e.g.*, dihydroartemisinin, notoginsenoside R1) regulate multiple HSC pathways including lipophagy, PPAR-γ/TGF-β	Whether single-node targeting is sufficient *vs* combinatorial strategies integrating NR1D1, ammonia-lowering therapy, bile acid modulation, and circadian-aligned dosing	[20,21,29,32,55,56]
Clinical translation challenges	Variability in circadian rhythms, fibrosis stage, etiology-specific metabolic alterations; existing models often do not reflect human heterogeneity	Optimal dosing schedules, patient stratification, and model selection; incorporation of patient-derived systems for translation	[4,16,21,30,57]

This table summarizes key issues, supporting evidence, and gaps in knowledge for the NR1D1–HIF-1α–ammonia axis, highlighting areas requiring further investigation. HSC: Hepatic stellate cell; NASH: Nonalcoholic steatohepatitis; MASH: Metabolic dysfunction-associated steatohepatitis; ALD: Alcoholic liver disease; NAFLD: Nonalcoholic fatty liver disease.

FUTURE DIRECTIONS

Despite substantial progress in elucidating the NR1D1–HIF-1α–ammonia axis, several critical avenues remain for advancing chrono-metabolic interventions toward clinical translation.

Molecular mapping of NR1D1-ligand interactions

Detailed structural characterization, binding kinetics, and ligand specificity studies of NR1D1 are essential to guide rational drug design and the development of potent agonists or modulators[22,32,58]. High-resolution techniques, including X-ray crystallography or cryo-EM, combined with computational docking and molecular dynamics simulations, can identify key residues for selective targeting and optimize ligand efficacy. In parallel, CRISPR/dCas9-based approaches can be employed to modulate NR1D1 expression or activity in HSCs, providing a precise experimental tool for validating structure–function relationships[58].

Transcriptional and epigenetic decoding

Comprehensive profiling of NR1D1-regulated genes in HSCs and hepatocytes using ChIP-seq, RNA-seq, and single-cell transcriptomics will delineate the network of chrono-metabolic control points[20,40,59]. Integration with epigenetic marks, ncRNA regulation (*e.g.*, MALAT1 and lnc-LFAR1), and circadian phase–dependent gene expression can uncover dynamic regulatory circuits governing fibrosis progression, providing mechanistic insight for multi-target interventions.

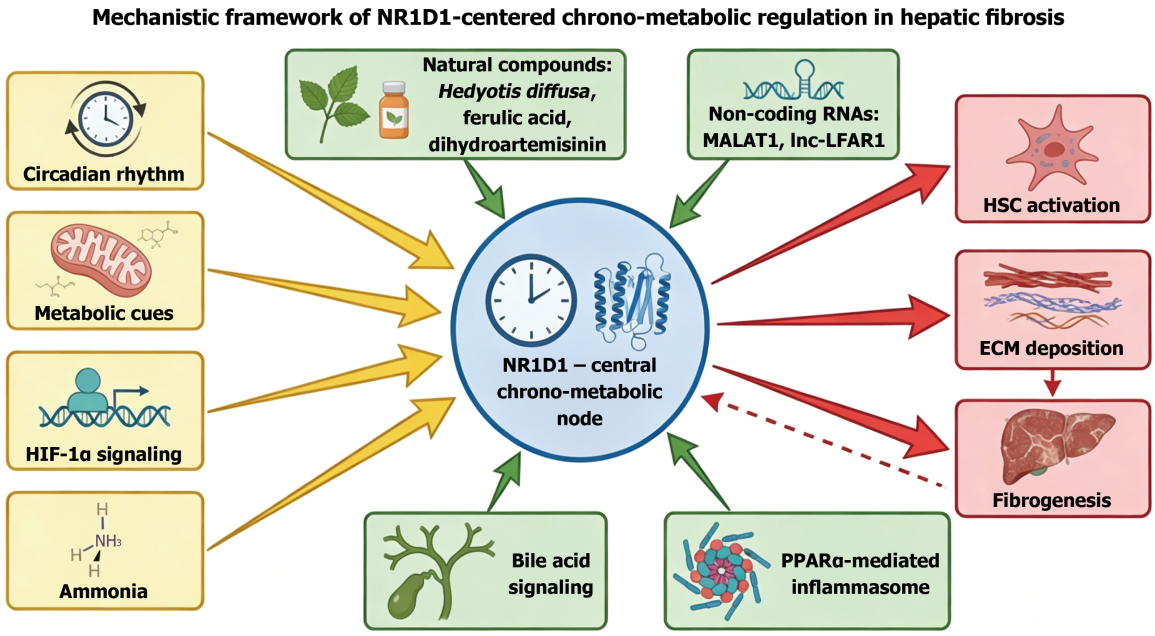


Figure 1 Mechanistic framework of NR1D1-centered chrono-metabolic regulation in hepatic fibrosis. NR1D1 acts as a central circadian-metabolic node, inhibiting HIF-1α signaling and reducing ammonia-induced hepatic stellate cell activation, thereby mitigating fibrogenesis. Pharmacological and natural compounds (*e.g.*, *Hedyotis diffusa*, ferulic acid, and dihydroartemisinin) restore NR1D1 expression and downstream pathways. Additional modulatory nodes, including bile acid signaling, non-coding RNAs (MALAT1 and lnc-LFAR1), and PPARα-mediated inflammasome regulation, further refine temporal and metabolic control. HSC: Hepatic stellate cell; ECM: Extracellular matrix.

Recent findings demonstrating that the hepatic CLOCK synergizes with HIF-1α to regulate nucleotide availability during liver damage repair further emphasize the importance of deciphering transcriptional networks at high spatiotemporal resolution[60].

Etiological expansion

Current evidence is largely derived from CCl4-induced fibrosis and NASH models. Extending NR1D1-focused studies to MASH, ALD, drug-induced fibrosis, and congenital HF will establish the generalizability of NR1D1–HIF-1α–ammonia targeting strategies[3,5,12]. Comparative analyses across etiologies can identify disease-specific modifiers, refine dosing regimens, and optimize therapeutic interventions. For instance, hypoxiainducible factor2α has been shown to promote fibrosis in NAFLD by enhancing glutamine catabolism and YAP signaling in HSCs, highlighting distinct metabolic cues across disease contexts[61].

Signal translation and microenvironmental crosstalk

Further research is needed to clarify how ammonia and other metabolic cues propagate within the hepatic microenvironment to activate HSCs, including crosstalk with hepatocytes, immune cells, and endothelial components[16,41]. Emerging evidence shows that metabolic regulators such as LOX1 can rewire glutamine–ammonia metabolism to drive liver fibrosis *via* HSC activation, suggesting additional targetable signaling nodes[62]. Understanding these interactions may reveal additional modulatory nodes for combinatorial chrono-metabolic therapies and allow targeted modulation of systemic and local signals.

Translational validation

Preclinical studies employing chronotherapy designs, coupled with early-phase clinical trials, are critical to establish optimal dosing schedules, therapeutic windows, and patient stratification strategies[30,34]. Incorporating circadian biomarkers, ammonia monitoring, and imaging-based fibrosis assessment can enhance precision interventions, improve reproducibility, and accelerate clinical translation.

Figure 2 presents a translational roadmap integrating molecular mapping, preclinical validation, and clinical application, emphasizing systematic chrono-metabolic interventions from bench to bedside. Collectively, these approaches will enhance mechanistic understanding, refine therapeutic targeting, and accelerate the translation of NR1D1-centered strategies into clinically effective treatments for HF.

CONCLUSION

HF is a progressive stage of chronic liver disease driven by persistent HSC activation, metabolic dysregulation, and

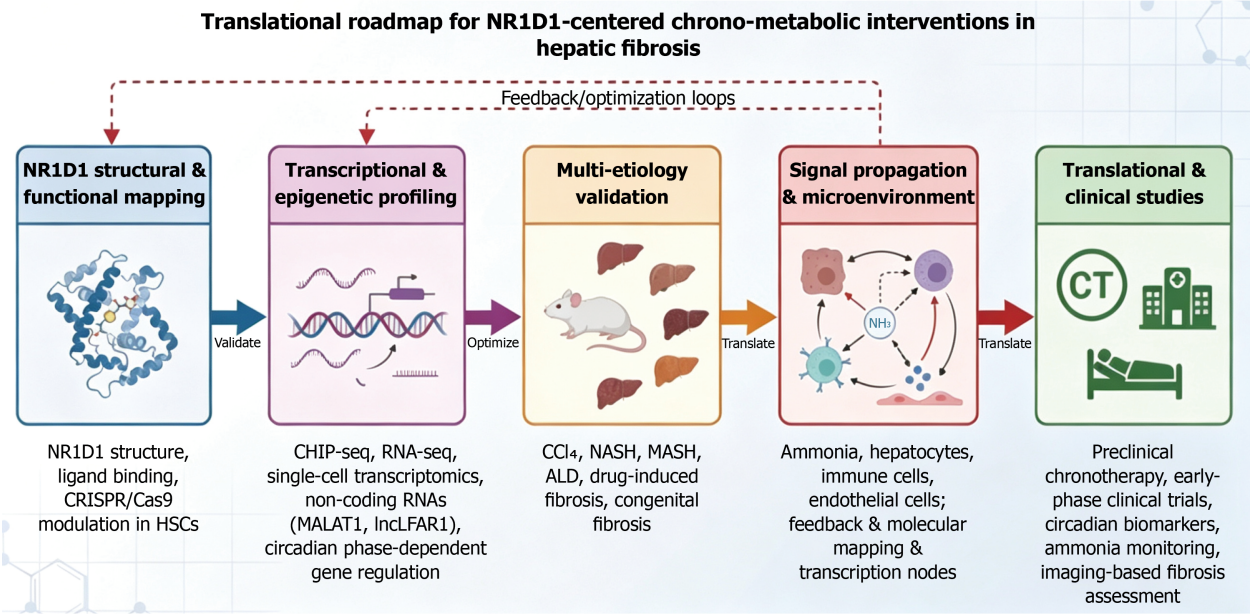


Figure 2 Translational roadmap for NR1D1-centered chrono-metabolic interventions in hepatic fibrosis. This framework integrates molecular mapping, transcriptional and epigenetic decoding, multi-etiology validation, signal translation within the hepatic microenvironment, and preclinical and early-phase clinical studies. Each stage represents a step toward translating mechanistic insights into chrono-metabolic therapies, highlighting points for intervention, validation, and optimization from bench to bedside. NASH: Nonalcoholic steatohepatitis; MASH: Metabolic dysfunction-associated steatohepatitis; ALD: Alcoholic liver disease.

inflammatory signaling. NR1D1 emerges as a central regulatory node linking circadian rhythm, HIF-1α signaling, and ammonia-induced HSC activation, providing a mechanistic framework for chrono-metabolic therapeutic strategies. By integrating temporal and metabolic regulation, these approaches enable optimization of dosing, timing, and target specificity, potentially surpassing conventional single-target interventions. Preclinical evidence with *Hedyotis diffusa* and related compounds supports the feasibility of modulating the NR1D1–HIF-1α–ammonia axis, and translational studies indicate potential for tailored interventions across diverse liver disease etiologies. Future research should refine chrono-metabolic targets, validate disease-specific applicability, and establish optimized chronotherapeutic regimens to improve clinical outcomes.

REFERENCES

1 **Friedman SL**, Pinzani M. Hepatic fibrosis 2022: Unmet needs and a blueprint for the future. *Hepatology* 2022; **75**: 473-488 [*RCA*] [PMID: 34923653 DOI: 10.1002/hep.32285] [FullText] [Full Text(PDF)]

2 **Roehlen N**, Crouchet E, Baumert TF. Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives. *Cells* 2020; **9**: 875 [*RCA*] [PMID: 32260126 DOI: 10.3390/cells9040875] [FullText] [Full Text(PDF)]

3 **Parola M**, Pinzani M. Liver fibrosis in NAFLD/NASH: from pathophysiology towards diagnostic and therapeutic strategies. *Mol Aspects Med* 2024; **95**: 101231 [*RCA*] [PMID: 38056058 DOI: 10.1016/j.mam.2023.101231] [FullText]

4 **Juanola A**, Pose E, Ginès P. Liver Cirrhosis: ancient disease, new challenge. *Med Clin (Barc)* 2025; **164**: 238-246 [*RCA*] [PMID: 39732564 DOI: 10.1016/j.medcli.2024.11.002] [FullText]

5 **Argenziano ME**, Kim MN, Montori M, Di Bucchianico A, Balducci D, Ahn SH, Svegliati Baroni G. Epidemiology, pathophysiology and clinical aspects of Hepatocellular Carcinoma in MAFLD patients. *Hepatol Int* 2024; **18**: 922-940 [*RCA*] [PMID: 39012579 DOI: 10.1007/s12072-024-10692-4] [FullText]

6 **Parola M**, Pinzani M. Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues. *Mol Aspects Med* 2019; **65**: 37-55 [*RCA*] [PMID: 30213667 DOI: 10.1016/j.mam.2018.09.002] [FullText]

7 **Higashi T**, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. *Adv Drug Deliv Rev* 2017; **121**: 27-42 [*RCA*] [PMID: 28506744 DOI: 10.1016/j.addr.2017.05.007] [FullText] [Full Text(PDF)]

8 **Khanam A**, Saleeb PG, Kottilil S. Pathophysiology and Treatment Options for Hepatic Fibrosis: Can It Be Completely Cured? *Cells* 2021; **10**: 1097 [*RCA*] [PMID: 34064375 DOI: 10.3390/cells10051097] [FullText] [Full Text(PDF)]

9 **Berumen J**, Baglieri J, Kisseleva T, Mekeel K. Liver fibrosis: Pathophysiology and clinical implications. *WIREs Mech Dis* 2021; **13**: e1499 [*RCA*] [PMID: 32713091 DOI: 10.1002/wsbm.1499] [FullText]

10 **Kamm DR**, McCommis KS. Hepatic stellate cells in physiology and pathology. *J Physiol* 2022; **600**: 1825-1837 [*RCA*] [PMID: 35307840 DOI: 10.1113/JP281061] [FullText]

11 **Yan T**, Yan N, Xia Y, Sawaswong V, Zhu X, Dias HB, Aibara D, Takahashi S, Hamada K, Saito Y, Li G, Liu H, Yan H, Velenosi TJ, Krausz KW, Huang J, Kimura S, Rotman Y, Qu A, Hao H, Gonzalez FJ. Hepatocyte-specific CCAAT/enhancer binding protein α restricts liver fibrosis progression. *J Clin Invest* 2024; **134**: e166731 [*RCA*] [PMID: 38557493 DOI: 10.1172/JCI166731] [FullText]

12 **Jalan R**, De Chiara F, Balasubramaniyan V, Andreola F, Khetan V, Malago M, Pinzani M, Mookerjee RP, Rombouts K. Ammonia produces pathological changes in human hepatic stellate cells and is a target for therapy of portal hypertension. *J Hepatol* 2016; **64**: 823-833 [*RCA*]

[PMID: 26654994 DOI: 10.1016/j.jhep.2015.11.019] [FullText]

13 **Dewidar B**, Meyer C, Dooley S, Meindl-Beinker AN. TGF-β in Hepatic Stellate Cell Activation and Liver Fibrogenesis-Updated 2019. *Cells* 2019; **8**: 1419 [*RCA*] [PMID: 31718044 DOI: 10.3390/cells8111419] [FullText] [Full Text(PDF)]

14 **Wu B**, Feng J, Guo J, Wang J, Xiu G, Xu J, Ning K, Ling B, Fu Q, Xu J. ADSCs-derived exosomes ameliorate hepatic fibrosis by suppressing stellate cell activation and remodeling hepatocellular glutamine synthetase-mediated glutamine and ammonia homeostasis. *Stem Cell Res Ther* 2022; **13**: 494 [*RCA*] [PMID: 36195966 DOI: 10.1186/s13287-022-03049-x] [FullText] [Full Text(PDF)]

15 **Gallego-Durán R**, Ampuero J, Pastor-Ramírez H, Álvarez-Amor L, Del Campo JA, Maya-Miles D, Montero-Vallejo R, Cárdenas-García A, Pareja MJ, Gato-Zambrano S, Millán R, Del Carmen Rico M, Luque-Sierra A, Gil-Gómez A, Rojas Á, Muñoz-Hernández R, García-Lozano M, Aller R, Andrade RJ, García-Monzón C, Andreola F, Martín F, Jalan R, Romero-Gómez M. Liver injury in non-alcoholic fatty liver disease is associated with urea cycle enzyme dysregulation. *Sci Rep* 2022; **12**: 3418 [*RCA*] [PMID: 35232986 DOI: 10.1038/s41598-022-06614-9] [FullText] [Full Text(PDF)]

16 **Balcar L**, Krawanja J, Scheiner B, Paternostro R, Simbrunner B, Semmler G, Jachs M, Hartl L, Stättermayer AF, Schwabl P, Pinter M, Szekeres T, Trauner M, Reiberger T, Mandorfer M. Impact of ammonia levels on outcome in clinically stable outpatients with advanced chronic liver disease. *JHEP Rep* 2023; **5**: 100682 [*RCA*] [PMID: 36873421 DOI: 10.1016/j.jhepr.2023.100682] [FullText] [Full Text(PDF)]

17 **Chen B**, Liu X, Wu S, Hou J, Shang P, Chamba Y, Mehmood K, Fouad D, Li Y, Zhang H. Inhalation of ammonia promotes apoptosis and induces autophagy in hepatocytes *via* Bax/BCl-2 and m-TOR/ATG5/LC-3bII axes. *Sci Total Environ* 2024; **912**: 169036 [*RCA*] [PMID: 38061639 DOI: 10.1016/j.scitotenv.2023.169036] [FullText]

18 **González-Fernández B**, Sánchez DI, Crespo I, San-Miguel B, de Urbina JO, González-Gallego J, Tuñón MJ. Melatonin Attenuates Dysregulation of the Circadian Clock Pathway in Mice With CCl(4)-Induced Fibrosis and Human Hepatic Stellate Cells. *Front Pharmacol* 2018; **9**: 556 [*RCA*] [PMID: 29892224 DOI: 10.3389/fphar.2018.00556] [FullText] [Full Text(PDF)]

19 **Crouchet E**, Dachraoui M, Jühling F, Roehlen N, Oudot MA, Durand SC, Ponsolles C, Gadenne C, Meiss-Heydmann L, Moehlin J, Martin R, Brignon N, Del Zompo F, Teraoka Y, Aikata H, Abe-Chayama H, Chayama K, Saviano A, Heide D, Onea M, Geyer L, Wolf T, Felli E, Pessaux P, Heikenwälder M, Chambon P, Schuster C, Lupberger J, Mukherji A, Baumert TF. Targeting the liver clock improves fibrosis by restoring TGF-β signaling. *J Hepatol* 2025; **82**: 120-133 [*RCA*] [PMID: 39173955 DOI: 10.1016/j.jhep.2024.07.034] [FullText] [Full Text (PDF)]

20 **Chen L**, Xia S, Wang F, Zhou Y, Wang S, Yang T, Li Y, Xu M, Zhou Y, Kong D, Zhang Z, Shao J, Xu X, Zhang F, Zheng S. m(6)A methylation-induced NR1D1 ablation disrupts the HSC circadian clock and promotes hepatic fibrosis. *Pharmacol Res* 2023; **189**: 106704 [*RCA*] [PMID: 36813093 DOI: 10.1016/j.phrs.2023.106704] [FullText]

21 **Wang C**, Luo M, Chen Q, Zheng L, Jiang T, Dai M. Hedyotis diffusa Willd. extract alleviates CCl(4)-induced liver fibrosis *via* modulation of the gut microbiota and FXR/SHP/CYP7A1-mediated bile acid metabolism. *J Ethnopharmacol* 2026; **363**: 121450 [*RCA*] [PMID: 41785727 DOI: 10.1016/j.jep.2026.121450] [FullText]

22 **Wang J**, Cao M, Li S, Pei W, Li J, Wang Z. Circadian clock genes: Their influence on liver metabolism, disease development and treatment (Review). *Mol Med Rep* 2026; **33**: 3 [*RCA*] [PMID: 41104881 DOI: 10.3892/mmr.2025.13713] [FullText] [Full Text(PDF)]

23 **Xiao B**, Yang W, Dong H, Liu T, Li C, Wang Y, Gao D, Han G, Kiran F, Wang A, Jin Y, Yuan Y, Chen H. Co-Exposure to Polystyrene Microplastics and Bisphenol A Contributes to the Formation of Liver Fibrosis in Mice through Inhibition of the BMAL1/E-Cad Signaling Pathway. *J Agric Food Chem* 2025; **73**: 7405-7422 [*RCA*] [PMID: 40073227 DOI: 10.1021/acs.jafc.4c08790] [FullText]

24 **Wang J**, Wang Y, Lin L, Pei W, Li Y. Reverbα: The circadian guardian against NLRP3driven liver fibrosis. *Mol Med Rep* 2025; **32**: 270 [*RCA*] [PMID: 40709400 DOI: 10.3892/mmr.2025.13635] [FullText] [Full Text(PDF)]

25 **Gachon F**, Bugianesi E, Castelnuovo G, Oster H, Pendergast JS, Montagnese S. Potential bidirectional communication between the liver and the central circadian clock in MASLD. *NPJ Metab Health Dis* 2025; **3**: 15 [*RCA*] [PMID: 40225783 DOI: 10.1038/s44324-025-00058-1] [Full Text] [Full Text(PDF)]

26 **Peng H**, Wan LY, Liang JJ, Zhang YQ, Ai WB, Wu JF. The roles of lncRNA in hepatic fibrosis. *Cell Biosci* 2018; **8**: 63 [*RCA*] [PMID: 30534359 DOI: 10.1186/s13578-018-0259-6] [FullText] [Full Text(PDF)]

27 **Wu Z**, Huang S, Zheng X, Gu S, Xu Q, Gong Y, Zhang J, Fu B, Tang L. Regulatory long non-coding RNAs of hepatic stellate cells in liver fibrosis (Review). *Exp Ther Med* 2021; **21**: 351 [*RCA*] [PMID: 33732324 DOI: 10.3892/etm.2021.9782] [FullText] [Full Text(PDF)]

28 **Zhang Y**, Wu Y, Shen W, Wang B, Yuan X. Crosstalk between NK cells and hepatic stellate cells in liver fibrosis (Review). *Mol Med Rep* 2022; **25**: 208 [*RCA*] [PMID: 35506449 DOI: 10.3892/mmr.2022.12724] [FullText] [Full Text(PDF)]

29 **Xia SW**, Liu H, Yang KY, Gao YJ, Zhang MR, Zhou JW, Kong DS, Wu HY, Zhang F, Chen L. Integrative study reveals NR1D1 mediates Hedyotis diffusa's antifibrosis *via* hypoxia inducible factor-1/ammonia axis. *World J Gastroenterol* 2026; **32**: 115334 [*RCA*] [PMID: 41809447 DOI: 10.3748/wjg.v32.i10.115334] [FullText] [Full Text(PDF)]

30 **Harada M**, Adam J, Han S, Shi M, Ge J, Lintelmann J, Cecil A, Zukunft S, Prehn C, Witting M, Scheerer MF, Neschen S, Irmler M, Beckers J, Adamski J, Teupser D, Linkohr B, Gieger C, de Angelis MH, Peters A, Wang-Sattler R. Urea cycle modulation by combined SGLT2 inhibitors and metformin. *BMC Med* 2026; **24**: 55 [*RCA*] [PMID: 41501827 DOI: 10.1186/s12916-025-04609-7] [FullText] [Full Text(PDF)]

31 **Mu M**, Zuo S, Wu RM, Deng KS, Lu S, Zhu JJ, Zou GL, Yang J, Cheng ML, Zhao XK. Ferulic acid attenuates liver fibrosis and hepatic stellate cell activation *via* inhibition of TGF-β/Smad signaling pathway. *Drug Des Devel Ther* 2018; **12**: 4107-4115 [*RCA*] [PMID: 30584275 DOI: 10.2147/DDDT.S186726] [FullText]

32 **Bao Z**, Xu M, Kan Y, Guo X, Li M, Wang J, Zhou Y, Zhang Z, Shao J, Zhang F, Chen L, Zheng S, Xuan J. Dihydroartemisinin requires NR1D1 mediated Rab7 ubiquitination to regulate hepatic stellate cells lipophagy in liver fibrosis. *Int J Biol Macromol* 2025; **305**: 141055 [*RCA*] [PMID: 39956231 DOI: 10.1016/j.ijbiomac.2025.141055] [FullText]

33 **Zhang JJ**, Zhang JX, Feng QY, Shi LQ, Guo X, Sun HM, Song J. Eriocitrin ameliorates hepatic fibrosis and inflammation: The involvement of PPARα-mediated NLRP1/NLRC4 inflammasome signaling cascades. *J Ethnopharmacol* 2025; **338**: 119119 [*RCA*] [PMID: 39557108 DOI: 10.1016/j.jep.2024.119119] [FullText]

34 **Shinn J**, Park S, Lee S, Park N, Kim S, Hwang S, Moon JJ, Kwon Y, Lee Y. Antioxidative Hyaluronic Acid-Bilirubin Nanomedicine Targeting Activated Hepatic Stellate Cells for Anti-Hepatic-Fibrosis Therapy. *ACS Nano* 2024; **18**: 4704-4716 [*RCA*] [PMID: 38288705 DOI: 10.1021/acsnano.3c06107] [FullText]

35 **Peng W**, Yang Y, Lu H, Shi H, Jiang L, Liao X, Zhao H, Wang W, Liu J. Network pharmacology combines machine learning, molecular simulation dynamics and experimental validation to explore the mechanism of acetylbinankadsurin A in the treatment of liver fibrosis. *J Ethnopharmacol* 2024; **323**: 117682 [*RCA*] [PMID: 38169205 DOI: 10.1016/j.jep.2023.117682] [FullText]

36 **Zhao M**, Wang L, Wang M, Zhou S, Lu Y, Cui H, Racanelli AC, Zhang L, Ye T, Ding B, Zhang B, Yang J, Yao Y. Targeting fibrosis, mechanisms and cilinical trials. *Signal Transduct Target Ther* 2022; **7**: 206 [*RCA*] [PMID: 35773269 DOI: 10.1038/s41392-022-01070-3] [Full Text] [Full Text(PDF)]

37 **Thomes PG**, Brandon-Warner E, Li T, Donohue TM Jr, Schrum LW. Rev-erb agonist and TGF-β similarly affect autophagy but differentially regulate hepatic stellate cell fibrogenic phenotype. *Int J Biochem Cell Biol* 2016; **81**: 137-147 [*RCA*] [PMID: 27840152 DOI: 10.1016/j.biocel.2016.11.007] [FullText]

38 **Kim JI**, Cheon HG. Melatonin ameliorates hepatic fibrosis *via* the melatonin receptor 2-mediated upregulation of BMAL1 and anti-oxidative enzymes. *Eur J Pharmacol* 2024; **966**: 176337 [*RCA*] [PMID: 38246330 DOI: 10.1016/j.ejphar.2024.176337] [FullText]

39 **Sato K**, Meng F, Francis H, Wu N, Chen L, Kennedy L, Zhou T, Franchitto A, Onori P, Gaudio E, Glaser S, Alpini G. Melatonin and circadian rhythms in liver diseases: Functional roles and potential therapies. *J Pineal Res* 2020; **68**: e12639 [*RCA*] [PMID: 32061110 DOI: 10.1111/jpi.12639] [FullText]

40 **Xu L**, Yang TY, Zhou YW, Wu MF, Shen J, Cheng JL, Liu QX, Cao SY, Wang JQ, Zhang L. Bmal1 inhibits phenotypic transformation of hepatic stellate cells in liver fibrosis *via* IDH1/α-KG-mediated glycolysis. *Acta Pharmacol Sin* 2022; **43**: 316-329 [*RCA*] [PMID: 33850278 DOI: 10.1038/s41401-021-00658-9] [FullText]

41 **Gallego-Durán R**, Hadjihambi A, Ampuero J, Rose CF, Jalan R, Romero-Gómez M. Ammonia-induced stress response in liver disease progression and hepatic encephalopathy. *Nat Rev Gastroenterol Hepatol* 2024; **21**: 774-791 [*RCA*] [PMID: 39251708 DOI: 10.1038/s41575-024-00970-9] [FullText]

42 **Yuan S**, Wei C, Liu G, Zhang L, Li J, Li L, Cai S, Fang L. Sorafenib attenuates liver fibrosis by triggering hepatic stellate cell ferroptosis *via* HIF-1α/SLC7A11 pathway. *Cell Prolif* 2022; **55**: e13158 [*RCA*] [PMID: 34811833 DOI: 10.1111/cpr.13158] [FullText] [Full Text(PDF)]

43 **Wang F**, Chen L, Kong D, Zhang X, Xia S, Liang B, Li Y, Zhou Y, Zhang Z, Shao J, Zheng S, Zhang F. Canonical Wnt signaling promotes HSC glycolysis and liver fibrosis through an LDH-A/HIF-1α transcriptional complex. *Hepatology* 2024; **79**: 606-623 [*RCA*] [PMID: 37733267 DOI: 10.1097/HEP.0000000000000569] [FullText] [Full Text(PDF)]

44 **Shi Y**, Lin Z, Zhang C, Cui J, Mao Y, Sun M, Zhao D, Nan Y, Sun D. 4EBP1 phosphorylation mediates TGF-β1-induced hepatic fibrosis and upregulates HIF-1α expression. *iScience* 2025; **28**: 113412 [*RCA*] [PMID: 41020147 DOI: 10.1016/j.isci.2025.113412] [FullText] [Full Text (PDF)]

45 **Zhu J**, Zhou T, Menggen M, Aimulajiang K, Wen H. Ghrelin regulating liver activity and its potential effects on liver fibrosis and Echinococcosis. *Front Cell Infect Microbiol* 2023; **13**: 1324134 [*RCA*] [PMID: 38259969 DOI: 10.3389/fcimb.2023.1324134] [FullText] [Full Text(PDF)]

46 **Xue T**, Yue L, Zhu G, Tan Z, Liu H, Gan C, Fan C, Su X, Xie Y, Ye T. An oral phenylacrylic acid derivative suppressed hepatic stellate cell activation and ameliorated liver fibrosis by blocking TGF-β1 signalling. *Liver Int* 2023; **43**: 718-732 [*RCA*] [PMID: 36448910 DOI: 10.1111/liv.15488] [FullText]

47 **Zhang R**, Li W, Jiang X, Cui X, You H, Tang Z, Liu W. Ferulic Acid Combined With Bone Marrow Mesenchymal Stem Cells Attenuates the Activation of Hepatic Stellate Cells and Alleviates Liver Fibrosis. *Front Pharmacol* 2022; **13**: 863797 [*RCA*] [PMID: 35721175 DOI: 10.3389/fphar.2022.863797] [FullText] [Full Text(PDF)]

48 **Tang R**, Sun T, Xing Z, Fan X, Jiang P, Le B, Jia K, Cai Y, Bi X, Zhang D, Lin R, He X. Glucocorticoid Receptor Inhibits the Progression of Schistosomiasis Hepatic Fibrosis Through Inducing Circadian Clock Gene Per1. *J Infect Dis* 2025; **232**: 901-911 [*RCA*] [PMID: 40183568 DOI: 10.1093/infdis/jiaf104] [FullText]

49 **Xiang D**, Zou J, Zhu X, Chen X, Luo J, Kong L, Zhang H. Physalin D attenuates hepatic stellate cell activation and liver fibrosis by blocking TGF-β/Smad and YAP signaling. *Phytomedicine* 2020; **78**: 153294 [*RCA*] [PMID: 32771890 DOI: 10.1016/j.phymed.2020.153294] [FullText]

50 **Shan L**, Liu Z, Ci L, Shuai C, Lv X, Li J. Research progress on the anti-hepatic fibrosis action and mechanism of natural products. *Int Immunopharmacol* 2019; **75**: 105765 [*RCA*] [PMID: 31336335 DOI: 10.1016/j.intimp.2019.105765] [FullText]

51 **Shen M**, Han W, Cai L, Gong W, Qian L. H3K9 acetylation-dependent CHAC1 transcription in dihydroartemisinin-induced hepatic stellate cell ferroptosis. *Chin Med J (Engl)* 2026; **139**: 93-107 [*RCA*] [PMID: 40947783 DOI: 10.1097/CM9.0000000000003788] [FullText] [Full Text (PDF)]

52 **Huan S**, Sun S, Song S, Dai J, Zhu G, Zhong Y, Ji Y, Zheng S, Yin G. Dihydroartemisinin inhibits the activation and proliferation of hepatic stellate cells by regulating miR29b3p. *Int J Mol Med* 2023; **51**: 40 [*RCA*] [PMID: 37026504 DOI: 10.3892/ijmm.2023.5243] [FullText]

53 **Hasbaoui BE**, Rifai Z, Saghir S, Ayad A, Lamalmi N, Abilkassem R, Agadr A. Congenital hepatic fibrosis: case report and review of literature. *Pan Afr Med J* 2021; **38**: 188 [*RCA*] [PMID: 33995794 DOI: 10.11604/pamj.2021.38.188.27941] [FullText] [Full Text(PDF)]

54 **Jokl E**, Llewellyn J, Simpson K, Adegboye O, Pritchett J, Zeef L, Donaldson I, Athwal VS, Purssell H, Street O, Bennett L, Guha IN, Hanley NA, Meng QJ, Piper Hanley K; ID-LIVER Consortium. Circadian Disruption Primes Myofibroblasts for Accelerated Activation as a Mechanism Underpinning Fibrotic Progression in Non-Alcoholic Fatty Liver Disease. *Cells* 2023; **12**: 1582 [*RCA*] [PMID: 37371052 DOI: 10.3390/cells12121582] [FullText]

55 **Yan M**, Cui Y, Xiang Q. Metabolism of hepatic stellate cells in chronic liver diseases: emerging molecular and therapeutic interventions. *Theranostics* 2025; **15**: 1715-1740 [*RCA*] [PMID: 39897543 DOI: 10.7150/thno.106597] [FullText] [Full Text(PDF)]

56 **Guo C**, Lai L, Ma B, Huang Q, Wang Z. Notoginsenoside R1 targets PPAR-γ to inhibit hepatic stellate cell activation and ameliorates liver fibrosis. *Exp Cell Res* 2024; **437**: 113992 [*RCA*] [PMID: 38492634 DOI: 10.1016/j.yexcr.2024.113992] [FullText]

57 **Sun M**, Wu X, Lin Z, Zhang C, Cui J, Mao Y, Shi Y, Zhang J, Nan Y. Investigation of HO-1 Regulation of Liver Fibrosis Related to Nonalcoholic Fatty Liver Disease Through the SIRT1/TGF-ß/Smad3 Pathway. *J Clin Transl Hepatol* 2025; **13**: 456-468 [*RCA*] [PMID: 40474887 DOI: 10.14218/JCTH.2024.00481] [FullText] [Full Text(PDF)]

58 **Luo N**, Zhong W, Li J, Lu J, Dong R. CRISPR/dCas9 for hepatic fibrosis therapy: implications and challenges. *Mol Biol Rep* 2022; **49**: 11403-11408 [*RCA*] [PMID: 35960410 DOI: 10.1007/s11033-022-07713-6] [FullText]

59 **Wang S**, Li K, Pickholz E, Dobie R, Matchett KP, Henderson NC, Carrico C, Driver I, Borch Jensen M, Chen L, Petitjean M, Bhattacharya D, Fiel MI, Liu X, Kisseleva T, Alon U, Adler M, Medzhitov R, Friedman SL. An autocrine signaling circuit in hepatic stellate cells underlies advanced fibrosis in nonalcoholic steatohepatitis. *Sci Transl Med* 2023; **15**: eadd3949 [*RCA*] [PMID: 36599008 DOI: 10.1126/scitranslmed.add3949] [FullText]

60 **Peng L**, Xiang S, Wang T, Yang M, Duan Y, Ma X, Li S, Yu C, Zhang X, Hu H, Liu Z, Sun J, Sun C, Wang C, Liu B, Wang Z, Qian M. The hepatic clock synergizes with HIF-1α to regulate nucleotide availability during liver damage repair. *Nat Metab* 2025; **7**: 148-165 [*RCA*] [PMID: 39775529 DOI: 10.1038/s42255-024-01184-8] [FullText]

61 **Yan R**, Cai H, Zhou X, Bao G, Bai Z, Ge RL. Hypoxia-inducible factor-2α promotes fibrosis in non-alcoholic fatty liver disease by enhancing glutamine catabolism and inhibiting yes-associated protein phosphorylation in hepatic stellate cells. *Front Endocrinol (Lausanne)* 2024; **15**: 1344971 [*RCA*] [PMID: 38501098 DOI: 10.3389/fendo.2024.1344971] [FullText] [Full Text(PDF)]

62 **Huang R**, Cui H, Yahya Ali Alshami MA, Fu C, Jiang W, Cai M, Zhou S, Zhu X, Hu C. LOX-1 rewires glutamine ammonia metabolism to drive liver fibrosis. *Mol Metab* 2025; **96**: 102132 [*RCA*] [PMID: 40180177 DOI: 10.1016/j.molmet.2025.102132] [FullText] [Full Text(PDF)]

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REVIEW

Second-line treatment of primary biliary cholangitis: To whom, which molecule, and when

Sylvia Drazilova, Peter Jarcuska, Tomas Koky, Martin Harhovsky, Slavomira Komarova, Jan Vasil, Martin Janicko

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Abstract

Ursodeoxycholic acid (UDCA) is the first-line treatment of primary biliary cholangitis (PBC). Approximately two-thirds of patients have a biochemical response to UDCA, but only one-third of treated patients achieve a complete biochemical response. UDCA nonresponse is associated with hepatic decompensation and higher liver-related mortality; therefore, UDCA nonresponders should be considered for second-line PBC treatment. Nonfibrate peroxisome proliferator-activated receptor (PPAR) agonists elafibranor and seladelpar are approved for second-line PBC treatment in UDCA nonresponders and in UDCA treatment intolerance. Fibrates are an alternative second-line PBC therapy, but they are not approved for second-line treatment. A significant benefit of PPAR receptor agonists therapy is the alleviation of pruritus in some patients. Second-line PBC treatment is usually indicated after 1 year of UDCA therapy; however, it may be considered after 6 months of UDCA treatment in high-risk PBC patients. The selection of drug for second-line PBC treatment and the timing of therapy initiation should be individualized. Many factors, including PBC complications, especially pruritus, should also be considered in the decision-making algorithm. It will be necessary in the coming years to evaluate the efficacy of PPAR agonists in real-world clinical practice, including monitoring the incidence of decompensated liver cirrhosis and liver-related mortality.

Key Words: Primary biliary cholangitis; Ursodeoxycholic acid; Second-line treatment; Elafibranor; Seladelpar; Therapeutic response

Core Tip: Primary biliary cholangitis (PBC) is a disease with increasing prevalence, particularly in developed countries. First-line treatment of choice is ursodeoxycholic acid (UDCA), but only about two-thirds of patients achieve biochemical response. UDCA nonresponders have a higher risk of liver cirrhosis decompensation and poorer transplant-free survival. These patients should be considered for second-line therapy. This review provides a comprehensive overview of second-line treatment indications, selection of the most appropriate medication, timing of therapy initiation, benefits, and adverse effects of therapy, as well as the administration of second-line treatment in specific groups of PBC patients.

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INTRODUCTION

Primary biliary cholangitis (PBC) is a nonsuppurative autoimmune liver disease. PBC is associated with destruction of the bile ducts, and liver fibrosis. PBC progresses to liver cirrhosis in some patients, and hepatocellular carcinoma is a rare complication in PBC[1,2]. PBC is a rare disease that occurs significantly more often in women. The PBC prevalence and incidence in recent years have been increasing in developed countries[3,4]. Two of the three criteria listed below are required to diagnose PBC: Increased alkaline phosphatase (ALP) above the upper limit of the normal lasting for ≥ 6 months; AMA M2 positivity in a titer of at least 1: 40 or specific ANA positivity (anti-sp100 or anti-gp210) in case of AMA negativity; histological findings consistent with PBC[1].

PBC may overlap with other autoimmune liver diseases including primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). These patients have a worse clinical course than patients with PBC alone. Patients with PBC/AIH overlap must meet the histological criteria for both diseases[5,6]. Pruritus, fatigue, dyslipidemia, and osteoporosis are the most common extrahepatic manifestations of PBC, and their presence worsens the quality of life of PBC patients[7].

The goals of PBC treatment include: (1) improvement of biochemical findings; (2) improvement of histological findings (reduction of inflammation and fibrosis); (3) reduction in the incidence of liver cirrhosis and decompensated liver cirrhosis; (4) reduction in the incidence of hepatocellular carcinoma; (5) improvement in the course of extrahepatic complications or their reduction; (6) improvement in quality of life; and (7) improvement in survival[8].

Today, we have effective treatments for PBC, which should be started immediately after diagnosis. If first-line treatment for PBC fails, second-line treatment should be considered. This is a narrative review summarizing current and emerging second-line therapies for PBC, with emphasis on patient selection, drug choice and timing of treatment escalation.

FIRST-LINE TREATMENT OF PBC

Ursodeoxycholic acid (UDCA) is the drug of choice in the first-line treatment of PBC. UDCA has been used in the treatment of PBC for almost four decades[9]. UDCA is a 7-β-epimer of chenodeoxycholic acid with hepatoprotective and choleretic effects[10]. UDCA reduces the absorption of toxic hydrophobic endogenous bile acids, stabilizes the hepatocyte membrane against toxic bile acid salts, and suppresses interleukin (IL)-2 production. During treatment, UDCA partially replaces hepatotoxic cholic, chenodeoxycholic, deoxycholic, and lithocholic acids. UDCA levels in bile correlate with biochemical findings in PBC patients[11]. UDCA leads to a reduction in fibrogenesis in the liver[12]. Treatment with UDCA in PBC patients is associated with a reduction in total cholesterol (TC) and low-density cholesterol (LDL-C); high-density cholesterol (HDL-C) and triglyceride (TG) levels do not change significantly during treatment[13].

The recommended dose of UDCA for the treatment of PBC is 13-15 mg/kg/day[1]. PBC patients tolerate UDCA treatment well. Rare adverse effects include pruritus, which is usually transient, diarrhea, and upper right abdominal quadrant pain[14]. Reduction of UDCA dose or premature discontinuation of treatment is rare[15].

An analysis of the Global PBC Study Group database, which included 3902 patients (90.4% were treated with UDCA) with mean follow-up of 7.8 (4.1-12.1) years, showed that UDCA improved transplant-free survival compared to untreated patients [hazard ratio (HR): 0.46; 95% confidence interval (CI): 0.40-0.52; $P < 0.001$]. Patients who had an inadequate response to UDCA treatment after 1 year of treatment had a significantly better transplant-free survival rate compared to the untreated patients (HR: 0.56; 95%CI: 0.45-0.69; $P < 0.001$)[16].

The definition of therapeutic response to UDCA in PBC patients is not clearly defined. Biochemical response to UDCA is evaluated by different qualitative binary scoring systems using simple laboratory parameters. Apart from the EHIME and Rotterdam Scoring System, these scoring systems evaluate ALP as a marker of cholestasis, and approximately half of them also evaluate bilirubin levels when assessing therapeutic response. The Toronto criteria are the most used in clinical practice. Approximately 70% of PBC patients treated with UDCA achieve a biochemical response according to the modified Toronto criteria: ALP ≤ 1.67 × upper limit of normal (ULN) after 1 year, and approximately one-third of patients have normal bilirubin and ALP levels[4]. Patients who achieve low ALP and bilirubin levels during UDCA treatment

have the best outcome. In an evaluation of > 2000 patients from the Global PBC Study Group database, the best survival rates were seen in patients who had normal ALP and bilirubin ≤ 0.6 × ULN after 1 year of UDCA treatment[17]. An overview of the most used qualitative binary scoring systems is provided in Table 1[17-26].

Carbone *et al*[27] constructed the UDCA Response Score, which uses parameters prior to the start of UDCA treatment. The authors included the following parameters in the scoring system: Age; bilirubin; aminotransferases; ALP, time from diagnosis to treatment; and worsening of ALP in the time interval between diagnosis and treatment. Modified Toronto criteria (ALP ≤ 1.67 × ULN after 1 year of treatment) were used to evaluate therapeutic response. The area under the receiver operating characteristic for the UDCA response score after 1 year of UDCA treatment was 0.83 (95%CI: 0.79-0.87).

Continuous scoring systems predict therapeutic response and patient prognosis more accurately than qualitative binary scoring systems. UK-PBC Risk Score uses the following variables: Pretreatment albumin and thrombocytes; 12 months post-treatment bilirubin; aminotransferase levels; and ALP[28]. GLOBE score evaluates pretreatment age and 12 months post-UDCA treatment bilirubin, ALP, albumin, and platelets[29]. GLOBE score and UK-PBC Risk Score are the most suitable for the real-world allocation of second-line therapies. However, ALP and total bilirubin normalization should be the primary outcome in clinical trials in PBC[30].

However, biochemical improvement in PBC patients does not correspond to histological improvement during long-term UDCA treatment. Paired liver biopsies showed that only a quarter of patients with a biochemical response according to the Toronto criteria had an improvement in fibrosis stage after 10 years of treatment. A quarter of patients had progression of fibrosis despite a biochemical response and approximately half had no change in fibrosis stage. Almost all UDCA nonresponders experienced fibrosis progression after 10 years[24].

Patients who do not achieve a biochemical response to UDCA treatment have a poorer prognosis and progress significantly more often to decompensated liver cirrhosis[4]. Findings of advanced fibrosis or even liver cirrhosis are associated with poor response to UDCA[24]. The presence of anti-sp 100 or anti-gp 210 antibodies is associated with poorer prognosis[31,32]. Younger PBC patients have poorer response to UDCA therapy and lower transplant-free survival compared to older patients[33]. Patients with PBC/AIH overlap have a poorer response to UDCA and a worse prognosis than patients with PBC alone[34].

Some factors are associated with poorer clinical course in treated PBC patients. As described above, nonresponders to UDCA treatment, patients with overlap PBC/AIH and young women with PBC have a worse prognosis. Histopathological or elastographic findings of advanced fibrosis or even liver cirrhosis are associated with poor response to UDCA and poor prognosis[24,35]. Fibrosis stage is an independent predictor of the outcome in PBC despite biochemical treatment response[36]. Transient elastography is an excellent method for assessing fibrosis stage. In a meta-analysis of 13 studies of PBC patients, sensitivity and specificity were estimated to be 0.76 and 0.93, 0.88 and 0.9, and 0.91 and 0.95 for ≥ F2, ≥ F3, and ≥ F4, respectively[37]. The 8- and 15-kPa cutoffs in vibration-controlled transient elastography selected low-, medium-, and high-risk groups of PBC patients[38]. Ductopenia, found by histological examination, is an independent risk factor for the occurrence of liver-related adverse events in PBC patients[39]. In men with PBC, the disease is diagnosed later than in women due to the absence of clinical symptoms. Men with PBC have a worse clinical course than women have[40,41]. However, the response to UDCA treatment is comparable in both sexes[33].

SECOND-LINE TREATMENT IN PBC

Progression of fibrosis and worse prognosis are observed in PBC complete and partial nonresponders to UDCA treatment. The loss of biochemical response to UDCA at any time is associated with worse prognosis in PBC patients[42]. These patients are indicated for the second-line PBC treatment. The most promising drugs in second-line treatment of PBC are peroxisome proliferator-activated receptor (PPAR) agonists, including fibrates which are still used off-label in this indication.

PPAR agonists in the second-line treatment of PBC

Nonfibrate PPAR agonists and fibrates are used in the management of PBC. Nonfibrate PPAR agonists include elafibranor, seladelpar, and saroglitazar, while fibrates used in the treatment of PBC include bezafibrate, fenofibrate, and ciprofibrate[43,44]. PPAR agonists, after binding to their ligands, form heterodimers with the retinoid X receptor and interact with specific DNA sequences to regulate the expression of target genes. Three isotypes of PPAR agonists may be identified: PPAR-α, PPAR-γ, and PPAR-β/δ agonists. PPAR-α agonists act on hepatocytes, reduce inflammation, regulate lipid and bile acid metabolism, and reduce liver fibrosis in rat models. PPAR-γ agonists act on Kupfer cells, reduce inflammation and liver fibrosis, but may increase fat accumulation. PPAR-β/δ agonists act on hepatocytes, Kupfer cells, hepatic stellate cells, and cholangiocytes; reduce inflammation and fat accumulation; and protect against carcinogenesis. Table 2 describes PPAR agonists used in the treatment of PBC.

Nonfibrate PPAR agonists in second-line treatment of PBC

Elafibranor is a pan-PPAR agonist. It led to an ~50% decrease in ALP after 12 weeks of treatment at a dose of 80 or 120 mg/day in a Phase 2 clinical study. The therapy was well tolerated[45]. Elafibranor at a dose of 80 mg daily was added to UDCA in nonresponders to UDCA treatment in the Phase 3 clinical trial ELATIVE. This was a randomized, double-blind, placebo-controlled trial where the primary outcome was defined as follows: ALP < 1.67 × ULN, with a decrease of ≥ 15% from baseline; and a normal total bilirubin level, assessed after 52 weeks of treatment. Primary endpoint was reached in 51% of patients treated with elafibranor and in 4% of patients in the placebo arm (P < 0.001). ALP normalization was present in 15% of patients in the elafibranor group and in any patient from the placebo group (P

Table 1 Overview of the most commonly used qualitative binary scoring systems in primary biliary cholangitis patients treated with ursodeoxycholic acid

Name of scoring system	Duration of treatment (mo)	Type of PBC population	UDCA treatment response definition	Ref.
Rochester	6	American	ALP < 2 × ULN and Mayo Risk Score < 4.5	[18]
Barcelona	12	European	Decrease in ALP > 40% or ALP ≤ × 1 ULN	[19]
Paris-I	12	European	ALP < 3 × ULN and AST < 2 × ULN and bilirubin ≤ 1 mg/dL	[20]
Paris-II	12	European	ALP ≤ × 1.5 ULN and AST ≤ × 1.5 ULN and bilirubin ≤ 1 × ULN	[21]
Rotterdam	12	European	Bilirubin ≤ 1 × ULN and albumin ≥ 1 × ULN	[22]
Ehime	6	Asian	Decrease in GGT > 70% or GGT ≤ 1 × ULN	[23]
Toronto	24	American	ALP ≤ 1.67 × ULN	[24]
Xi'an	1	Asian	ALP ≤ 2.5 × ULN, AST ≤ 2 × ULN and total bilirubin ≤ 1 × ULN	[25]
Monza	12	European	GGT < 3.2 × ULN or ALP < 2 × ULN	[26]
Global PBC study group	12	International	Bilirubin ≤ 0.6 × ULN and ALP ≤ 1 × ULN	[17]

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; GGT: Gamma glutamyl transferase; mo: Months; PBC: Primary biliary cholangitis; UDCA: Ursodeoxycholic acid; ULN: Upper limit of normal.

= 0.002). The intensity of pruritus in patients with baseline moderate-to-severe pruritus was comparable in both groups at the end of treatment[46]. Interim results from the open label extension up to 3 years showed sustained biochemical response in elafibranor-treated PBC patients. The intensity of pruritus was reduced in patients who were switched from placebo to elafibranor after 52 weeks of follow-up. Long-term treatment with elafibranor led to further significant improvement in fatigue and sleep disorders in patients with baseline moderate to severe fatigue or excessive sleepiness [47].

Seladelpar is a PPAR-β/δ agonist that has demonstrated clinical efficacy in UDCA nonresponders in a Phase 2 clinical trial[48]. The registration Phase 3, multicenter, double-blind, randomized, placebo-controlled clinical trial also included nonresponders to UDCA. Seladelpar was added to UDCA treatment at a dose of 10 mg daily. The primary objective was to achieve a biochemical response after 12 months of treatment. The biochemical response was defined in the same way as in the ELATIVE trial with elafibranor. Secondary objectives were normalization of ALP after 12 months of treatment and improvement in itching intensity. The primary outcome was achieved by 61.1% of patients treated with seladelpar and in 20% of patients in the placebo group ($P < 0.001$). ALP normalization was achieved by a quarter of patients in the seladelpar group, but by none of the patients in the placebo group ($P < 0.001$). Treatment with seladelpar led to a significant reduction in pruritus intensity[49]. The effect of seladelpar in second-line treatment of PBC lasts a long time [50]. After 30 months of treatment, 81% of patients treated with seladelpar achieved a biochemical response and 41% of patients achieved normalization of ALP[51]. Real-world clinical experience confirmed the efficacy of seladelpar as a second-line treatment for PBC. More than half of the patients achieved a biochemical response, and nearly one-third of the patients had normalized ALP levels after just 1 month of treatment with seladelpar[52].

A beneficial effect of seladelpar treatment is a reduction in pruritus intensity, which was associated with a decrease in IL-31 and serum bile acid levels[53]. Seladelpar treatment for PBC led to a reduction in pruritus intensity, and to improved sleep. No patient treated with seladelpar developed *de novo* pruritus[54]. Seladelpar also reduces fatigue in PBC patients[54,55].

Elafibranor and seladelpar were approved by the Food and Drug Administration and European Medicines Agency (EMA) for second-line PBC treatment.

Saroglitazar is a dual PPAR agonist (α and γ). It is approved for treatment of metabolic dysfunction-associated steatotic liver disease and noncirrhotic metabolic dysfunction-associated steatohepatitis in India. The cost of the drug is relatively low[56]. Saroglitazar was evaluated for second-line treatment of PBC in a Phase 2 clinical trial. Treatment with saroglitazar at a dose of 2 or 4 mg led to a rapid decrease in ALP after 4 weeks of treatment and persisted at the end of the study after 16 weeks of treatment. In four of 17 patients, treatment was discontinued prematurely due to aminotransferase elevation, which promptly resolved after discontinuation of treatment[57].

Fibrates in the second-line treatment of PBC

Fibrates are a group of drugs developed for the treatment of dyslipidemia. They have a strong agonistic effect on PPAR-α, leading to a significant reduction in serum TG levels and a slight increase in HDL-C. This is associated with the cardioprotective effect of fibrates. Creatinine increase is the most common adverse effect of fibrate treatment, occurring mainly in diabetics, elderly patients, men, and patients with cardiovascular disease[58]. Fibrates, like statins, have many pleiotropic effects. One of the most important is the reduction of cholestasis, hepatic inflammation, and fibrosis[8].

Table 2 Overview of peroxisome proliferator-activated receptor agonists used in the treatment of primary biliary cholangitis

Name of PPAR agonist	Fibrate/non-fibrate	Isotypes of PPAR	Status of regulatory approval	Clinical results
Elafibranor	Non-fibrate	PPAR-α and PPAR-β/δ	Approved	Improvement of cholestasis
				Biochemical response and ALP normalization
				Reduction of pruritus
				Improvement of fatigue and sleep disturbance
Seladelpar	Non-fibrate	PPAR-β/δ	Approved	Improvement of cholestasis
				Biochemical response
				ALP normalization
				Reduction of pruritus
				Improvement of fatigue and sleep disturbance
Saroglitazar	Non-fibrate	PPAR-α and PPAR-γ	Phase 3 study complete	Improvement of cholestasis
				Biochemical response and ALP decrease
Bezafibrate	Fibrate	PPAR-α, PPAR-γ and PPAR-β/δ	Off-label	Improvement of cholestasis
				Biochemical response and ALP normalization
				Reduction of pruritus
Fenofibrate	Fibrate	PPAR-α	Off-label	Improvement of cholestasis
				Biochemical response and ALP normalization
				Reduction of pruritus

ALP: Alkaline phosphatase; PPAR: Peroxisome proliferator-activated receptor.

Corpechot *et al*[59] conducted the double-blind, placebo-controlled, randomized BEZURSO trial comparing combination therapy with UDCA + bezafibrate at a dose of 400 mg daily *vs* UDCA + placebo in PBC nonresponders to first-line UDCA treatment. The duration of the study was 24 months. The primary outcome was a complete biochemical response (CBR), which was defined as normal levels of total bilirubin, ALP, aminotransferases, and albumin, as well as a normal prothrombin time at the end of follow-up. The primary outcome was achieved in 31% of patients in the bezafibrate *vs* 0% patients in the placebo group ($P < 0.001$). ALP normalization at the end of treatment was observed in 67% of patients treated with bezafibrate *vs* 2% of patients in the placebo group. Creatinine level increase and myalgia were the most common adverse effects during treatment with bezafibrate[59].

A meta-analysis comparing combined treatment with UDCA + bezafibrate with UDCA alone, which included the BEZURSO trial and several Japanese studies, was recently published[59-65]. Combined treatment with UDCA and bezafibrate significantly reduced ALP and-glutamyl transferase (GGT) levels, but not total bilirubin and IgM, compared with UDCA monotherapy. Insufficient data were available to assess mortality[60]. Data from the Japanese National PBC Database showed that long-term combination therapy with UDCA + bezafibrate reduces all-cause and liver-related mortality approximately threefold compared to UDCA monotherapy[66].

Nonresponse to combined treatment with UDCA and bezafibrate in PBC patients is associated with a higher incidence of decompensation of liver cirrhosis[67].

Bezafibrate is not available in some countries; a possible alternative is the use of fenofibrate in second-line treatment of PBC when bezafibrate is not available. A low dose of fenofibrate (80 mg daily) was comparably effective in treating PBC in one small study compared to bezafibrate at a dose of 200 mg daily[68]. Chinese authors compared combination therapy with UDCA and fenofibrate *vs* UDCA monotherapy. Patients receiving combination therapy achieved normalization of ALP and a CBR; defined as normalization of ALP, GGT, and bilirubin, after 1 year of treatment significantly more often compared to PBC patients treated with UDCA monotherapy[69].

A meta-analysis showed that combined treatment with UDCA + fibrate improves laboratory findings and pruritus compared to UDCA monotherapy. Combined treatment does not result in more frequent adverse effects[70]. Despite the beneficial effect of fibrates on biochemical findings in PBC patients and on mortality data in multiple studies, fibrates have not yet been approved for the treatment of PBC and their use remains off-label.

Obeticholic acid in the second-line treatment of PBC

Obeticholic acid (OCA) is semisynthetic derivate of chenodeoxycholic acid (6α-ethyl-chenodeoxycholic acid), it acts as an agonist of the farnesoid X receptor (FXR). OCA regulates impaired enterohepatic circulation and bile acid composition through FXR activation[71]. OCA activates sinusoidal endothelial cells in the liver and Kupffer cells, leading to an anti-inflammatory effect and, through reduced stellate cell activity, also to an antifibrotic effect[71,72]. OCA modulates fibroblast growth factor (FGF)-19, which is associated with hepatoprotective and anticholestatic effects[73].

PBC nonresponders to UDCA were enrolled in the POISE Phase 3 registration study. One group was treated with a combination of OCA at a dose of 5-10 mg daily + UDCA, while the control group received UDCA + placebo. The primary outcome was the same as in the ELATIVE study mentioned above and was achieved by almost half of the patients treated with OCA + UDCA and only by 10% of patients in the placebo control group ($P < 0.001$). The most serious adverse effect of OCA treatment was pruritus. Patients treated with OCA suffered from pruritus more frequently, with pruritus being more common at a dose of 10 mg/day than at 5 mg/day[74]. The biochemical response to OCA treatment persisted after 4 years of treatment, but paired biopsies showed improvement in fibrosis stage in approximately one quarter of patients and worsening in approximately another quarter of patients, with no change in fibrosis stage after 3 years in half of the treated patients[75,76].

Three randomized placebo-controlled studies were included in a meta-analysis[74,77,78] documenting the superiority of combined OCA + UDCA treatment compared to UDCA and placebo in achieving therapeutic response. Combined treatment led to partial correction of dyslipidemia[79].

Data from real-world clinical practice using OCA as second-line treatment for PBC showed poorer results, especially in patients with compensated liver cirrhosis, and confirmed the frequent occurrence of pruritus in treated patients[80-83].

OCA is contraindicated in advanced cirrhosis because it increases the risk of decompensation of liver cirrhosis and liver-related mortality[2,84].

COBALT, a double-blind, randomized, Phase 3b/4 clinical trial compared UDCA nonresponders who were treated with OCA and placebo. The primary composite endpoint was time to death, liver transplant, model for end-stage liver disease score ≥ 15, uncontrolled ascites, or hospitalization for hepatic decompensation. The study did not demonstrate a difference in the achievement of the primary endpoint between the two groups of PBC patients. Among PBC patients in the COBALT trial treated with OCA, the primary endpoint occurred less frequently compared to the external control arm from the Komodo Healthcare Map database: PBC patients who did not respond to UDCA treatment and were not treated with OCA (HR = 0.39; 95%CI: 0.22-0.69; $P = 0.001$)[85]. However, a limitation of the COBALT trial was its short duration and small number of enrolled patients (< 170 in each trial arm). Another significant limitation of the study was the advanced stage of the disease at the time of enrollment in the OCA arm. The original inclusion criteria were mean ALP > 5 × ULN and mean total bilirubin > ULN and ≤ 3 × ULN. Subsequently, these criteria were revised to ALP > 3 × ULN and mean total bilirubin > ULN and ≤ 5 × ULN. All patients treated with OCA in the COBALT trial had elevated baseline bilirubin levels, which are associated with a poorer biochemical response to OCA treatment[86]. The HEROES trial used real clinical data from the Komodo Healthcare Map and demonstrated superiority of the OCA treatment compared to non-OCA treatment. OCA treatment reduced the risk of hospitalization for hepatic decompensation, liver transplantation, or death by 63% compared to untreated patients[87].

OCA is not currently a routine second-line option, as the adverse effects of treatment may outweigh its benefits, especially in patients with advanced PBC. Based on the results of the COBALT trial, EMA revoked the conditional marketing authorization for OCA. The European Union General Court upheld the revocation of the conditional authorization of OCA on November 26, 2024. OCA is currently officially listed in the Union-register of nonactive medicinal products. OCA was officially withdrawn from the US market on November 14, 2025. OCA remains licensed and available in many other regions, including Canada (conditional notice of compliance was granted by Health Canada on May 25, 2017) and the UK (conditional marketing authorization was recommended by National Institute for Health and Care Excellence on March 2, 2017). Second-line treatment for PBC should begin with PPAR agonists in these countries as well.

Budesonide in second-line treatment of PBC

Budesonide is a combined glucocorticoid receptor and pregnane X receptor agonist with higher affinity for the glucocorticoid receptor compared to prednisolone[88]. PBC nonresponders to UDCA with liver inflammatory activity confirmed by liver biopsy treated with budesonide achieved biochemical response more frequently than nonresponders treated with placebo[89]. Combined treatment with UDCA + budesonide is recommended in first line for patients with PBC/AIH overlap based on the results of a meta-analysis published by Chinese researchers[90]. The initial dose of budesonide for PBC/AIH overlap syndrome is 9 mg daily (divided into three doses) in combination with UDCA and azathioprine. Once biochemical remission is achieved, the dose may be reduced to 3 mg over a period of 6 months. In cases of long-term remission, budesonide may be withdrawn while continuing treatment with UDCA and azathioprine; however, this approach will need to be confirmed in clinical studies[91].

Novel molecules in second-line treatment of PBC

Several molecules are being studied as second-line treatment for PBC: Nonsteroidal FXR agonists, nor-UDCA, fibroblast growth factor 19 (FGF-19) modulators, and dual nicotinamide adenine dinucleotide phosphate oxidase 1/4 (NOX 1/4) inhibitors. Tropifexor and cilofexor are nonsteroidal FXR agonists, which improve cholestasis in the second-line PBC treatment. Common adverse effects that limit the potential use of both molecules are pruritus and atherogenic dyslipidemia, which are dose dependent. TQA3526 and ASC42 are other promising molecules for the treatment of PBC from this group[92]. Nor-UDCA is a side-chain-shortened derivative of UDCA; it has anti-inflammatory, anti-cholestatic, and anti-fibrotic properties[93]. FGF-19 analogs decrease toxic bile acids production and have anti-inflammatory, anticholes-

static and antifibrotic properties without pruritogenic effect[94].

Setanaxib is a dual NOX 1/4 inhibitor that improves cholestasis and alleviates fatigue in patients with PBC[95]. Patients treated with setanaxib experienced an improvement in quality of life compared with those receiving placebo[96].

An overview of drugs in clinical trials for PBC is summarized in Table 3. Phase 3 clinical trials are required for these drugs to be incorporated into clinical practice.

Personalized second-line treatment for PBC

Elafibranor and seladelpar are approved second-line options for selected patients with an inadequate response or intolerance to UDCA. Their approval is based primarily on biochemical endpoints; therefore, long-term data on clinical outcomes remain important.

Indirect comparisons suggest potential differences between agents. Elafibranor seems to be more effective in achieving a biochemical response, while seladelpar shows particularly consistent antipruritic effects; however, there is a lack of head-to-head comparative studies[97]. Data from real-world clinical practice in the future will need to be analyzed to confirm these preliminary findings. Cost-effectiveness, drug tolerance, and adverse effects will play a crucial role in the selection of a nonfibrate PPAR agonist for second-line treatment of PBC.

Fibrates are used as an off-label treatment, but may be indicated as a second-line therapy in low-income countries; countries where elafibranor or seladelpar are not available; and countries where the cost of these drugs is not reimbursed. Bezafibrate is the preferred fibrate PPAR agonist; if it is unavailable, fenofibrate or ciprofibrate may be administered as second-line treatment for PBC[43]. It should be noted that although fibrates have an anticholestatic and likely also an antipruritic effect, this is an off-label treatment with potential adverse effects - most commonly renal insufficiency and myalgia.

The question of how to manage PBC patients who do not achieve a therapeutic response to second-line treatment with PPAR agonists remains unanswered. A combination of UDCA, OCA, and PPAR agonists appears promising. Treatment with UDCA-OCA-fibrate led to normalization of ALP levels in 30% of nonresponders to first-line UDCA therapy after 6 months. The treatment was also effective in older patients. Triple therapy alleviated pruritus in 65% of patients, was associated with worsening of the pruritus in 12% of patients, and the remaining patients reported no change in the intensity of pruritus[98]. Another multicenter study evaluated the combination therapy of OCA and fibrates in nonresponders to second-line PBC treatment. In PBC patients with failure of second-line OCA therapy, addition of a fibrate (bezafibrate or fenofibrate) led to a significant decrease in ALP. In contrast, addition of OCA in nonresponders to fibrates did not significantly affect ALP levels. The results of this study may significantly limit the future use of OCA in patients who do not respond to UDCA and fibrate[99]. Recent observations suggest that seladelpar may improve cholestasis and pruritus in OCA or fibrate-experienced PBC patients[100].

Disease stage, PBC complications, comorbidities, and potential drug-drug interactions also play a role in the selection of second-line PBC treatment. OCA and nonfibrate PPAR agonists are contraindicated in decompensated liver cirrhosis[2,84,101]. Although there have been reports about the benefits of using fibrates in decompensated cirrhosis in PBC patients, no studies on the efficacy and safety of fibrates for this indication are available[102,103]. Nephrotoxicity is a serious adverse effect of fibrates and may limit their use in patients with advanced liver cirrhosis[59,102]. PBC patients with decompensated cirrhosis should be placed on the waiting list for liver transplantation. Prophylactic administration of UDCA following liver transplantation for PBC reduces the risk of recurrence of PBC and improves the prognosis of transplant recipients[104,105].

Although head-to-head comparisons of the antipruritic effects of PPAR agonists are lacking, available clinical data have described a strong antipruritic effect of seladelpar[97]. Ileal bile acid transporter (IBAT) inhibitors prevent reabsorption of bile acids from the intestine and decrease bile acid enterohepatic circulation. Linerixibat and maralixibat show promising antipruritic effects[106,107]. Treatment with linerixibat was associated with an improvement in pruritus without worsening of ALP levels in the randomized, multicenter, double-blind, placebo-controlled Phase 3 GLISTEIN trial. Nearly two-thirds of patients treated with linerixibat experienced gastrointestinal adverse effects, primarily diarrhea and abdominal pain, and 7% of patients discontinued treatment early due to adverse effects[108]. The discovery of a potent antipruritic effect in nonfibrate PPAR agonists and IBAT inhibitors will reduce the future use of currently prescribed pruritus-relieving medications (cholestyramine, rifampicin, naltrexone, and sertraline)[7].

Fibrates are used not only to treat PBC, but also to treat atherogenic dyslipidemia, which is often associated with PBC [7,109]. The use of statins in PBC has a protective effect on the prevalence of hepatic decompensation[110]. Statins have a low incidence of drug-induced liver injury and are safe for patients with PBC[110,111]. Treatment of osteoporosis in PBC is the same as in patients without PBC, and bisphosphonates are the drugs of choice[1].

UDCA is a safe medication during pregnancy, and the clinical course of PBC stabilizes or even improves during pregnancy[112]. Fibrates, elafibranor, and seladelpar are not generally recommended during pregnancy because of insufficient safety data[113-116].

Clinical aspects of second-line treatment for PBC patients

Treatment with PPAR agonists leads to an improvement in biochemical parameters: A decrease or even normalization of ALP and normalization of bilirubin; therefore, the evidence for newer drugs is currently based on surrogate endpoints. To date, there is no objective evidence linking treatment with PPAR agonists to improvements in histological findings, a reduction in the incidence of decompensated liver cirrhosis, and a decrease in liver-related mortality. To definitively assess the clinical efficacy of these drugs, long-term clinical trials will be needed that focus on laboratory parameters but primarily on the clinical endpoints mentioned above.

Table 3 Overview of drugs in clinical trials for primary biliary cholangitis

Drug name	Drug class	Therapeutic indication	Study phase
Tropifexor	FXR agonist	PBC	Phase 2 complete
Cilofexor	FXR agonist	PBC	Phase 2 complete
TQA3526	FXR agonist	PBC	Phase 3 ongoing
ASC42	FXR agonist	PBC	Phase 2 closed prematurely
Nor-UDCA	UDCA homolog	PBC	Phase 2 ongoing
Aldafermin	FGF-19 analogue	PBC	Phase 2 complete
Setanaxib	NOX 1/4 inhibitor	PBC	Phase 2b complete
Linerixibat	IBAT inhibitor	Pruritus in PBC	Phase 3 complete; FDA approved for pruritus in PBC
Maralixibat	IBAT inhibitor	Pruritus in PBC	Phase 2 complete
Volixibat	IBAT inhibitor	Pruritus in PBC	Phase 2b ongoing

FDA: Food and drug administration; FGF: Fibroblast growth factor; FXR: Farnesoid X receptor; IBAT: Ileal bile acid transporter; NOX1/4: Dual nicotinamide adenine dinucleotide phosphate oxidase 1/4; PBC: Primary biliary cholangitis; UDCA: Ursodeoxycholic acid.

Patients receiving second-line treatment for PBC must be carefully monitored before and during treatment. Liver biochemistry bilirubin, total protein, albumin, renal parameters, creatine kinase, lipoprotein metabolism parameters, and blood count should be monitored regularly. The therapeutic response is evaluated every 6 months. An elastography examination should be performed once a year; an abdominal ultrasound should be performed every six months in patients with compensated cirrhosis; and a densitometry examination should be performed once every 2 years. It is important to actively look for clinical manifestations of the disease, such as pruritus and fatigue, as well as for treatment-related complications, such as myalgia. Decompensated cirrhosis should prompt transplant evaluation and specialist management rather than routine pharmacological escalation.

When should second-line PBC treatment be indicated?

Approximately one-third of PBC patients treated with UDCA achieve a CBR with normalization of ALP and bilirubin. Although another third of treated patients do not achieve a CBR, they do achieve a biochemical response according to the Toronto criteria, which can be described as an incomplete UDCA response[15]. Nonresponders to UDCA, according to the Toronto criteria, have poorer transplant-free survival compared to UDCA responders[117]. A loss of biochemical response at any time during UDCA treatment is associated with a poorer prognosis in PBC patients[42].

Second-line treatment for PBC is typically initiated in patients who have not responded to UDCA therapy after 12 months or in patients who did not tolerate UDCA therapy. However, in some UDCA nonresponders, the underlying disease may worsen over the course of a year. The risk of PBC progression is particularly high among young women; males; patients with positive anti-sp100 and anti-gp 210 antibodies; patients with overlap syndrome involving AIH or PSC; patients with high baseline ALP level; patients with ductopenic form of PBC; and patients with advanced fibrosis or even liver cirrhosis at the time of diagnosis.

Nonresponse to UDCA treatment can be predicted based on baseline parameters - the UDCA response score[27,118] or detected after a short course of treatment using the Xi'an score[25]; these patients are candidates for early second-line therapy. Determining the biochemical response to UDCA according to the modified Toronto criteria is the optimal approach at an earlier time frame for the assessment of the effectiveness of first-line treatment. UDCA nonresponders after 6 months of treatment have approximately a 12-fold increased risk of developing decompensated liver cirrhosis in the future[4]. UDCA nonresponders after 6 months of treatment have a minimal chance of achieving CBR[119], which is associated with the best prognosis[17]. Assessing the therapeutic response to UDCA treatment after 6 months may be the optimal time frame.

The indication for second-line treatment in cases of incomplete biochemical response remains unclear. On the one hand, these patients have a better chance of achieving a CBR, which may slightly improve their prognosis; on the other hand, there is a risk of treatment-related adverse effects, and the cost of treatment and monitoring is also significant. Second-line therapy for incomplete UDCA responders should be considered primarily in high-risk patients, as described above. PBC patients with fibrosis progression exceeding 2.1 kPa/year on repeated vibration-controlled transient elastography[35] and an incomplete UDCA response may also be considered for second-line treatment.

The consideration of the indication for second-line PBC treatment and its timing is summarized in Figure 1.

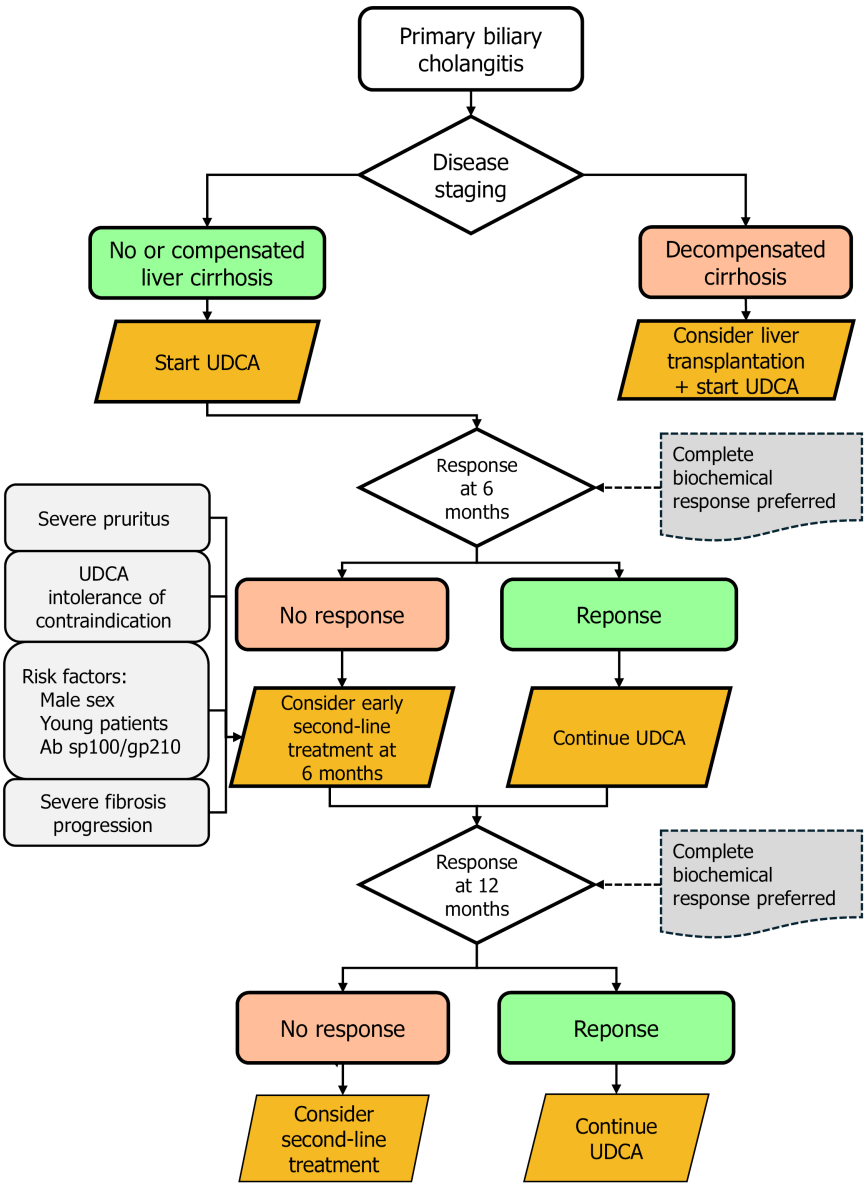


Figure 1 Considerations of the indication for second-line primary biliary cholangitis treatment and its timing. Elafibranor and seladelpar are approved drugs for second-line primary biliary cholangitis treatment. Fibrates should be considered in countries where approved agents are unavailable or not reimbursed. UDCA: Ursodeoxycholic acid.

CONCLUSION

PPAR agonists are approved for second-line treatment of PBC. The results of Phase 3 clinical trials are promising, but long-term clinical outcome data and real-world validation remain necessary. If these drugs are unavailable, replacing elafibranor or seladelpar with bezafibrate or other fibrates may be considered; however, this therapeutic option is off-label. No second-line treatment is available for PBC patients with decompensated cirrhosis or for pregnant patients. Second-line PBC treatment should be personalized, considering not only the benefits of treatment but also its side effects [120].

REFERENCES

1 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *J Hepatol* 2017; **67**: 145-172 [*RCA*] [PMID: 28427765 DOI: 10.1016/j.jhep.2017.03.022] [FullText]

2 **Lindor KD**, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2021 practice guidance update from the American Association for the Study of Liver Diseases. *Hepatology* 2022; **75**: 1012-1013 [*RCA*] [PMID: 34431119 DOI: 10.1002/hep.32117] [FullText]

3 **de Veer RC**, van Hooff MCB, Werner E, Beuers U, Drenth JPH, Cuperus FJC, van Hoek B, Veldt BJ, Klemt-Kropp M, van Meer S, Verdonk RC, Flink HJ, Vrolijk JM, Gevers TJG, Ponsioen CY, Ter Borg MJ, Soufidi K, Boersma F, de Jonge HJM, Wolfhagen FHJ, Baak LC, Onderwater SL, van Bergeijk JD, van Putten PG, de Bruin GJ, Adang RPR, Aparicio-Pages MN, de Boer W, Borg FT, van Soest H, Janssen

HLA, Hansen BE, Erler NS, van der Meer AJ; Dutch PBC Study Group. Incidence and prevalence of primary biliary cholangitis in the Netherlands - A nationwide cohort study. *JHEP Rep* 2024; **6**: 101132 [*RCA*] [PMID: 39113899 DOI: 10.1016/j.jhepr.2024.101132] [FullText] [Full Text(PDF)]

4 **Gazda J**, Drazilova S, Janicko M, Grgurevic I, Filipec Kanizaj T, Koller T, Bodorovska B, Bozin T, Mijic M, Rob Z, Mikolasevic I, Madir A, Kucinsky B, Jarcuska P. Prognostic Factors in Primary Biliary Cholangitis: A Retrospective Study of Joint Slovak and Croatian Cohort of 249 Patients. *J Pers Med* 2021; **11**: 495 [*RCA*] [PMID: 34205918 DOI: 10.3390/jpm11060495] [FullText] [Full Text(PDF)]

5 **Boberg KM**, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E; International Autoimmune Hepatitis Group. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. *J Hepatol* 2011; **54**: 374-385 [*RCA*] [PMID: 21067838 DOI: 10.1016/j.jhep.2010.09.002] [FullText]

6 **Muratori P**, Granito A, Pappas G, Muratori L. Validation of simplified diagnostic criteria for autoimmune hepatitis in Italian patients. *Hepatology* 2009; **49**: 1782-3; author reply 1783 [*RCA*] [PMID: 19402135 DOI: 10.1002/hep.22825] [FullText]

7 **Drazilova S**, Koky T, Macej M, Janicko M, Simkova D, Tsedendamba A, Komarova S, Jarcuska P. Pruritus, Fatigue, Osteoporosis and Dyslipoproteinemia in Pbc Patients: A Clinician's Perspective. *Gastroenterol Insights* 2024; **15**: 419-432 [*RCA*] [DOI: 10.3390/gastroent15020030] [FullText]

8 **Sylvia D**, Tomas K, Marian M, Martin J, Dagmar S, Peter J. The treatment of primary biliary cholangitis: from shadow to light. *Ther Adv Gastroenterol* 2024; **17**: 17562848241265782 [*RCA*] [PMID: 39081664 DOI: 10.1177/17562848241265782] [FullText] [Full Text(PDF)]

9 **Poupon R**, Chrétien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? *Lancet* 1987; **1**: 834-836 [*RCA*] [PMID: 2882236 DOI: 10.1016/s0140-6736(87)91610-2] [FullText]

10 **Ishizaki K**, Imada T, Tsurufuji M. Hepatoprotective bile acid 'ursodeoxycholic acid (UDCA)' Property and difference as bile acids. *Hepatol Res* 2005; **33**: 174-177 [*RCA*] [PMID: 16214392 DOI: 10.1016/j.hepres.2005.09.029] [FullText]

11 **Angulo P**, Lindor KD. Management of primary biliary cirrhosis and autoimmune cholangitis. *Clin Liver Dis* 1998; **2**: 333-351, ix [*RCA*] [PMID: 15560036 DOI: 10.1016/s1089-3261(05)70011-8] [FullText]

12 **Corpechot C**, Carrat F, Bonnand AM, Poupon RE, Poupon R. The effect of ursodeoxycholic acid therapy on liver fibrosis progression in primary biliary cirrhosis. *Hepatology* 2000; **32**: 1196-1199 [*RCA*] [PMID: 11093724 DOI: 10.1053/jhep.2000.20240] [FullText]

13 **Simental-Mendía LE**, Simental-Mendía M, Sánchez-García A, Banach M, Serban MC, Cicero AFG, Sahebkar A. Impact of ursodeoxycholic acid on circulating lipid concentrations: a systematic review and meta-analysis of randomized placebo-controlled trials. *Lipids Health Dis* 2019; **18**: 88 [*RCA*] [PMID: 30954082 DOI: 10.1186/s12944-019-1041-4] [FullText] [Full Text(PDF)]

14 **Hempfling W**, Dilger K, Beuers U. Systematic review: ursodeoxycholic acid--adverse effects and drug interactions. *Aliment Pharmacol Ther* 2003; **18**: 963-972 [*RCA*] [PMID: 14616161 DOI: 10.1046/j.1365-2036.2003.01792.x] [FullText]

15 **Drazilova S**, Babinska I, Gazda J, Halanova M, Janicko M, Kucinsky B, Safcak D, Martinkova D, Tarbajova L, Cekanova A, Jarcuska P; Eastern Slovakia PBC Group. Epidemiology and clinical course of primary biliary cholangitis in Eastern Slovakia. *Int J Public Health* 2020; **65**: 683-691 [*RCA*] [PMID: 32500239 DOI: 10.1007/s00038-020-01391-6] [FullText]

16 **Harms MH**, van Buuren HR, Corpechot C, Thorburn D, Janssen HLA, Lindor KD, Hirschfield GM, Parés A, Floreani A, Mayo MJ, Invernizzi P, Battezzati PM, Nevens F, Ponsioen CY, Mason AL, Kowdley KV, Lammers WJ, Hansen BE, van der Meer AJ. Ursodeoxycholic acid therapy and liver transplant-free survival in patients with primary biliary cholangitis. *J Hepatol* 2019; **71**: 357-365 [*RCA*] [PMID: 30980847 DOI: 10.1016/j.jhep.2019.04.001] [FullText]

17 **Murillo Perez CF**, Harms MH, Lindor KD, van Buuren HR, Hirschfield GM, Corpechot C, van der Meer AJ, Feld JJ, Gulamhusein A, Lammers WJ, Ponsioen CY, Carbone M, Mason AL, Mayo MJ, Invernizzi P, Battezzati PM, Floreani A, Lleo A, Nevens F, Kowdley KV, Bruns T, Dalekos GN, Gatselis NK, Thorburn D, Trivedi PJ, Verhelst X, Parés A, Janssen HLA, Hansen BE; GLOBAL PBC Study Group. Goals of Treatment for Improved Survival in Primary Biliary Cholangitis: Treatment Target Should Be Bilirubin Within the Normal Range and Normalization of Alkaline Phosphatase. *Am J Gastroenterol* 2020; **115**: 1066-1074 [*RCA*] [PMID: 32618657 DOI: 10.14309/ajg.0000000000000557] [FullText]

18 **Angulo P**, Lindor KD, Therneau TM, Jorgensen RA, Malinchoc M, Kamath PS, Dickson ER. Utilization of the Mayo risk score in patients with primary biliary cirrhosis receiving ursodeoxycholic acid. *Liver* 1999; **19**: 115-121 [*RCA*] [PMID: 10220741 DOI: 10.1111/j.1478-3231.1999.tb00020.x] [FullText]

19 **Parés A**, Caballería L, Rodés J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. *Gastroenterology* 2006; **130**: 715-720 [*RCA*] [PMID: 16530513 DOI: 10.1053/j.gastro.2005.12.029] [FullText]

20 **Corpechot C**, Abenavoli L, Rabahi N, Chrétien Y, Andréani T, Johanet C, Chazouillères O, Poupon R. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology* 2008; **48**: 871-877 [*RCA*] [PMID: 18752324 DOI: 10.1002/hep.22428] [FullText]

21 **Corpechot C**, Chazouillères O, Poupon R. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. *J Hepatol* 2011; **55**: 1361-1367 [*RCA*] [PMID: 21703194 DOI: 10.1016/j.jhep.2011.02.031] [FullText]

22 **Kuiper EM**, Hansen BE, de Vries RA, den Ouden-Muller JW, van Ditzhuijsen TJ, Haagsma EB, Houben MH, Witteman BJ, van Erpecum KJ, van Buuren HR; Dutch PBC Study Group. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. *Gastroenterology* 2009; **136**: 1281-1287 [*RCA*] [PMID: 19208346 DOI: 10.1053/j.gastro.2009.01.003] [FullText]

23 **Azemoto N**, Abe M, Murata Y, Hiasa Y, Hamada M, Matsuura B, Onji M. Early biochemical response to ursodeoxycholic acid predicts symptom development in patients with asymptomatic primary biliary cirrhosis. *J Gastroenterol* 2009; **44**: 630-634 [*RCA*] [PMID: 19370305 DOI: 10.1007/s00535-009-0051-9] [FullText]

24 **Kumagi T**, Guindi M, Fischer SE, Arenovich T, Abdalian R, Coltescu C, Heathcote EJ, Hirschfield GM. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. *Am J Gastroenterol* 2010; **105**: 2186-2194 [*RCA*] [PMID: 20502446 DOI: 10.1038/ajg.2010.216] [FullText]

25 **Yang C**, Guo G, Li B, Zheng L, Sun R, Wang X, Deng J, Jia G, Zhou X, Cui L, Guo C, Zhou X, Leung PSC, Gershwin ME, Shang Y, Han Y. Prediction and evaluation of high-risk patients with primary biliary cholangitis receiving ursodeoxycholic acid therapy: an early criterion. *Hepatol Int* 2023; **17**: 237-248 [*RCA*] [PMID: 36309918 DOI: 10.1007/s12072-022-10431-7] [FullText] [Full Text(PDF)]

26 **Gerussi A**, Bernasconi DP, O'Donnell SE, Lammers WJ, Van Buuren H, Hirschfield G, Janssen H, Corpechot C, Reig A, Pares A, Battezzati PM, Zuin MG, Cazzagon N, Floreani A, Nevens F, Gatselis N, Dalekos G, Mayo MJ, Thorburn D, Bruns T, Mason AL, Verhelst X, Kowdley K, van der Meer A, Niro GA, Beretta-Piccoli BT, Marzioni M, Belli LS, Marra F, Valsecchi MG, Lindor KD, Invernizzi P, Hansen BE, Carbone M; Italian PBC Study Group and the GLOBAL PBC Study Group. Measurement of Gamma Glutamyl Transferase to Determine Risk

of Liver Transplantation or Death in Patients With Primary Biliary Cholangitis. *Clin Gastroenterol Hepatol* 2021; **19**: 1688-1697.e14 [*RCA*] [PMID: 32777554 DOI: 10.1016/j.cgh.2020.08.006] [FullText]

27 **Carbone M**, Nardi A, Flack S, Carpino G, Varvaropoulou N, Gavrila C, Spicer A, Badrock J, Bernuzzi F, Cardinale V, Ainsworth HF, Heneghan MA, Thorburn D, Bathgate A, Jones R, Neuberger JM, Battezzati PM, Zuin M, Taylor-Robinson S, Donato MF, Kirby J, Mitchell-Thain R, Floreani A, Sampaziotis F, Muratori L, Alvaro D, Marzioni M, Miele L, Marra F, Giannini E, Gaudio E, Ronca V, Bonato G, Cristoferi L, Malinverno F, Gerussi A, Stocken DD, Cordell HJ, Hirschfield GM, Alexander GJ, Sandford RN, Jones DE, Invernizzi P, Mells GF; Italian PBC Study Group and the UK-PBC Consortium. Pretreatment prediction of response to ursodeoxycholic acid in primary biliary cholangitis: development and validation of the UDCA Response Score. *Lancet Gastroenterol Hepatol* 2018; **3**: 626-634 [*RCA*] [PMID: 30017646 DOI: 10.1016/S2468-1253(18)30163-8] [FullText]

28 **Carbone M**, Sharp SJ, Flack S, Paximadas D, Spiess K, Adgey C, Griffiths L, Lim R, Trembling P, Williamson K, Wareham NJ, Aldersley M, Bathgate A, Burroughs AK, Heneghan MA, Neuberger JM, Thorburn D, Hirschfield GM, Cordell HJ, Alexander GJ, Jones DE, Sandford RN, Mells GF; UK-PBC Consortium. The UK-PBC risk scores: Derivation and validation of a scoring system for long-term prediction of end-stage liver disease in primary biliary cholangitis. *Hepatology* 2016; **63**: 930-950 [*RCA*] [PMID: 26223498 DOI: 10.1002/hep.28017] [FullText] [Full Text(PDF)]

29 **Lammers WJ**, Hirschfield GM, Corpechot C, Nevens F, Lindor KD, Janssen HL, Floreani A, Ponsioen CY, Mayo MJ, Invernizzi P, Battezzati PM, Parés A, Burroughs AK, Mason AL, Kowdley KV, Kumagi T, Harms MH, Trivedi PJ, Poupon R, Cheung A, Lleo A, Caballeria L, Hansen BE, van Buuren HR; Global PBC Study Group. Development and Validation of a Scoring System to Predict Outcomes of Patients With Primary Biliary Cirrhosis Receiving Ursodeoxycholic Acid Therapy. *Gastroenterology* 2015; **149**: 1804-1812.e4 [*RCA*] [PMID: 26261009 DOI: 10.1053/j.gastro.2015.07.061] [FullText]

30 **Gazda J**, Drazilova S, Gazda M, Janicko M, Koky T, Macej M, Carbone M, Jarcuska P. Treatment response to ursodeoxycholic acid in primary biliary cholangitis: A systematic review and meta-analysis. *Dig Liver Dis* 2023; **55**: 1318-1327 [*RCA*] [PMID: 36593158 DOI: 10.1016/j.dld.2022.12.010] [FullText]

31 **Chen Q**, Zhong R, Dong K, Wang Y, Kui Y, Ma B, Wen X, Jin Q. The prognostic value of antibodies to gp210 among patients with primary biliary cholangitis in Northeast China. *Dig Liver Dis* 2022; **54**: 1094-1100 [*RCA*] [PMID: 34789400 DOI: 10.1016/j.dld.2021.10.011] [Full Text]

32 **Juliusson G**, Imam M, Björnsson ES, Talwalkar JA, Lindor KD. Long-term outcomes in antimitochondrial antibody negative primary biliary cirrhosis. *Scand J Gastroenterol* 2016; **51**: 745-752 [*RCA*] [PMID: 26776319 DOI: 10.3109/00365521.2015.1132337] [FullText]

33 **Cheung AC**, Lammers WJ, Murillo Perez CF, van Buuren HR, Gulamhusein A, Trivedi PJ, Lazaridis KN, Ponsioen CY, Floreani A, Hirschfield GM, Corpechot C, Mayo MJ, Invernizzi P, Battezzati PM, Parés A, Nevens F, Thorburn D, Mason AL, Carbone M, Kowdley KV, Bruns T, Dalekos GN, Gatselis NK, Verhelst X, Lindor KD, Lleo A, Poupon R, Janssen HLA, Hansen BE; Global PBC Study Group. Effects of Age and Sex of Response to Ursodeoxycholic Acid and Transplant-free Survival in Patients With Primary Biliary Cholangitis. *Clin Gastroenterol Hepatol* 2019; **17**: 2076-2084.e2 [*RCA*] [PMID: 30616022 DOI: 10.1016/j.cgh.2018.12.028] [FullText]

34 **Wen M**, Men R, Fan X, Shen Y, Ni P, Hu Z, Yang L. Worse Response to Ursodeoxycholic Acid in Primary Biliary Cholangitis Patients with Autoimmune Hepatitis Features. *Dig Dis* 2021; **39**: 366-374 [*RCA*] [PMID: 33238269 DOI: 10.1159/000513331] [FullText]

35 **Corpechot C**, Carrat F, Poujol-Robert A, Gaouar F, Wendum D, Chazouillères O, Poupon R. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. *Hepatology* 2012; **56**: 198-208 [*RCA*] [PMID: 22271046 DOI: 10.1002/hep.25599] [FullText]

36 **Murillo Perez CF**, Hirschfield GM, Corpechot C, Floreani A, Mayo MJ, van der Meer A, Ponsioen CY, Lammers WJ, Parés A, Invernizzi P, Carbone M, Maria Battezzati P, Nevens F, Kowdley KV, Thorburn D, Mason AL, Trivedi PJ, Lindor KD, Bruns T, Dalekos GN, Gatselis NK, Verhelst X, Janssen HLA, Hansen BE, Gulamhusein A; GLOBAL PBC Study Group. Fibrosis stage is an independent predictor of outcome in primary biliary cholangitis despite biochemical treatment response. *Aliment Pharmacol Ther* 2019; **50**: 1127-1136 [*RCA*] [PMID: 31621931 DOI: 10.1111/apt.15533] [FullText]

37 **Marcos Carrasco N**, López Jerez A, Garrido E, García González M. Estimation of liver fibrosis using elastography in cholestatic diseases: systematic review and meta-analysis. *Rev Esp Enferm Dig* 2025; **117**: 265-273 [*RCA*] [PMID: 37366032 DOI: 10.17235/reed.2023.9254/2022] [FullText]

38 **Corpechot C**, Carrat F, Gaouar F, Chau F, Hirschfield G, Gulamhusein A, Montano-Loza AJ, Lytvyak E, Schramm C, Pares A, Olivas I, Eaton JE, Osman KT, Dalekos G, Gatselis N, Nevens F, Cazzagon N, Zago A, Russo FP, Abbas N, Trivedi P, Thorburn D, Saffioti F, Barkai L, Roccarina D, Calvaruso V, Fichera A, Delamarre A, Medina-Morales E, Bonder A, Patwardhan V, Rigamonti C, Carbone M, Invernizzi P, Cristoferi L, van der Meer A, de Veer R, Zigmond E, Yehezkel E, Kremer AE, Deibel A, Dumortier J, Bruns T, Große K, Pageaux GP, Wetten A, Dyson J, Jones D, Chazouillères O, Hansen B, de Lédinghen V; Global & ERN Rare-Liver PBC Study Groups. Liver stiffness measurement by vibration-controlled transient elastography improves outcome prediction in primary biliary cholangitis. *J Hepatol* 2022; **77**: 1545-1553 [*RCA*] [PMID: 35777587 DOI: 10.1016/j.jhep.2022.06.017] [FullText]

39 **Wang W**, Zhang G, Liu H, Lv L, Chang J, Liu Z, Ye H, Ding H, Han Y. Clinical outcomes and early- prognostic biomarkers of primary biliary cholangitis with ductopenia. *Front Immunol* 2025; **16**: 1680942 [*RCA*] [PMID: 41409297 DOI: 10.3389/fimmu.2025.1680942] [FullText] [Full Text(PDF)]

40 **Abdulkarim M**, Zenouzi R, Sebode M, Schulz L, Quaas A, Lohse AW, Schramm C, Weiler-Normann C. Sex differences in clinical presentation and prognosis in patients with primary biliary cholangitis. *Scand J Gastroenterol* 2019; **54**: 1391-1396 [*RCA*] [PMID: 31692389 DOI: 10.1080/00365521.2019.1683226] [FullText]

41 **Marschall HU**, Henriksson I, Lindberg S, Söderdahl F, Thuresson M, Wahlin S, Ludvigsson JF. Incidence, prevalence, and outcome of primary biliary cholangitis in a nationwide Swedish population-based cohort. *Sci Rep* 2019; **9**: 11525 [*RCA*] [PMID: 31395896 DOI: 10.1038/s41598-019-47890-2] [FullText] [Full Text(PDF)]

42 **Roberts SB**, Choi WJ, Worobetz L, Vincent C, Flemming JA, Cheung A, Qumosani K, Swain M, Grbic D, Ko HH, Peltekian KM, Abrahamyan L, Saini M, Tirona K, Aziz B, Lytvyak E, Invernizzi P, Ponsioen CY, Bruns T, Cazzagon N, Lindor K, Dalekos GN, Gatselis NK, Verhelst X, Floreani A, Corpechot C, Mayo MJ, Levy C, Londoño MC, Battezzati PM, Pares A, Nevens F, van der Meer A, Kowdley KV, Trivedi PJ, Lleo A, Thorburn D, Carbone M, Selzner N, Gulamhusein AF, Janssen H, Montano-Loza AJ, Mason AL, Hirschfield GM, Hansen BE; Canadian Network for Autoimmune Liver disease (CaNAL). Loss of biochemical response at any time worsens outcomes in UDCA-treated patients with primary biliary cholangitis. *JHEP Rep* 2024; **6**: 101168 [*RCA*] [PMID: 39380718 DOI: 10.1016/j.jhepr.2024.101168] [Full Text] [Full Text(PDF)]

43 **Cançado GGL**, Couto CA, Guedes LV, Braga MH, Terrabuio DRB, Cançado ELR, Ferraz MLG, Villela-Nogueira CA, Nardelli MJ, Faria LC,

de Oliveira EMG, Rotman V, Mazo DFC, Borges VFAE, Mendes LSC, Codes L, Pessoa MG, Signorelli IV, Levy C, Bittencourt PL. Fibrates for the Treatment of Primary Biliary Cholangitis Unresponsive to Ursodeoxycholic Acid: An Exploratory Study. *Front Pharmacol* 2021; **12**: 818089 [*RCA*] [PMID: 35126149 DOI: 10.3389/fphar.2021.818089] [FullText] [Full Text(PDF)]

44 **Colapietro F**, Gershwin ME, Lleo A. PPAR agonists for the treatment of primary biliary cholangitis: Old and new tales. *J Transl Autoimmun* 2023; **6**: 100188 [*RCA*] [PMID: 36684809 DOI: 10.1016/j.jtauto.2023.100188] [FullText] [Full Text(PDF)]

45 **Schattenberg JM**, Pares A, Kowdley KV, Heneghan MA, Caldwell S, Pratt D, Bonder A, Hirschfield GM, Levy C, Vierling J, Jones D, Tailleux A, Staels B, Megnien S, Hanf R, Magrez D, Birman P, Luketic V. A randomized placebo-controlled trial of elafibranor in patients with primary biliary cholangitis and incomplete response to UDCA. *J Hepatol* 2021; **74**: 1344-1354 [*RCA*] [PMID: 33484775 DOI: 10.1016/j.jhep.2021.01.013] [FullText]

46 **Kowdley KV**, Bowlus CL, Levy C, Akarca US, Alvares-da-Silva MR, Andreone P, Arrese M, Corpechot C, Francque SM, Heneghan MA, Invernizzi P, Jones D, Kruger FC, Lawitz E, Mayo MJ, Shiffman ML, Swain MG, Valera JM, Vargas V, Vierling JM, Villamil A, Addy C, Dietrich J, Germain JM, Mazain S, Rafailovic D, Taddé B, Miller B, Shu J, Zein CO, Schattenberg JM; ELATIVE Study Investigators' Group; ELATIVE Study Investigators' Group. Efficacy and Safety of Elafibranor in Primary Biliary Cholangitis. *N Engl J Med* 2024; **390**: 795-805 [*RCA*] [PMID: 37962077 DOI: 10.1056/NEJMoa2306185] [FullText]

47 Elafibranor Long-Term Efficacy and Safety and Impact on Fatigue in Primary Biliary Cholangitis: Interim Results From the Open-Label Extension of the ELATIVE Trial Up to 3 Years. *Gastroenterol Hepatol (N Y)* 2024; **20**: 3-4 [*RCA*] [PMID: 39897512] [FullText]

48 **Jones D**, Boudes PF, Swain MG, Bowlus CL, Galambos MR, Bacon BR, Doerffel Y, Gitlin N, Gordon SC, Odin JA, Sheridan D, Wörns MA, Clark V, Corless L, Hartmann H, Jonas ME, Kremer AE, Mells GF, Buggisch P, Freilich BL, Levy C, Vierling JM, Bernstein DE, Hartleb M, Janczewska E, Rochling F, Shah H, Shiffman ML, Smith JH, Choi YJ, Steinberg A, Varga M, Chera H, Martin R, McWherter CA, Hirschfield GM. Seladelpar (MBX-8025), a selective PPAR-δ agonist, in patients with primary biliary cholangitis with an inadequate response to ursodeoxycholic acid: a double-blind, randomised, placebo-controlled, phase 2, proof-of-concept study. *Lancet Gastroenterol Hepatol* 2017; **2**: 716-726 [*RCA*] [PMID: 28818518 DOI: 10.1016/S2468-1253(17)30246-7] [FullText]

49 **Hirschfield GM**, Bowlus CL, Mayo MJ, Kremer AE, Vierling JM, Kowdley KV, Levy C, Villamil A, Ladrón de Guevara Cetina AL, Janczewska E, Zigmond E, Jeong SH, Yilmaz Y, Kallis Y, Corpechot C, Buggisch P, Invernizzi P, Londoño Hurtado MC, Bergheanu S, Yang K, Choi YJ, Crittenden DB, McWherter CA; RESPONSE Study Group. A Phase 3 Trial of Seladelpar in Primary Biliary Cholangitis. *N Engl J Med* 2024; **390**: 783-794 [*RCA*] [PMID: 38381664 DOI: 10.1056/NEJMoa2312100] [FullText]

50 **Levy C**, Trivedi PJ, Kowdley KV, Gordon SC, Bowlus CL, Londoño MC, Hirschfield GM, Gulamhusein A, Lawitz EJ, Vierling JM, Mayo MJ, Jacobson IM, Kremer AE, Corpechot C, Jones D, Buggisch P, Zhuo S, Proehl S, Heusner C, McWherter CA, Crittenden DB; ASSURE Investigators. Long-Term Efficacy and Safety of Selective PPARδ Agonist Seladelpar in Primary Biliary Cholangitis: ASSURE Interim Study Results. *Am J Gastroenterol* 2025; **121**: 1140-1153 [*RCA*] [PMID: 40553148 DOI: 10.14309/ajg.0000000000003603] [FullText] [Full Text (PDF)]

51 Long-Term Efficacy and Safety of Open-Label Seladelpar Treatment in Patients With Primary Biliary Cholangitis: Pooled Interim Results for Up to 3 Years From the ASSURE Study. *Gastroenterol Hepatol (N Y)* 2024; **20**: 8-9 [*RCA*] [PMID: 39897518] [FullText]

52 **Kuo SZ**, Yin J, Loomba R. Research Communication: Real-World Clinical Experience With Seladelpar in Primary Biliary Cholangitis. *Aliment Pharmacol Ther* 2026; **63**: 728-731 [*RCA*] [PMID: 41163419 DOI: 10.1111/apt.70435] [FullText] [Full Text(PDF)]

53 **Kremer AE**, Mayo MJ, Hirschfield GM, Levy C, Bowlus CL, Jones DE, Johnson JD, McWherter CA, Choi YJ. Seladelpar treatment reduces IL-31 and pruritus in patients with primary biliary cholangitis. *Hepatology* 2024; **80**: 27-37 [*RCA*] [PMID: 38117036 DOI: 10.1097/HEP.0000000000000728] [FullText] [Full Text(PDF)]

54 Attenuation, Near Resolution, and Prevention of Pruritus in Patients With Primary Biliary Cholangitis Treated With Seladelpar: A Secondary Analysis of Patterns of Pruritus Change in the RESPONSE Trial. *Gastroenterol Hepatol (N Y)* 2024; **20**: 4-6 [*RCA*] [PMID: 39897516] [Full Text]

55 **Kremer AE**, Mayo MJ, Hirschfield G, Levy C, Bowlus CL, Jones DE, Steinberg A, McWherter CA, Choi YJ. Seladelpar improved measures of pruritus, sleep, and fatigue and decreased serum bile acids in patients with primary biliary cholangitis. *Liver Int* 2022; **42**: 112-123 [*RCA*] [PMID: 34403559 DOI: 10.1111/liv.15039] [FullText]

56 **Zargar AH**, Bhansali A, Majumdar A, Maheshwari A, Bhattacharyya A, Dasgupta A, Saboo BD, Sethi BK, Sanyal D, Seshadri KG, Deshpande NR, Kapoor N, Lakhani OJ, Talwalkar PG, Kalra P, Mehrotra RN, Sahay RK, Shukla R, Kant S, Das S, Agarwal SC, Phatak SR, G S, Joshi SR, Shaikh SS, Aravind SR, Goswami S, Ghosh S, Panikar VK, Mohan V. Management of metabolic dysfunction-associated steatotic liver disease (MASLD)-An expert consensus statement from Indian diabetologists' perspective. *Diabetes Obes Metab* 2025; **27** Suppl 4: 3-20 [*RCA*] [PMID: 40457532 DOI: 10.1111/dom.16496] [FullText] [Full Text(PDF)]

57 **Vuppalanchi R**, Caldwell SH, Pyrsopoulos N, deLemos AS, Rossi S, Levy C, Goldberg DS, Mena EA, Sheikh A, Ravinuthala R, Shaikh F, Bainbridge JD, Parmar DV, Chalasani NP. Proof-of-concept study to evaluate the safety and efficacy of saroglitazar in patients with primary biliary cholangitis. *J Hepatol* 2022; **76**: 75-85 [*RCA*] [PMID: 34487750 DOI: 10.1016/j.jhep.2021.08.025] [FullText]

58 **Katsiki N**, Nikolic D, Montalto G, Banach M, Mikhailidis DP, Rizzo M. The role of fibrate treatment in dyslipidemia: an overview. *Curr Pharm Des* 2013; **19**: 3124-3131 [*RCA*] [PMID: 23317397 DOI: 10.2174/1381612811319170020] [FullText]

59 **Corpechot C**, Chazouillères O, Rousseau A, Le Gruyer A, Habersetzer F, Mathurin P, Goria O, Potier P, Minello A, Silvain C, Abergel A, Debette-Gratien M, Larrey D, Roux O, Bronowicki JP, Boursier J, de Ledinghen V, Heurgue-Berlot A, Nguyen-Khac E, Zoulim F, Ollivier-Hourmand I, Zarski JP, Nkontchou G, Lemoinne S, Humbert L, Rainteau D, Lefèvre G, de Chaisemartin L, Chollet-Martin S, Gaouar F, Admane FH, Simon T, Poupon R. A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis. *N Engl J Med* 2018; **378**: 2171-2181 [*RCA*] [PMID: 29874528 DOI: 10.1056/NEJMoa1714519] [FullText]

60 **Khakoo NS**, Sultan S, Reynolds JM, Levy C. Efficacy and Safety of Bezafibrate Alone or in Combination with Ursodeoxycholic Acid in Primary Biliary Cholangitis: Systematic Review and Meta-Analysis. *Dig Dis Sci* 2023; **68**: 1559-1573 [*RCA*] [PMID: 36180756 DOI: 10.1007/s10620-022-07704-4] [FullText]

61 **Hosonuma K**, Sato K, Yamazaki Y, Yanagisawa M, Hashizume H, Horiguchi N, Kakizaki S, Kusano M, Yamada M. A prospective randomized controlled study of long-term combination therapy using ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis and dyslipidemia. *Am J Gastroenterol* 2015; **110**: 423-431 [*RCA*] [PMID: 25732417 DOI: 10.1038/ajg.2015.20] [FullText]

62 **Itakura J**, Izumi N, Nishimura Y, Inoue K, Ueda K, Nakanishi H, Tsuchiya K, Hamano K, Asahina Y, Kurosaki M, Uchihara M, Miyake S. Prospective randomized crossover trial of combination therapy with bezafibrate and UDCA for primary biliary cirrhosis. *Hepatol Res* 2004; **29**: 216-222 [*RCA*] [PMID: 15288014 DOI: 10.1016/j.hepres.2004.04.001] [FullText]

63 **Iwasaki S**, Ohira H, Nishiguchi S, Zeniya M, Kaneko S, Onji M, Ishibashi H, Sakaida I, Kuriyama S, Ichida T, Onishi S, Toda G; Study Group

of Intractable Liver Diseases for Research on a Specific Disease, Health Science Research Grant, Ministry of Health, Labour and Welfare of Japan. The efficacy of ursodeoxycholic acid and bezafibrate combination therapy for primary biliary cirrhosis: A prospective, multicenter study. *Hepatol Res* 2008; **38**: 557-564 [*RCA*] [PMID: 18452482 DOI: 10.1111/j.1872-034X.2007.00305.x] [FullText]

64 **Kanda T**, Yokosuka O, Imazeki F, Saisho H. Bezafibrate treatment: a new medical approach for PBC patients? *J Gastroenterol* 2003; **38**: 573-578 [*RCA*] [PMID: 12825134 DOI: 10.1007/s00535-002-1102-7] [FullText]

65 **Nakai S**, Masaki T, Kurokohchi K, Deguchi A, Nishioka M. Combination therapy of bezafibrate and ursodeoxycholic acid in primary biliary cirrhosis: a preliminary study. *Am J Gastroenterol* 2000; **95**: 326-327 [*RCA*] [PMID: 10638623 DOI: 10.1111/j.1572-0241.2000.01667.x] [FullText]

66 **Tanaka A**, Hirohara J, Nakano T, Matsumoto K, Chazouillères O, Takikawa H, Hansen BE, Carrat F, Corpechot C. Association of bezafibrate with transplant-free survival in patients with primary biliary cholangitis. *J Hepatol* 2021; **75**: 565-571 [*RCA*] [PMID: 33882268 DOI: 10.1016/j.jhep.2021.04.010] [FullText]

67 **Takano K**, Saeki C, Oikawa T, Hidaka A, Mizuno Y, Ishida J, Takakura K, Nakano M, Torisu Y, Amano K, Ishikawa T, Zeniya M, Tsubota A, Saruta M. IgM response is a prognostic biomarker of primary biliary cholangitis treated with ursodeoxycholic acid and bezafibrate. *J Gastroenterol Hepatol* 2020; **35**: 663-672 [*RCA*] [PMID: 31677185 DOI: 10.1111/jgh.14900] [FullText]

68 **Dohmen K**, Tanaka H, Haruno M. Effectiveness of fenofibrate in comparison to bezafibrate for patients with asymptomatic primary biliary cirrhosis. *Fukuoka Igaku Zasshi* 2013; **104**: 350-361 [*RCA*] [PMID: 24511666] [FullText]

69 **Li C**, Zheng K, Chen Y, He C, Liu S, Yang Y, Li M, Zeng X, Wang L, Zhang F. A randomized, controlled trial on fenofibrate in primary biliary cholangitis patients with incomplete response to ursodeoxycholic acid. *Ther Adv Chronic Dis* 2022; **13**: 20406223221114198 [*RCA*] [PMID: 35924008 DOI: 10.1177/20406223221114198] [FullText] [Full Text(PDF)]

70 **Zhang H**, Li S, Feng Y, Zhang Q, Xie B. Efficacy of fibrates in the treatment of primary biliary cholangitis: a meta-analysis. *Clin Exp Med* 2023; **23**: 1741-1749 [*RCA*] [PMID: 36318376 DOI: 10.1007/s10238-022-00904-2] [FullText]

71 **Floreani A**, Gabbia D, De Martin S. Obeticholic Acid for Primary Biliary Cholangitis. *Biomedicines* 2022; **10**: 2464 [*RCA*] [PMID: 36289726 DOI: 10.3390/biomedicines10102464] [FullText] [Full Text(PDF)]

72 **Verbeke L**, Mannaerts I, Schierwagen R, Govaere O, Klein S, Vander Elst I, Windmolders P, Farre R, Wenes M, Mazzone M, Nevens F, van Grunsven LA, Trebicka J, Laleman W. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. *Sci Rep* 2016; **6**: 33453 [*RCA*] [PMID: 27634375 DOI: 10.1038/srep33453] [FullText] [Full Text(PDF)]

73 **Chapman RW**, Lynch KD. Obeticholic acid-a new therapy in PBC and NASH. *Br Med Bull* 2020; **133**: 95-104 [*RCA*] [PMID: 32282030 DOI: 10.1093/bmb/ldaa006] [FullText]

74 **Nevens F**, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, Drenth JP, Pockros PJ, Regula J, Beuers U, Trauner M, Jones DE, Floreani A, Hohenester S, Luketic V, Shiffman M, van Erpecum KJ, Vargas V, Vincent C, Hirschfield GM, Shah H, Hansen B, Lindor KD, Marschall HU, Kowdley KV, Hooshmand-Rad R, Marmon T, Sheeron S, Pencek R, MacConell L, Pruzanski M, Shapiro D; POISE Study Group. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *N Engl J Med* 2016; **375**: 631-643 [*RCA*] [PMID: 27532829 DOI: 10.1056/NEJMoa1509840] [FullText]

75 **Bowlus CL**, Pockros PJ, Kremer AE, Parés A, Forman LM, Drenth JPH, Ryder SD, Terracciano L, Jin Y, Liberman A, Pencek R, Iloeje U, MacConell L, Bedossa P. Long-Term Obeticholic Acid Therapy Improves Histological Endpoints in Patients With Primary Biliary Cholangitis. *Clin Gastroenterol Hepatol* 2020; **18**: 1170-1178.e6 [*RCA*] [PMID: 31606455 DOI: 10.1016/j.cgh.2019.09.050] [FullText]

76 **Trauner M**, Nevens F, Shiffman ML, Drenth JPH, Bowlus CL, Vargas V, Andreone P, Hirschfield GM, Pencek R, Malecha ES, MacConell L, Shapiro D. Long-term efficacy and safety of obeticholic acid for patients with primary biliary cholangitis: 3-year results of an international open-label extension study. *Lancet Gastroenterol Hepatol* 2019; **4**: 445-453 [*RCA*] [PMID: 30922873 DOI: 10.1016/S2468-1253(19)30094-9] [FullText]

77 **Hirschfield GM**, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M, Kowdley KV, Vincent C, Bodhenheimer HC Jr, Parés A, Trauner M, Marschall HU, Adorini L, Sciacca C, Beecher-Jones T, Castelloe E, Böhm O, Shapiro D. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. *Gastroenterology* 2015; **148**: 751-61.e8 [*RCA*] [PMID: 25500425 DOI: 10.1053/j.gastro.2014.12.005] [FullText]

78 **Kowdley KV**, Luketic V, Chapman R, Hirschfield GM, Poupon R, Schramm C, Vincent C, Rust C, Parés A, Mason A, Marschall HU, Shapiro D, Adorini L, Sciacca C, Beecher-Jones T, Böhm O, Pencek R, Jones D; Obeticholic Acid PBC Monotherapy Study Group. A randomized trial of obeticholic acid monotherapy in patients with primary biliary cholangitis. *Hepatology* 2018; **67**: 1890-1902 [*RCA*] [PMID: 29023915 DOI: 10.1002/hep.29569] [FullText] [Full Text(PDF)]

79 **Gao Y**, Li L, Li B, Zhan Y. Response Rate and Impact on Lipid Profiles of Obeticholic Acid Treatment for Patients with Primary Biliary Cholangitis: A Meta-Analysis. *Can J Gastroenterol Hepatol* 2021; **2021**: 8829510 [*RCA*] [PMID: 33511089 DOI: 10.1155/2021/8829510] [FullText] [Full Text(PDF)]

80 **D'Amato D**, De Vincentis A, Malinverno F, Viganò M, Alvaro D, Pompili M, Picciotto A, Palitti VP, Russello M, Storato S, Pigozzi MG, Calvaruso V, De Gasperi E, Lleo A, Castellaneta A, Pellicelli A, Cazzagon N, Floreani A, Muratori L, Fagiuoli S, Niro GA, Feletti V, Cozzolongo R, Terreni N, Marzioni M, Pellicano R, Pozzoni P, Baiocchi L, Chessa L, Rosina F, Bertino G, Vinci M, Morgando A, Vanni E, Scifo G, Sacco R, D'Antò M, Bellia V, Boldizzoni R, Casella S, Omazzi B, Poggi G, Cristoferi L, Gerussi A, Ronca V, Venere R, Ponziani F, Cannavò M, Mussetto A, Fontana R, Losito F, Frazzetto E, Distefano M, Colapietro F, Labanca S, Marconi G, Grassi G, Galati G, O'Donnell SE, Mancuso C, Mulinacci G, Palermo A, Claar E, Izzi A, Picardi A, Invernizzi P, Carbone M, Vespasiani-Gentilucci U; Italian PBC Registry and the Club Epatologi Ospedalieri (CLEO)/Associazione Italiana Gastroenterologi ed Endoscopisti Digestivi Ospedalieri (AIGO) PBC Study Group. Real-world experience with obeticholic acid in patients with primary biliary cholangitis. *JHEP Rep* 2021; **3**: 100248 [*RCA*] [PMID: 33681748 DOI: 10.1016/j.jhepr.2021.100248] [FullText] [Full Text(PDF)]

81 **De Vincentis A**, D'Amato D, Cristoferi L, Gerussi A, Malinverno F, Lleo A, Colapietro F, Marra F, Galli A, Fiorini C, Coco B, Brunetto M, Niro GA, Cotugno R, Saitta C, Cozzolongo R, Losito F, Giannini EG, Labanca S, Marzioni M, Marconi G, Morgando A, Pellicano R, Vanni E, Cazzagon N, Floreani A, Chessa L, Morelli O, Muratori L, Pellicelli A, Pompili M, Ponziani F, Tortora A, Rosina F, Russello M, Cannavò M, Simone L, Storato S, Viganò M, Abenavoli L, D'Antò M, De Gasperi E, Distefano M, Scifo G, Zolfino T, Calvaruso V, Cuccorese G, Palitti VP, Sacco R, Bertino G, Frazzetto E, Alvaro D, Mulinacci G, Palermo A, Scaravaglio M, Terracciani F, Galati G, Ronca V, Zuin M, Claar E, Izzi A, Picardi A, Invernizzi P, Vespasiani-Gentilucci U, Carbone M; on behalf of the Club Epatologi Ospedalieri (CLEO)/Associazione Italiana Gastroenterologi ed Endoscopisti Digestivi Ospedalieri (AIGO) PBC Study Group and, the Italian PBC Registry. Predictors of serious adverse events and non-response in cirrhotic patients with primary biliary cholangitis treated with obeticholic acid. *Liver Int* 2022; **42**: 2453-2465 [*RCA*] [PMID: 35932095 DOI: 10.1111/liv.15386] [FullText] [Full Text(PDF)]

82 **Gomez E**, Garcia Buey L, Molina E, Casado M, Conde I, Berenguer M, Jorquera F, Simón MA, Olveira A, Hernández-Guerra M, Mesquita M, Presa J, Costa-Moreira P, Macedo G, Arenas JI, Manuel Sousa J, Ampuero J, Morillas RM, Santos A, De Carvalho A, Uriz J, Carrión JA, Luisa Gutiérrez M, Pérez-Fernández E, Fernández-Rodríguez CM; IBER-PBC leading Cooperative Group. Effectiveness and safety of obeticholic acid in a Southern European multicentre cohort of patients with primary biliary cholangitis and suboptimal response to ursodeoxycholic acid. *Aliment Pharmacol Ther* 2021; **53**: 519-530 [*RCA*] [PMID: 33314220 DOI: 10.1111/apt.16181] [FullText]

83 **Roberts SB**, Ismail M, Kanagalingam G, Mason AL, Swain MG, Vincent C, Yoshida EM, Tsien C, Flemming JA, Janssen HLA, Hirschfield GM, Hansen BE, Gulamhusein AF; Canadian Network for Autoimmune Liver Disease. Real-World Effectiveness of Obeticholic Acid in Patients with Primary Biliary Cholangitis. *Hepatol Commun* 2020; **4**: 1332-1345 [*RCA*] [PMID: 32923836 DOI: 10.1002/hep4.1518] [Full Text] [Full Text(PDF)]

84 **Londoño MC**, Díaz-González Á. Predicting liver-related serious adverse events in patients with primary biliary cholangitis-related cirrhosis treated with obeticholic acid. *Liver Int* 2022; **42**: 2356-2359 [*RCA*] [PMID: 36162085 DOI: 10.1111/liv.15379] [FullText]

85 **Kowdley KV**, Hirschfield GM, Coombs C, Malecha ES, Bessonova L, Li J, Rathnayaka N, Mells G, Jones DE, Trivedi PJ, Hansen BE, Smith R, Wason J, Hiu S, Kareithi DN, Mason AL, Bowlus CL, Muller K, Carbone M, Berenguer M, Milkiewicz P, Adekunle F, Villamil A. COBALT: A Confirmatory Trial of Obeticholic Acid in Primary Biliary Cholangitis With Placebo and External Controls. *Am J Gastroenterol* 2025; **120**: 390-400 [*RCA*] [PMID: 39140490 DOI: 10.14309/ajg.0000000000003029] [FullText] [Full Text(PDF)]

86 **Eaton JE**, Vuppalanchi R, Reddy R, Sathapathy S, Ali B, Kamath PS. Liver Injury in Patients With Cholestatic Liver Disease Treated With Obeticholic Acid. *Hepatology* 2020; **71**: 1511-1514 [*RCA*] [PMID: 31680292 DOI: 10.1002/hep.31017] [FullText]

87 **Brookhart MA**, Mayne TJ, Coombs C, Breskin A, Ness E, Bessonova L, Chu YJ, Li J, Fried MW, Hansen BE, Kowdley KV, Jones D, Mells G, Trivedi PJ, Hiu S, Kareithi DN, Wason J, Smith R, Seeger JD, Hirschfield GM. Hepatic real-world outcomes with obeticholic acid in primary biliary cholangitis (HEROES): A trial emulation study design. *Hepatology* 2025; **81**: 1647-1659 [*RCA*] [PMID: 39630028 DOI: 10.1097/HEP.0000000000001174] [FullText] [Full Text(PDF)]

88 **Silveira MG**, Lindor KD. Obeticholic acid and budesonide for the treatment of primary biliary cirrhosis. *Expert Opin Pharmacother* 2014; **15**: 365-372 [*RCA*] [PMID: 24382005 DOI: 10.1517/14656566.2014.873404] [FullText]

89 **Hirschfield GM**, Beuers U, Kupcinskas L, Ott P, Bergquist A, Färkkilä M, Manns MP, Parés A, Spengler U, Stiess M, Greinwald R, Pröls M, Wendum D, Drebber U, Poupon R. A placebo-controlled randomised trial of budesonide for PBC following an insufficient response to UDCA. *J Hepatol* 2021; **74**: 321-329 [*RCA*] [PMID: 32950590 DOI: 10.1016/j.jhep.2020.09.011] [FullText]

90 **Zhang H**, Yang J, Zhu R, Zheng Y, Zhou Y, Dai W, Wang F, Chen K, Li J, Wang C, Li S, Liu T, Abudumijiti H, Zhou Z, Wang J, Lu W, Wang J, Xia Y, Zhou Y, Lu J, Guo C. Combination therapy of ursodeoxycholic acid and budesonide for PBC-AIH overlap syndrome: a meta-analysis. *Drug Des Devel Ther* 2015; **9**: 567-574 [*RCA*] [PMID: 25632224 DOI: 10.2147/DDDT.S74515] [FullText] [Full Text(PDF)]

91 **Olivas I**, Cobreros M, Londoño MC, Díaz-González Á. Budesonide in the first line treatment of patients with autoimmune hepatitis. *Gastroenterol Hepatol* 2022; **45**: 561-570 [*RCA*] [PMID: 34923033 DOI: 10.1016/j.gastrohep.2021.11.012] [FullText]

92 **Li Q**, Liu Z, Shang W, Zhou X, Mao WY, Cao Y, Shan L. FXR-targeted drug discovery: Recent advances and therapeutic perspectives. *Eur J Med Chem* 2026; **302**: 118332 [*RCA*] [PMID: 41205516 DOI: 10.1016/j.ejmech.2025.118332] [FullText]

93 **Naffouj S**, Wang J. Current and future opportunities for the management of primary biliary cholangitis. *Front Gastroenterol (Lausanne)* 2023; **2**: 1241901 [*RCA*] [PMID: 41821804 DOI: 10.3389/fgstr.2023.1241901] [FullText] [Full Text(PDF)]

94 **Nevens F**, Trauner M, Manns MP. Primary biliary cholangitis as a roadmap for the development of novel treatments for cholestatic liver diseases(†). *J Hepatol* 2023; **78**: 430-441 [*RCA*] [PMID: 36272496 DOI: 10.1016/j.jhep.2022.10.007] [FullText]

95 **Invernizzi P**, Carbone M, Jones D, Levy C, Little N, Wiesel P, Nevens F; study investigators. Setanaxib, a first-in-class selective NADPH oxidase 1/4 inhibitor for primary biliary cholangitis: A randomized, placebo-controlled, phase 2 trial. *Liver Int* 2023; **43**: 1507-1522 [*RCA*] [PMID: 37183520 DOI: 10.1111/liv.15596] [FullText]

96 **Jones D**, Carbone M, Invernizzi P, Little N, Nevens F, Swain MG, Wiesel P, Levy C. Impact of setanaxib on quality of life outcomes in primary biliary cholangitis in a phase 2 randomized controlled trial. *Hepatol Commun* 2023; **7**: e0057 [*RCA*] [PMID: 36809195 DOI: 10.1097/HC9.0000000000000057] [FullText] [Full Text(PDF)]

97 **Giannini EG**, Pasta A, Calabrese F, Labanca S, Marenco S, Pieri G, Plaz Torres MC, Strazzabosco M. Second-Line Treatment for Patients With Primary Biliary Cholangitis: A Systematic Review With Network Meta-Analysis. *Liver Int* 2025; **45**: e16222 [*RCA*] [PMID: 39720853 DOI: 10.1111/liv.16222] [FullText] [Full Text(PDF)]

98 **Cançado GGL**, Chen B, Cameron M, Houri I, Leung KK, Gulamhusein AF, Hansen B, Hirschfield GM. High biochemical remission rates in patients with primary biliary cholangitis treated with "triple" anticholestatic therapy. *Hepatol Commun* 2025; **9**: e0798 [*RCA*] [PMID: 41021281 DOI: 10.1097/HC9.0000000000000798] [FullText]

99 **Soret PA**, Lam L, Carrat F, Smets L, Berg T, Carbone M, Invernizzi P, Leroy V, Trivedi P, Cazzagon N, Weiler-Normann C, Alric L, Rosa-Hezode I, Heurgué A, Cervoni JP, Dumortier J, Potier P, Roux O, Silvain C, Bureau C, Anty R, Larrey D, Levy C, Pares A, Schramm C, Nevens F, Chazouillères O, Corpechot C. Combination of fibrates with obeticholic acid is able to normalise biochemical liver tests in patients with difficult-to-treat primary biliary cholangitis. *Aliment Pharmacol Ther* 2021; **53**: 1138-1146 [*RCA*] [PMID: 33764590 DOI: 10.1111/apt.16336] [FullText]

100 **Gordon SC**, Proehl S, Pratt D, Qi X, Levy C, Crittenden DB. Seladelpar Improves Cholestasis and Pruritus in Obeticholic Acid and Fibrate-Experienced Patients With Primary Biliary Cholangitis. *Clin Gastroenterol Hepatol* 2026; S1542-3565(26)00039 [*RCA*] [PMID: 41577065 DOI: 10.1016/j.cgh.2026.01.011] [FullText]

101 **Jamal F**, Elshaer A, Alatout MH, Odeh NB, Youssef A, Abo Abdullah H, Elmasry S, Shahin T, Abdul Nabi H, Worden AR, Malik TA, Lizaola-Mayo BC. Second-Line Therapies in Primary Biliary Cholangitis: A Comparative Review of Obeticholic Acid, Fibrates, Seladelpar, and Elafibranor. *Biomedicines* 2025; **13**: 2335 [*RCA*] [PMID: 41153623 DOI: 10.3390/biomedicines13102335] [FullText] [Full Text(PDF)]

102 **Ao X**, Zeng Y, Wang X, Fan X. Letter: Fibrates may be safe and effective in patients with primary biliary cholangitis and decompensated cirrhosis. *Aliment Pharmacol Ther* 2024; **60**: 105-106 [*RCA*] [PMID: 38803269 DOI: 10.1111/apt.18044] [FullText]

103 **Burghart L**, Hofer BS, Halilbasic E, Simbrunner B, Staettermayer AF, Trauner M, Reiberger T. Letter: Fibrates may be safe and effective in patients with primary biliary cholangitis and decompensated cirrhosis-Authors' reply. *Aliment Pharmacol Ther* 2024; **60**: 107-108 [*RCA*] [PMID: 38803242 DOI: 10.1111/apt.18067] [FullText]

104 **Corpechot C**, Chazouillères O, Belnou P, Montano-Loza AJ, Mason A, Ebadi M, Eurich D, Chopra S, Jacob D, Schramm C, Sterneck M, Bruns T, Reuken P, Rauchfuss F, Roccarina D, Thorburn D, Gerussi A, Trivedi P, Hirschfield G, McDowell P, Nevens F, Boillot O, Bosch A, Giostra E, Conti F, Poupon R, Parés A, Reig A, Donato MF, Malinverno F, Floreani A, Russo FP, Cazzagon N, Verhelst X, Goet J, Harms M,

van Buuren H, Hansen B, Carrat F, Dumortier J; Global PBC Study Group. Long-term impact of preventive UDCA therapy after transplantation for primary biliary cholangitis. *J Hepatol* 2020; **73**: 559-565 [*RCA*] [PMID: 32275981 DOI: 10.1016/j.jhep.2020.03.043] [Full Text]

105 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [*RCA*] [PMID: 26597456 DOI: 10.1016/j.jhep.2015.10.006] [FullText]

106 **Levy C**, Kendrick S, Bowlus CL, Tanaka A, Jones D, Kremer AE, Mayo MJ, Haque N, von Maltzahn R, Allinder M, Swift B, McLaughlin MM, Hirschfield GM; GLIMMER Study Group. GLIMMER: A Randomized Phase 2b Dose-Ranging Trial of Linerixibat in Primary Biliary Cholangitis Patients With Pruritus. *Clin Gastroenterol Hepatol* 2023; **21**: 1902-1912.e13 [*RCA*] [PMID: 36343847 DOI: 10.1016/j.cgh.2022.10.032] [FullText]

107 **Mayo MJ**, Pockros PJ, Jones D, Bowlus CL, Levy C, Patanwala I, Bacon B, Luketic V, Vuppalanchi R, Medendorp S, Dorenbaum A, Kennedy C, Novak P, Gu J, Apostol G, Hirschfield GM. A Randomized, Controlled, Phase 2 Study of Maralixibat in the Treatment of Itching Associated With Primary Biliary Cholangitis. *Hepatol Commun* 2019; **3**: 365-381 [*RCA*] [PMID: 30859149 DOI: 10.1002/hep4.1305] [Full Text] [Full Text(PDF)]

108 **Hirschfield GM**, Bowlus CL, Jones DEJ, Kremer AE, Mayo MJ, Tanaka A, Andreone P, Jia J, Jin Q, Macías-Rodríguez RU, Cobitz AR, Currie BM, Gorey C, Lazic I, Podmore D, Ribeiro A, Shannon JB, Swift B, McLaughlin MM, Levy C; GLISTEN Study Group. Linerixibat in patients with primary biliary cholangitis and cholestatic pruritus (GLISTEN): a randomised, multicentre, double-blind, placebo-controlled, phase 3 trial. *Lancet Gastroenterol Hepatol* 2026; **11**: 22-33 [*RCA*] [PMID: 41173016 DOI: 10.1016/S2468-1253(25)00192-X] [FullText]

109 **Okopień B**, Buldak L, Bołdys A. Fibrates in the management of atherogenic dyslipidemia. *Expert Rev Cardiovasc Ther* 2017; **15**: 913-921 [*RCA*] [PMID: 29183206 DOI: 10.1080/14779072.2017.1408410] [FullText]

110 **Choi J**, Xu J, Nguyen VH, Przybyszewski E, Pratt DS, Song J, Carroll A, Michta M, Almazan E, Simon TG, Chung RT. Association between statin use and hepatic decompensation in patients with primary biliary cholangitis: A target trial emulation study. *Hepatology* 2026; **84**: 367-377 [*RCA*] [PMID: 41627867 DOI: 10.1097/HEP.0000000000001701] [FullText]

111 **Björnsson ES**. Hepatotoxicity of statins and other lipid-lowering agents. *Liver Int* 2017; **37**: 173-178 [*RCA*] [PMID: 27860156 DOI: 10.1111/liv.13308] [FullText]

112 **Trivedi PJ**, Kumagi T, Al-Harthy N, Coltescu C, Ward S, Cheung A, Hirschfield GM. Good maternal and fetal outcomes for pregnant women with primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 2014; **12**: 1179-1185.e1 [*RCA*] [PMID: 24321209 DOI: 10.1016/j.cgh.2013.11.030] [FullText]

113 Elafibranor (Iqirvo) for primary biliary cholangitis. *Med Lett Drugs Ther* 2024; **66**: 202-204 [*RCA*] [PMID: 39762188 DOI: 10.58347/tml.2024.1718b] [FullText]

114 Seladelpar (Livdelzi) for primary biliary cholangitis. *Med Lett Drugs Ther* 2025; **67**: 13-15 [*RCA*] [PMID: 39819989 DOI: 10.58347/tml.2025.1720d] [FullText]

115 **Kay HY**, Jang HY, Kim IW, Oh JM. Fibrates and risk of congenital malformations: a nationwide cohort study in South Korea. *Arch Gynecol Obstet* 2024; **310**: 1967-1973 [*RCA*] [PMID: 38553644 DOI: 10.1007/s00404-023-07357-2] [FullText]

116 **Kosmas CE**, Rallidis LS, Hoursalas I, Zoumi EA, Kostara CE. Lipid Profile and Management of Dyslipidemias in Pregnancy. *J Cardiovasc Dev Dis* 2025; **12**: 445 [*RCA*] [PMID: 41295371 DOI: 10.3390/jcdd12110445] [FullText] [Full Text(PDF)]

117 **Lammert C**, Juran BD, Schlicht E, Chan LL, Atkinson EJ, de Andrade M, Lazaridis KN. Biochemical response to ursodeoxycholic acid predicts survival in a North American cohort of primary biliary cirrhosis patients. *J Gastroenterol* 2014; **49**: 1414-1420 [*RCA*] [PMID: 24317935 DOI: 10.1007/s00535-013-0903-1] [FullText]

118 **Chang JI**, Kim JH, Sinn DH, Cho JY, Kim KM, Oh JH, Park Y, Sohn W, Goh MJ, Kang W, Gwak GY, Paik YH, Choi MS, Lee JH, Koh KC, Paik SW. Clinical Outcomes and Validation of Ursodeoxycholic Acid Response Scores in Patients with Korean Primary Biliary Cholangitis: A Multicenter Cohort Study. *Gut Liver* 2023; **17**: 620-628 [*RCA*] [PMID: 36999383 DOI: 10.5009/gnl220420] [FullText] [Full Text(PDF)]

119 **Koky T**, Drazilova S, Janicko M, Toporcerova D, Gazda J, Jarcuska P. Early assessment of treatment response in primary biliary cholangitis: key to timely management. *BMC Gastroenterol* 2025; **25**: 571 [*RCA*] [PMID: 40783513 DOI: 10.1186/s12876-025-04138-w] [FullText] [Full Text(PDF)]

120 **Levy C**, Bowlus CL. Primary biliary cholangitis: Personalizing second-line therapies. *Hepatology* 2025; **82**: 895-910 [*RCA*] [PMID: 39707635 DOI: 10.1097/HEP.0000000000001166] [FullText]

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REVIEW

Peribiliary and colonic crypt glands in primary sclerosing cholangitis and inflammatory bowel disease pathogenesis: New research perspectives

Vasiliy Ivanovich Reshetnyak, Alexandra Andreevna Reshetnyak, Igor Veniaminovich Maev

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Abstract

Primary sclerosing cholangitis (PSC) is a chronic, continuously progressive cholestatic liver disease of unknown etiology. It is characterized by inflammation, local foci of obliterating fibrosis with proximal segmental dilatation of the bile ducts, which is caused by the formation of multifocal biliary concentric strictures. The classic form of PSC affects mainly men and occurs with damage to the extrahepatic and large intrahepatic (segmental and lobar) bile ducts. The disease naturally progresses to secondary biliary cirrhosis of the liver, portal hypertension, liver failure with the possible development of cholangiocellular carcinoma. In 60%-80% of cases, it is associated with inflammatory bowel disease (IBD). In recent years, a rarer form of PSC with involvement of small (septal) intrahepatic bile ducts has been highlighted separately. However, in any form of PSC, the small intralobular and interlobular bile ducts remain intact. The mechanism of PSC development remains unclear, as well as why the pathological process develops mainly in the extrahepatic and large intrahepatic bile ducts. The question of the mechanism of formation of multiple local irregularly distributed concentric strictures alternating with proximal dilatation of the bile ducts also remains unanswered. Furthermore, the strong association between PSC and IBD remains unexplained. In the proposed review, the authors try for the first time to answer these questions based on the assumption that in the bile ducts and colon the presence of a common morpho-functional structure, and damage to or dysfunction of these structures may provoke the development of a pathological process in PSC. Such a unifying structure is the glands that produce a protective secretion con-

taining mucin for the bile ducts and colon. In the bile ducts, these are the peribiliary glands (PBGs), and in the large intestine, these are the glands that form the crypts. It is known that PBGs are absent in small intralobular and interlobular bile ducts. PBGs and the glands that form the crypts of the colon mucosa have a similar structure and consist of two types of cells: Secretory cells, which produce secretions, and stem cells, which play an important role in the processes of repair and regeneration. Structural damage and/or functional disorders to cells of the PBGs and glands of the colon crypts are most likely trigger factor in the development of the pathological process in bile duct and colon in PSC with often concomitant IBD.

Key Words: Primary sclerosing cholangitis; Inflammatory bowel disease; Peribiliary glands; Glands of colon crypts; Mucins of the bile ducts; Mucins of the colon mucosa; Fucosyltransferase 2

Core Tip: This review presents a novel hypothesis explaining the pathogenesis of primary sclerosing cholangitis (PSC), focusing on the key role of peribiliary glands (PBGs) and colonic crypt glands as a common morphofunctional structure linking pathology of bile ducts and colon. The hypothesis proposes that simultaneous structural and functional impairment of these mucin-producing glands may trigger PSC development. The unifying mechanism, which links PSC and inflammatory bowel disease (IBD) through dysfunction PBGs and colonic crypt glands, probably closely related to mutations in the *FUT2* gene affecting fucosylation of protective mucins in bile ducts and colonic mucosa. This hypothesis explains key clinical features of PSC: (1) Selective involvement of extrahepatic and large intrahepatic bile ducts; (2) Formation of characteristic concentric strictures; (3) Strong association between PSC in IBD; (4) Sparing of small intralobular and interlobular bile ducts; and (5) Increased risk of cholangiocellular carcinoma and colorectal cancer. The proposed mechanism provides a comprehensive framework for understanding PSC pathogenesis and identifies potential therapeutic targets.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic, cholestatic, continuously progressive liver disease of unknown etiology, characterized by localized inflammation, fibrosis, and segmental dilation of the extrahepatic and large intrahepatic bile ducts. The natural course of PSC leads to cholestasis, secondary biliary cirrhosis, portal hypertension, liver failure, and, quite often, cholangiocellular carcinoma (CCC)[1-3].

The causes of PSC remain unknown, and its pathogenesis has not been well studied[4]. It is hypothesized that genetic factors (particularly *HLA* genes), as well as environmental and immunological factors, play a role in its development[3,5,6]. There is evidence of a hereditary predisposition to this disease. However, according to available data, less than 10% of cases of PSC inheritance can be explained by genes that have been identified as being associated with this disease[3].

Notably, only about half of the genes associated with PSC are also linked to ulcerative colitis (UC) or Crohn's disease (CD), or both of these conditions[2,3,7]. The most widely accepted current hypothesis regarding the pathogenesis of PSC suggests that it arises as an immune response in individuals with a genetic predisposition following exposure to certain environmental factors or toxic substances, including lipopolysaccharides, which are structural components of bacterial cell walls[8-10].

However, it remains unclear why the pathological process is localized primarily in the extrahepatic and large intrahepatic bile ducts and does not affect the small intralobular and interlobular biliary ducts. The question of the mechanism of formation of multiple, unevenly spaced, concentric strictures, with proximal dilatation of the bile ducts, in these ducts, also remains unanswered. Furthermore, there is no explanation for the strong association between PSC and inflammatory bowel disease (IBD).

To answer these questions, it is logical to assume that there are structures in the bile ducts and colon with similar architecture and function. Simultaneous damage or dysfunction of these structures, caused by certain exogenous or endogenous factors, may trigger the simultaneous development of a pathological process in both the bile ducts and the colon. Identifying these structures would help to understand and explain the association between PSC and IBD, as well as the formation of multifocal biliary concentric strictures in the bile ducts, which develop predominantly in large bile ducts in classic PSC.

We hypothesize that such structures could be peribiliary glands (PBGs) in the bile ducts and crypt-forming glands in the colon. Both types of glands have a similar morphological and functional structure and produce mucin-rich secretions that protect cholangiocytes and colonic epithelial cells. This review compiles materials supporting this hypothesis.

Biliary epithelial cells (BECs) and colonocytes are constantly exposed to various aggressive damaging factors. BECs, which line the bile ducts, are affected by bile components, while colonocytes are exposed to fecal matter and toxic products formed during digestion and the vital activity of microorganisms. This requires specific and effective defense mechanisms to protect against these threats.

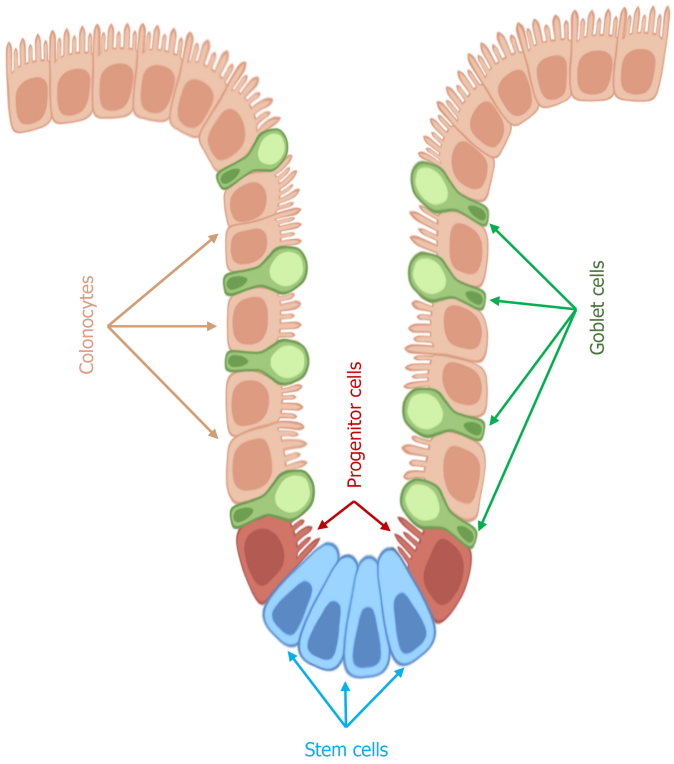


Figure 1 Schematic representation of the structural organization and cellular composition of colonic crypts, including the main cell types: Colonocytes, progenitor cells, goblet cells, and stem cells. The figure demonstrates the tubular organization of the crypt and the distribution of different cell populations.

FACTORS OF AGGRESSION AND DEFENSE OF COLONOCYTES

Aggressive factors include: (1) Toxic metabolites produced by opportunistic bacteria, which can alter colon wall permeability and colonocyte regeneration processes; (2) The composition and changes in the abundance and ratio of microorganisms in the colon microflora can also affect the permeability and barrier function of the mucous membrane; (3) Endogenous factors, such as bile acids, products of impaired metabolism, pro-inflammatory and vasoactive mediators, can have a damaging effect on the crypt-forming glands and colon epithelium; and (4) Exogenous factors, such as irritating food components and/or medications, can also have a damaging effect on colonocytes.

A pre-epithelial mucus-bicarbonate barrier provides balanced protection of the colon mucosal epithelium against damaging factors. It effectively protects colonocytes from the aggressive effects of antigens, acids, alkalis, enzymes, and other toxic substances, as well as from mechanical damage associated with the passage of fecal matter. The main component of the pre-epithelial mucus protective barrier is mucins. They play a key role in protecting colonocytes from bacteria and other damaging agents. Mucins are glycoproteins synthesized by secretory cells located in the glands forming the crypts.

COLON CRYPTS: CELLULAR COMPOSITION AND GLANDULAR ORGANIZATION

The glands of the large intestine are vital structures responsible for producing mucus. This secret provides reliable protection for colonocytes against various aggressive factors[9-12]. There are between 7.5 million and 12.0 million crypts, which are located between the villi and extend from the surface of the mucous membrane to the underlying muscular layer throughout the entire length of the large intestine, all the way to the anal opening[9]. In adults, the wall of the cecum contains approximately 4.5% of the total number of these glands, the ascending colon contains about 22%, the transverse colon 27%, the descending colon 21%, in the sigmoid colon - 20%, and in the rectum - 5.5% of the glands[9]. The crypts in the right half of the large intestine are deeper, but their density per unit area is slightly lower than in the left sections[9]. The left half of the large intestine, especially the rectum, is characterized by a high density of crypts per unit area. Despite the lower density of crypts, the right half of the large intestine compensates for this with greater depth and total surface area of the crypts.

Normally, crypts are microscopic, straight, blind-ended tubular structures arranged parallel to one another (Figure 1). Their average length is approximately 0.5 mm: Longer in the proximal and shorter in the distal sections of the large intestine[13]. The walls of the glands are lined with a single-layer of epithelium, which is located on the basal membrane. The crypts contain various types of cells[9,14]: (1) Absorptive cells participate in the absorption process; (2) Goblet cells produce mucus containing mucins; (3) Undifferentiated (stem, mesenchymal) cells responsible for regeneration; and (4)

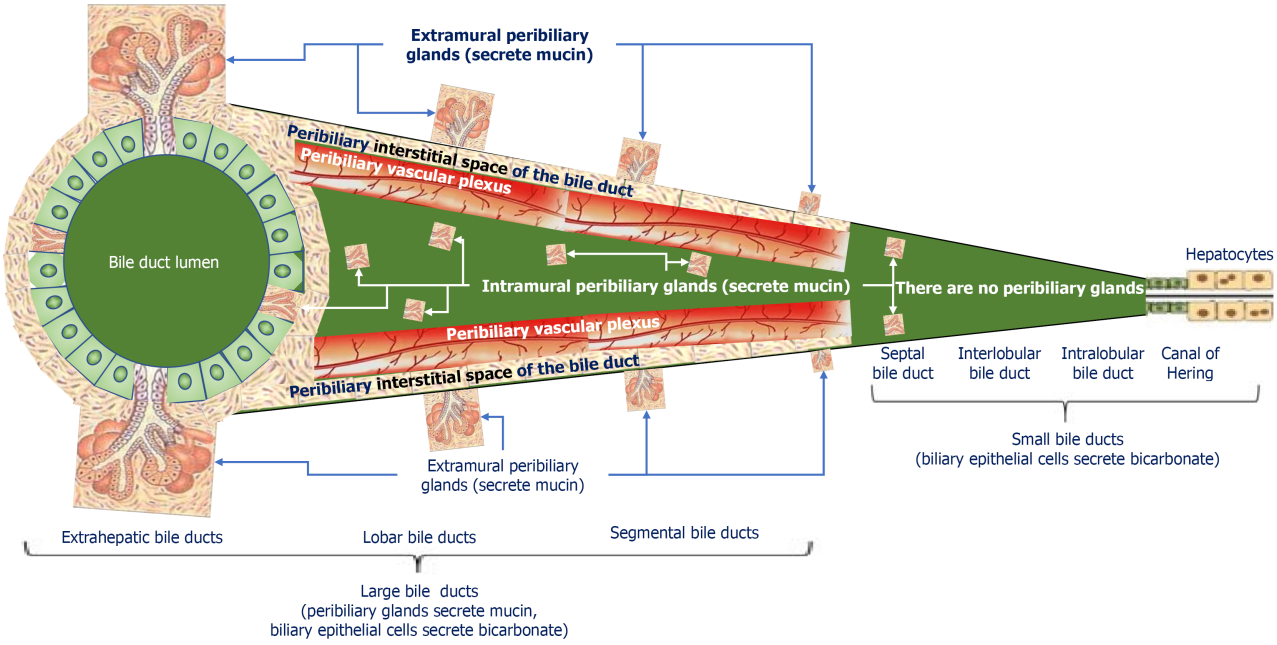


Figure 2 Schematic representation of the location of intramural and extramural peribiliary glands in the large bile ducts.

Progenitor cells, which develop into mature, differentiated cells from stem cells during their maturation.

In the crypts of the cecum and ascending colon, goblet cells account for 44%-49% of all epithelial cells[9]. In the sigmoid colon, their proportion decreases to 33%-44%. The number of undifferentiated cells increases as one approaches the rectum: From 12%-19% in the glands of the cecum to 19%-26% in the glands of the rectum[9]. Stem cells play a key role in regenerating both the glandular and covering epithelium of the mucous membrane. Regenerative processes are particularly important in the distal colon, given that fecal matter becomes denser in this region, increasing the risk of intestinal wall damage.

FACTORS OF AGGRESSION AND DEFENSE OF CHOLANGIOCYTES IN THE BILE DUCTS

Bile constitutes an aggressive environment for the cholangiocytes lining the intra- and extrahepatic bile ducts. The bile acids present in bile possess strong detergent properties, which can damage the cell membranes of cholangiocytes. Bile acids have a cytotoxic effect on many types of cells[15]. However, under physiological conditions, despite exposure to very high (millimolar) concentrations of bile acids, the epithelial cells of human bile ducts remain resistant to their cytotoxic effects[16]. This resistance indicates the presence of mechanisms that protect cholangiocytes from the detergent action of bile acids.

The conjugation of bile acids with glycine and taurine, as well as the formation of mixed micelles with cholesterol and phospholipids - these are protective mechanisms that act at the level of hepatocytes, and bile capillaries[16]. A well-known protective factor of the bile duct epithelium is the glycocalyx, which covers cholangiocytes and consists of mucin glycoproteins and bicarbonate (HCO_3^-)[17]. Under physiological conditions, the primary function of cholangiocytes is the biliary secretion of HCO_3^-[18]. Cholangiocytes produce HCO_3^- throughout the entire biliary tree. Mucin glycoproteins are produced by the PBGs, which are found only in the large bile ducts[19].

PBGs

PBGs are small tubule-alveolar structures that are located within and outside the walls of the extrahepatic and large intrahepatic bile ducts (Figure 2). The excretory ducts of these glands open directly into the bile duct lumen (Figure 2). Figure 3 shows how densely the PBGs are located along the biliary tree. The largest number of PBGs (shown in dark green in Figure 3) are located at the point where the cystic duct branches off, in the region of the hepatopancreatic ampulla, as well as in the area of the liver porta[20,21] (Figure 3). Light green indicates areas with low content of PBGs, while uncolored branches of the biliary tree indicate the absence of PBGs. In approximately 40% of cases, intramural PBGs can be detected in the septal bile ducts, which are located between the interlobular and larger (segmental) intrahepatic bile ducts[17,20]. However, PBGs are absent in the gallbladder and in the small intralobular and interlobular bile ducts[20,21] (Figures 2 and 3).

Based on their location relative to the wall of the bile duct, PBGs are divided into two types: Intramural and extramural [22,23]. Intramural PBGs are simple tubular mucinous glands. They take the form of shallow invaginations and are

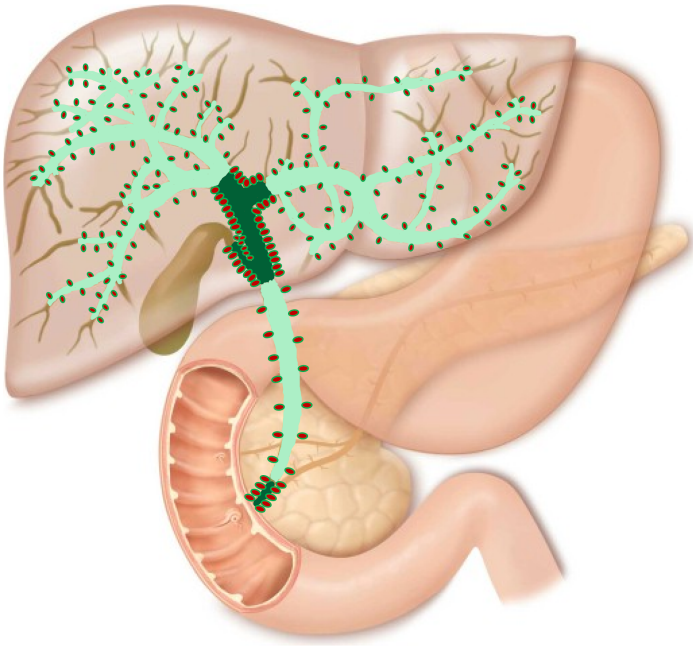


Figure 3 Density of peribiliary glands distribution in the biliary tree. Dark green indicates high peribiliary glands (PBGs) density, light green indicates areas with fewer PBGs, and uncolored branches of the biliary tree indicate the absence of PBGs.

randomly distributed within the walls of the extrahepatic and large intrahepatic bile ducts[24] (Figure 2). The mucinous secretion they produce flows directly into the lumen of the bile ducts[22]. Extramural glands consist of branched tubulo-alveolar acini, which may be serous, mucinous, or mixed, and have a lobular structure. These glands are located outside the wall of the bile duct, in the periductal space, and open into the lumen of the extrahepatic and large intrahepatic bile ducts. In the intrahepatic bile ducts, extramural PBGs are found only in the large lobar and segmental ducts[20,25] (Figure 2). Due to their small size, PBGs are usually not detected using standard clinical imaging methods, except in cases of obvious pathological conditions.

PBGs consist of mature and undifferentiated cells that can actively divide and proliferate, both under normal conditions and in various hepatobiliary pathologies[25]. Three main cell types can be identified within these glands[20]: (1) Secretory [mucinous, (PAS+)] cells, which form the seromucinous portion of the PBGs acinus and are responsible for mucin production; (2) Stem (CD34+) cells, which are located on the bottom of PBGs near the fibromuscular layer and involved in the renewal of secretory cells and cholangiocytes of the extrahepatic and large intrahepatic bile ducts[19,24,26]; and (3) Epithelial cells (cholangiocytes), which are located at the place where the PBGs confluence with the lumen of the bile duct. For a long time, it was believed that the sole function of the PBGs was mucin production. However, recent data suggest that these glands contain multipotent stem cells which can differentiate into hepatocytes, cholangiocytes and pancreatic cells[20,21].

Progenitor (intermediate) cells are located in the middle portion of the glandular acini and develop into mature, differentiated cells during maturation from stem cells. The number of proliferating cells decreases as they approach the surface of the gland, as they transform into goblet cells and cholangiocytes[19,27]. The PBGs and their stem cells actively participate in restoring the integrity of extrahepatic and large intrahepatic bile ducts, both under normal conditions and in pathologies of the biliary tract. This active differentiation process occurs during reparative regeneration following duct damage caused by viral infections, cholestasis, or other factors, and is aimed at restoring the structure and function of the biliary tract[22,24,25]. It is hypothesized that PBGs stem cells are involved in the initiation and progression of CCC through the transformation of stem and progenitor cells into cancer cells[28].

MUCINS: THE ROLE OF GOBLET CELLS IN THE PBGs AND COLON CRYPTS

Goblet cells, which are found in the PBGs and the glands of the colon mucosa crypts, are important producers of mucins.

The mucins synthesized by the glands of the colon crypts

The large intestinal mucosa provides reliable protection against the penetration of bacteria and damaging aggressive factors thanks to mucus and the glycocalyx, which are produced by the glands of the colon crypts. The mucus covering the epithelium of the colon consists of two layers: (1) The outer layer is loose, low-adhesive, and abundant in microorganisms; and (2) The inner layer is dense, highly adhesive, and impermeable to microorganisms.

The main structural components of colonic mucus and the glycocalyx are mucins, which constitute a family of highly glycosylated glycoproteins[29,30]. They consist of a protein component (apomucin) and numerous O-linked oligosaccharide chains[29,31]. Two types of mucins are distinguished: Secretory and membrane-associated.

The inner mucus layer consists of a dense network of polymerized type 2 mucin (MUC2) molecules, which are synthesized by goblet cells of the colon crypt glands[29]. This layer adheres tightly to the colon epithelium and cannot be removed by simple aspiration. Under normal conditions, it is impermeable to bacteria and particles larger than 0.5 μm [32].

The outer mucus layer is looser, easily removed, and densely populated by bacteria. It forms as a result of partial degradation and loosening of the mucin network of the inner layer. This process is likely mediated by the intestine's own proteases, as it can be observed in mice lacking intestinal microflora, and protease inhibitors prevent the formation of the outer mucus layer.

The carbohydrates that make up mucins serve as a nutrient source for commensal bacteria. Many of these bacteria have specialized operons for various types of complex carbohydrates, meaning that the patterns of glycosylated mucins can influence bacterial selection[32,33]. Beneath the mucus layer in the colon lies the glycocalyx - a kind of "sieve" composed of molecules bound to the plasma membrane of the colonocytes. This "sieve" does not allow large molecules and bacteria to pass through.

The glycocalyx also contains mucins that have a transmembrane domain and protrude 200-1500 nm above the surface of the epithelial cell[30]. The stability of the mucus layer is maintained by a balance between its secretion by goblet cells of colon crypts and its degradation by proteases and glycosidases, as well as by mechanical flushing with intestinal contents [29,30]. The glycocalyx performs several important functions: It prevents bacterial adhesion and invasion, protects the epithelium from mechanical damage, and participates in cellular signal transmission.

In the large intestine of a healthy person, the primary secretory mucin produced by goblet cells throughout the crypts is MUC2. Another secretory mucin that plays an important role in the intestine is MUC5B, which possesses unique antimicrobial properties. However, its expression in the colon is weak and remains at a low basal level. Along with the dominant MUC2, a group of membrane-bound mucins - MUC1, MUC3, MUC4, MUC12, MUC13 and MUC17 - is actively synthesized in the glands of the crypts in the large intestine under normal conditions. These mucins form an important protective glycocalyx layer.

The mucins synthesized by the PBGs

One important function of the PBGs is to produce mucin glycoproteins. These glycoproteins, together with bicarbonate (HCO_3^-), protect the cholangiocytes that line the large bile ducts from the aggressive, detergent-like effects of bile acids[18,20]. Experimental data indicate that the glycocalyx covering the apical surface of large cholangiocyte membranes contains glycosylated mucins and other glycan-containing membrane glycoproteins. The glycocalyx stabilizes the "biliary bicarbonate umbrella", thereby helping to protect cholangiocytes from the detergent action of bile acids[34].

The key mucins comprising the glycocalyx of large bile ducts are MUC3 and, possibly, MUC5B, synthesized by the PBGs. The mucins produced by the PBGs, together with HCO_3^-, provide protection for cholangiocytes only in extrahepatic and large intrahepatic bile ducts[17]. Intralobular, interlobular, and many septal bile ducts lack PGBs, resulting in the absence of a protective mucin-containing secretion in these ducts[19] (Figure 2). As a result, at the level of small bile ducts, cholangiocytes are protected only by HCO_3^-[17-19,34-36].

Under normal conditions, there is a balance between aggressive factors, such as bile acids, and protective mechanisms, such as the secretion of bicarbonate and mucin glycoproteins. Therefore, the mucins produced by the PBGs and the glands of the colon crypts are highly specialized secretions, perfectly adapted to their location. Mucins from the PBGs, together with bicarbonate, provide protection for the cholangiocytes, while mucins from the colon crypts serve not only an important protective function for colonocytes but also act as a "lubricant" and nutrient medium for commensal microbes.

It is worth noting that two common types of mucins can be distinguished in the glycocalyx covering the epithelial cells of the bile ducts, as well as in the glycocalyx covering colonocytes: Membrane-bound MUC3 and secreted MUC5B. Impaired secretion or a change in the structure and conformation of these mucins can play a role in damage to both the PBGs of the bile ducts and the colon crypt glands. This, in turn, can lead to the simultaneous development of a pathological process in the liver and the large intestine.

Current data indicate an association of PSC with a mutation of the *FUT2* gene, encoding the enzyme fucosyltransferase 2, which is involved in the synthesis of mucin - a component that ensures the stability of glycocalyx[31,37]. Dysregulation of expression of the *FUT2* gene, leading to the synthesis of abnormal mucin and destabilization of the glycocalyx, can initiate in the PBGs a cascade of pathological changes, including uncontrolled cell proliferation, impaired apoptosis, and pathological fibrogenesis. In genetically predisposed individuals, these mechanisms may represent key pathogenic factors in the development of both isolated PSC and PSC-IBD. Furthermore, it is likely that the *FUT2* mutation may serve as a potential biomarker for assessing the risk of occurrence and progression of both isolated PSC and PSC-IBD, requiring further validation in prospective studies.

Fucosyltransferase 2 and mucins in PSC

The FUT2 enzyme plays a key role in the incorporation of fucose into the sugars of mucin glycoproteins and glycolipids, which are components of secreted and membrane-bound mucins synthesized by PBGs and glands of the colon crypts[31]. Fucosylation of glycoproteins promotes cell-cell interactions, including those between host cells and bacteria[38-40].

Mucins that are fucosylated by FUT2 serve as a primary nutrient source and, consequently, as a selective substrate for commensal bacteria. These bacteria break down complex carbohydrates and contribute to the formation of a healthy gut microbiota[40]. Fucose also influences the properties of mucins, such as their hydrophilicity, charge, and intermolecular interactions.

Since the same enzyme, FUT2, is involved in mucin synthesis in both the PBGs and the glands of the colon crypts, disruption of fucosylation processes may weaken the protective properties of the glycocalyx mucin layer in both the bile ducts and the colon mucosa. Therefore, the described mutation in the *FUT2* gene in PSC poses a common threat to two critically important mucosal barriers: The colonic barrier and the biliary barrier in the large bile ducts. This dual vulnerability may explain the high frequency of PSC co-occurrence with IBD.

The *FUT2* gene encodes the enzyme fucosyltransferase 2, which is likely to play an important role in the pathogenesis of PSC in genetically predisposed individuals[37,41,42]. Nonsynonymous mutations, known as missense mutations, have been identified in this gene in patients with PSC[31,37]. These mutations lead to changes in the protein's structure, which, in turn, can result in the loss or alteration of its function.

The most common mutation in the gene is rs601338 (G428A), which renders the enzyme inactive[37]. Mutated alleles (A) of this gene (AA or AG genotypes) were identified in 67.9% of patients with PSC, which significantly exceeds the frequency in the general population (approximately 20%). This underscores the importance of this genetic risk factor in the development of the disease[31,37]. In carriers of inactive *FUT2* alleles, the risk of developing PSC increases by approximately 1.5-fold[31,37]. This is one of the most reproducible genetic associations in PSC[31,37].

It can be hypothesized that the relationship between a mutation in the *FUT2* gene and the pathogenesis of PSC may occur due to impaired mucins synthesis by the PBGs and the colonic crypt glands. A mutation in the *FUT2* gene presumably can lead to disruption of terminal fucosylation, which, in turn, may cause the synthesis and incorporation of structurally abnormal mucins into the glycocalyx of the colon and bile ducts in genetically predisposed individuals.

To restore the protective properties of the glycocalyx layer, the PBGs and the colon crypts glands begin to compensatorily intensively produce additional mucins. Increased mucin synthesis activates stem cells to increase the number of goblet cells that produce mucin glycoproteins. Since the *FUT2* gene mutation is a constantly active factor causing the formation of defective mucins, this leads to continuous activation of stem cells and an increase in their differentiation into goblet cells. This process results in the proliferation of the PBGs and the colon crypt glands.

Thus, a mutation in the *FUT2* gene, which is present in both the PBGs and the colon crypt glands, can lead to a defect in mucin synthesis in the liver and the large intestine, creating two independent sites of vulnerability. Unfortunately, data on the *FUT2* genetic mutation in PSC are scarce. For example, we were unable to find data in the scientific literature on the prevalence of the *FUT2* genetic mutation in isolated PSC, in PSC co-occurring with UC, and in isolated UC without PSC.

If this hypothesis is correct, then the combination of PSC and IBD caused by a mutation in the *FUT2* gene represents a single systemic pathological process. Perhaps this assumption explains the unique IBD phenotype in patients with PSC, which is actively discussed by the scientific community[7,43,44].

In addition to the key *FUT2* gene, associations have been identified in PSC with mutations in other genes that also may significantly impact the synthesis, maturation, and function of mucins: (1) Galactose-3-O-sulfotransferase 2 - participates in the sulfation of mucin oligosaccharide chains; and (2) Glucosaminyl (N-acetyl) transferase 4 - responsible for the synthesis of branched O-glycans (complex sugar chains) in mucins.

Mutations in these genes are not as common in PSC and do not have as significant an impact on its development as mutations in the *FUT2* gene. Nevertheless, they indicate a genetic predisposition to defects in the synthesis of fully functional protective mucin glycoproteins in both the colon and the bile ducts. When the proliferation process becomes chronic and progressive, it can lead to the development of fibrotic and neoplastic processes, which are frequently observed in patients with PSC and PSC co-occurring with IBD.

CONCENTRIC STRICTURES IN PSC: PATHOGENETIC SIGNIFICANCE OF PBGs

The clinical presentation of PSC is highly variable. Most often, the onset of clinical signs is driven by disease complications, such as strictures with proximal dilatation of the bile ducts, and the resulting cholestasis, bacterial cholangitis, and liver cirrhosis[2,45-47]. The formation of bile duct strictures is a common complication of the classic form of PSC, which affects the large bile ducts. In 45%-60% of patients with PSC, the formation of a dominant stricture is observed[37]. Notably, the fibrotic process in the bile ducts correlates spatially with the locations of the PBGs, suggesting their involvement in PSC pathogenesis.

In the classic form of the disease, strictures typically form in the extrahepatic and larger intrahepatic bile ducts, where both intramural and extramural PBGs are located. PSC with involvement of small (septal) intrahepatic bile ducts, where intramural PBGs are predominantly present, is less common. Small intralobular and interlobular bile ducts, which lack PBGs completely, are never affected by PSC.

Multifocal strictures indicate focal rather than diffuse damage to bile ducts. Local concentric stenoses caused by strictures result from periductal fibrosis, which, according to the proposed hypothesis, originates in the PBGs. The exact mechanism of periductal fibrosis in PSC remains incompletely understood.

Scientific evidence indicates that PBGs are key to the development of the fibrosis and underlies multifocal concentric strictures. Under physiological conditions, PBGs maintain a homeostatic balance between stem cell proliferation and goblet cell apoptosis. In PSC, persistent and prolonged proliferation in the PBGs, caused by impaired synthesis of protective mucins, may lead to an imbalance of these processes. This imbalance can lead to fibrogenesis, directly linking PBGs dysfunction to the development of fibrosis[48].

Periductal fibrosis in PSC: Key mechanisms

According to the theory of fibrogenesis, the mechanism of fibrosis development in PSC can be explained by the

progressive proliferation and uncontrolled hyperplasia of the PBGs in large bile ducts[24,48]. Carpino *et al*[24] convincingly demonstrated that, in PSC, stem cells of the PBGs are activated and acquire the myofibroblast phenotype. Their data indicate a significant increase in the area occupied by α-smooth muscle actin (α-SMA)-positive myofibroblasts in large bile ducts affected by fibrosis (15.8 ± 2.3 *vs* 0.8 ± 1.1 in normal ducts; $P < 0.05$). The expansion of the PBGs area and the area occupied by α-SMA-positive myofibroblasts in fibrotic large bile ducts indirectly indicates active local proliferation processes.

Myofibroblasts exhibit high fibrogenic activity: They proliferate rapidly and, due to α-SMA expression, become contractile, enabling them to migrate and colonize the interstitial space[49]. The locally formed numerous myofibroblast populations activate type I collagen synthesis, which forms a fibrous structure, thickening the wall around the bile duct, forming a stricture that leads to bile duct stenosis. Notably, the wall thickness of bile ducts affected by fibrosis (861.1 ± 299.8 μm; $P < 0.05$) in PSC is statistically significantly greater than the wall thickness in normal bile ducts (100.3 ± 15.0 μm) and in adjacent bile ducts without fibrosis (103.7 ± 20.5 μm)[24]. Local increased proliferation and hyperplasia of PBGs in PSC are confirmed by a statistically significant elevation of the area occupied by these glands in large fibrotic bile ducts (5.9 ± 2.1 *vs* 1.64 ± 0.51 in the control and 1.8 ± 0.7 in large ducts without fibrosis; $P < 0.02$)[24]. Moreover, the wall thickness of the bile duct with fibrosis positively correlates with the area occupied by α-SMA-positive myofibroblasts ($r = 0.855$; $P = 0.014$) and the PBGs area ($r = 0.811$; $P = 0.015$)[24].

Excessive myofibroblast activity leads to remodeling of the periductal space. Local periductal fibrosis affects specific bile duct segments, resulting in stricture formation and ductal stenosis. The formation of bile duct strictures in PSC appears to be the final stage of chronic proliferation and fibrosis of the extramural PBGs.

Studies by Carpino *et al*[24] indicate that the primary pathological process in PSC develops in the PBGs, with subsequent involvement of cholangiocytes. As the disease progresses, the area of proliferating PBGs expands. Cholangiography reveals multifocal, ring-shaped strictures alternating with segments of normal or slightly dilated bile ducts containing sac-like protrusions resembling diverticula[45,50].

Fibrosis of the PBGs in PSC of small (septal) bile ducts

In clinical practice, a special form of PSC of small bile ducts is distinguished. This form is characterized by signs of cholestasis and a typical histological pattern. However, it shows no strictures in the large bile ducts on cholangiography [46,51,52]. This type of PSC is less common, occurring in approximately 10%-20% of cases, and has a more favorable prognosis[52]. We hypothesize that in this form of PSC, the intramural PBGs located within the wall of small septal bile ducts are damaged. However, this assumption remains hypothetical and requires rigorous clinical and morphological validation through further research. Since intramural PBGs are present in only 40% of septal bile ducts, the prevalence of this disease form is significantly lower.

The mechanism of fibrosis development in PSC of the small bile ducts is similar to that developed in the extramural PBGs during classic form of PSC: (1) Activation of stem cells and uncontrolled hyperplasia in intramural PBGs of small septal bile ducts; (2) Acquisition of the myofibroblast phenotype by stem cells; (3) Synthesis of type I collagen; and (4) Formation of local concentric fibrosis, which is described histologically as "onion-skin"[1,52].

In the PSC of small bile ducts, damage to the intramural PBGs is accompanied by the formation of a concentric type "onion-skin" fibrosis surrounding the bile duct. This leads to obstruction of its lumen and development of ductulopenia. In a small number of patients (12%) with PSC of small bile ducts, the disease progresses to the classic form involving large bile ducts[53-55]. Based on the proposed hypothesis, in this case, both intramural and extramural PBGs of extrahepatic and large intrahepatic bile ducts become involved in the pathological process. The appearance of a morphological picture of fibrosis of the "onion-skin" type in large bile ducts probably indicates damage to the intramural PBGs in large intrahepatic bile ducts. Additionally, extramural PBGs are also involved in the pathological process, leading to the formation of local strictures.

Development of cholestasis in PSC

In PSC, bile duct narrowing occurs due to both local fibrous strictures and the development of ductulopenia. This impedes the normal bile flow, causing cholestasis and dilation of the bile ducts proximal of the stricture and/or ductulopenia. Studies show that areas of bile duct dilation in PSC are not associated with damage to extra- or intramural PBGs or cholangiocytes[24]. For this reason, in bile ducts without fibrosis, the area of PBGs, the number of α-SMA-positive myofibroblasts and wall thickness of bile duct remain within normal limits[24]. This suggests that non-fibrotic bile ducts are only minimally involved in the pathological process, particularly in the early stages of PSC[46,56]. This is precisely why a blind liver biopsy in patients with PSC can yield ambiguous results, which can vary significantly depending on the tissue sampling site[46,56]. For this reason, biopsy often proves insufficiently informative[46,56]. It is important to note that dysplastic cells are not detected in the intralobular and interlobular bile ducts, where PBGs is absent[24].

The presented data convincingly demonstrate that in PSC, the pathological process may begin in the PBGs. Local bile duct damage in the form of multifocal biliary concentric strictures and ductulopenia is secondary. Most likely, strictures form at the sites where the PBGs open into the lumen of the bile duct. Cholestasis, which develops proximal to the site of stricture, eventually leads to damage to cholangiocytes and hepatocytes. This is accompanied by cellular senescence, apoptosis and autophagy, as confirmed by the presence of the relevant markers in liver samples and blood plasma from patients with PSC[24,49]. Over time, persistent cholestasis drives disease progression to cirrhosis, with consequent hepatocellular failure and portal hypertension. Furthermore, cholestasis, associated with strictures and ductulopenia, frequently raises the risk and increases the likelihood of the development of cholangitis.

Cholangitis and FUT2 mutations in PSC

The synthesis of physiologically defective fucosylated glycans in PSC predisposes to an increased risk of microbial invasion of bile. Mutations in the *FUT2* gene, identified in patients with PSC, are a significant genetic factor that links impaired fucosylation with the development and spread of various microorganisms in bile. The *rs601338-FUT2* genotype is closely associated with episodes of cholangitis, fungal infection, and the frequency of dominant strictures, which are three clinical features of PSC[37,57,58]. The *FUT2* mutation in these patients directly correlates with an elevated risk of both dominant strictures and cholangitis caused by bacterial and *Candida* infections of the bile. An inactivating mutation in the *FUT2* gene leads to the synthesis of functionally abnormal fucosylated glycans for the glycocalyx, which disrupts its protective properties. As a result, patients with mutant *FUT2* alleles (rs601338) exhibit a statistically significant increase in the frequency of cholangitis episodes (P = 0.0025)[37,57]. Carriers of mutant alleles also show a marked increase in the frequency of *Candida* fungi detection in bile (P = 0.025), which is an additional factor complicating the course of the disease[37,57]. Thus, the *FUT2* gene mutation not only contributes to the formation of a defective protective barrier for the bile ducts, but actively shapes the biliary microbiome, promoting an aggressive fungal-bacterial community that exacerbates disease severity[10,37,57].

THE UNIQUE PHENOTYPE OF IBD IN PSC

Given the frequent co-occurrence of PSC with IBD, intestinal symptoms often precede or coincide the onset of liver disease[4,7,51]. The prevalence of IBD in patients with PSC reaches 90%[7,10]. Patients with PSC exhibit a specific IBD phenotype characterized by pancolitis with relative sparing of the rectum, as well as reverse ileitis[7,43]. Typical symptoms observed in PSC-associated IBD include: Loose stools, sometimes with blood, abdominal discomfort, a rise in temperature, and weight loss. However, these symptoms are less pronounced than in isolated UC and CD. In some patients, IBD onset in combination with PSC may be asymptomatic or present with minimal clinical manifestations until the late stages of the disease[59-61]. More than half of patients with IBD and PSC have a mild course of colitis[62,63]. Furthermore, the endoscopic activity of IBD in PSC is usually lower than in isolated forms of UC and CD[62-64]. Another endoscopic feature of IBD in PSC is that rectal involvement is less frequent and less severe than in isolated UC[3,7].

However, histological examination of biopsy material from patients with IBD and PSC reveals high inflammatory activity in 97% of cases, accompanied by marked infiltration, crypt damage, and an increase in the number of goblet cells in the right parts of the colon[3,65]. Thus, a characteristic feature of colitis in patients with combined PSC and IBD is a proximal inflammatory gradient in the colon, with less pronounced endoscopic activity and more pronounced morphological activity in the caecum and ascending colon[63,66,67]. There is evidence of high expression of angiotensin-converting enzyme (ACE) and ACE2, the main components of the renin-angiotensin system (RAS), in the ileum and colon [68-71]. Dysregulation of these RAS components may have potential implications for inflammation and fibrosis in patients with IBD[68-71].

Interestingly, *FUT2* gene expression is also higher in the proximal colon[72]. This is believed to be associated with the need to produce more glycans, which serve as a nutrient substrate for the resident microbiota in this region. In PSC-associated IBD, a decrease in *FUT2* gene expression is observed. It is logical to assume that this causes structural and functional changes that are more pronounced in the proximal and less pronounced in the distal colon. Therefore, more pronounced morphological changes are observed in these regions, thereby determining disease activity predominantly in the right colon. The degree of involvement of the intestinal crypt glands in the pathological process may vary. Therefore, damage to the colonic mucosa stroma may not be diffuse but focal (zonal). A distinctive feature of colon involvement in PSC is the minimal clinical and endoscopic manifestations, which are discordant with the high morphological activity of colitis. Often, this colitis is an incidental finding during ileocolonoscopy[73].

FIBROSIS OF THE COLONIC MUCOSA STROMA IN IBD CO-OCCURRENCE WITH PSC

Fibrosis of the colonic mucosal stroma is a common feature of PSC-associated IBD. Notably, colonic mucosal fibrosis is observed in many patients with classical PSC, even in the absence of overt IBD signs[73]. This fibrosis can be detected *via* histological examination of colon biopsy specimens and is often associated with eosinophilia in peripheral blood[3,66,74]. The association between IBD and PSC is so strong that the term "PSC-IBD phenotype" has appeared in the scientific literature[7,75,76]. However, in isolated UC, colonic mucosal fibrosis occurs in only 5% of cases[76]. Despite the frequent development of colonic mucosal fibrosis in PSC, the clinical course of IBD in these patients is relatively mild[66,77]. This may be due to the fact that, in the development of both classic PSC and PSC combined with IBD, the glands of the colon crypts are involved in the pathological process not diffusely, but zonally. The mechanism of fibrosis development can be schematically represented as follows: *FUT2* mutation → apoptosis/regeneration imbalance → stem cell activation → proliferation → transdifferentiation into myofibroblasts → collagen synthesis → fibrosis.

Since myofibroblasts are resistant to apoptotic stimuli, and the fibrous collagenous tissue they produce is resistant to degradative enzymes, their excessive activity is a major driver of fibrosis development and of the remodeling of the colonic mucosa stroma[78]. Nielsen *et al*[78] analyzed four highly specific serological markers reflecting extracellular matrix remodeling processes in PSC. These markers reflect the dynamic balance between two opposing processes: (1) Fibrogenesis: *PRO-C3* (interstitial matrix collagen type III) and *PRO-C5* (interstitial matrix collagen type V); and (2) Collagen degradation: *C3M* (marker of collagen type III degradation) and *C4M* (marker of collagen type IV degradation).

The authors demonstrated that in PSC, markers of fibrosis formation are important prognostic factors and are stronger predictors of fibrosis development than degradation markers. Furthermore, they noted that initial bile duct injury in PSC is accompanied by an elevation in C4M levels, which is a marker of collagen degradation. This may reflect the predominance of collagen degradation processes over fibrogenesis in the early stages of the disease, representing a compensatory response aimed at preventing the development and progression of fibrosis in the PBGs of bile ducts and colonic crypt glands. Conversely, levels of interstitial matrix formation markers (*PRO-C3* and *PRO-C5*) increase as the disease progresses, reflecting the advancement of fibrosis in later stages[78].

MESENCHYMAL STEM CELLS OF PBGs AND COLON CRYPT GLANDS IN THE DEVELOPMENT OF CCC AND COLORECTAL CANCER

The development of CCC and colorectal cancer (CRC) in PSC is associated with the activation of mesenchymal stem cells. Activation of stem cells in PBGs and colon crypt glands can stimulate the Hedgehog (Hh) signaling pathway, which plays a crucial role in embryonic development. In adult tissues, this pathway is largely inactive, except in cases where it is required to actively maintain regenerative processes[79]. In PSC, persistent regenerative and proliferative processes in PBGs and colon crypt glands lead to pathological autocrine activation of the Hh signaling pathway. As a result, this pathway is involved not only in fibrogenesis but also in carcinogenesis[80,81]. Within stem cells, an uncontrolled intracellular signaling cascade is triggered, leading to alterations in processes associated with continuous proliferation. This results in hyperplasia and dysplasia of cells[82]. Continuous, uncontrolled autocrine stimulation of the Hh pathway causes persistent, unrestrained self-renewal of stem cells in PBGs and colon crypt glands, which may block differentiation. As a result, this can lead to malignant transformation of stem cells, accompanied by the development of CCC or CRC[80-82].

Development of CCC in PSC

A growing body of scientific evidence suggests that PBGs in PSC play a crucial role in active cell proliferation, which can lead to the malignant transformation of stem cells[24,83,84]. Liver biopsy specimens from patients with PSC reveal progressive hyperplasia and mucinous metaplasia of the PBGs[24]. These processes are observed exclusively in large bile ducts with fibrosis and are absent in ducts without fibrosis[24]. In PSC, activation of stem cells in the depth of the acini of PBGs promotes metaplasia development, which may initiate CCC[24,85]. It should be noted that PSC is associated with a very high incidence of CCC, whereas hepatocellular carcinoma and gallbladder cancer are extremely rare in this disease [86]. This can be explained by the absence of PBGs in the gallbladder; therefore, the gallbladder is not involved in the process of malignant transformation in PSC. These data indirectly suggest that the cause of CCC in PSC is most likely the malignant transformation of stem cells in the PBGs.

CCC has not been reported in patients with small-duct PSC[54,87]. It is likely that the proliferative activity and the number of stem cells in the intramural PBGs of the small septal bile ducts are not so large as to cause a malignant transformation. They may be sufficient for the development of periductular fibrosis and ductulopenia. Schematically, the development of CCC can be described as: Activation of stem cells in the acini of the PBGs → hyperplasia/metaplasia of the PBGs → initiation of cholangiocarcinogenesis.

Development of CRC in PSC with concomitant IBD

CRC is the most dangerous and common complication of IBD combined with PSC[88-92]. Although these patients exhibit a relatively mild clinical course of IBD, including a lower rate of colectomy, they have a higher incidence of CRC[43]. One of the early predictors of potential CRC development is the detection of non-conventional forms of dysplasia (hypermucinous, crypt-cell, and goblet-cell-deficient) in histological examination of colonic mucosa biopsy specimens[93]. A distinctive feature of CRC in IBD-PSC patients is its location in the proximal colon, where the density of goblet cells in the crypt glands is highest[94,95]. In both isolated PSC and PSC combined with IBD, CRC development is most likely associated with dysplasia, which results from stem cell transformation in the glands of the colon crypts[96]. Crypts are part of the transformation zone, and metaplastic processes within them in the IBD with PSC carry a risk of CRC development[97]. As colitis progresses, structural changes occur in the crypts: They become shorter, and the distance between them becomes irregular[13,96,98,99]. Progressive proliferative processes in the colon crypt glands lead to the development of non-conventional forms of dysplasia[93].

According to the World Health Organization definition, dysplasia is a morphological manifestation of neoplastic transformation without signs of invasive growth and is the most important risk marker for cancer development in patients with IBD and PSC[96,100]. Changes in the crypts in the form of non-conventional dysplasia become increasingly noticeable as the disease progresses. These non-conventional dysplasia forms, on the one hand, indicate involvement of the crypts in the pathological process, and, on the other hand, signal a high risk of progressive neoplasia[96,100]. The vast majority (up to 96%) of non-conventional dysplasia in patients with IBD and PSC, as well as in isolated PSC, manifests as flat, invisible lesions, which may subsequently progress to CRC[94]. Patients with isolated PSC without IBD have a lower risk of CRC development compared to those with combined pathology[93]. The presented data indirectly suggest that stem cells of the colon crypt glands are involved in the process of malignant transformation and CRC development.

SEROLOGICAL MARKERS OF PSC

The most extensively studied autoantibodies in PSC are antineutrophil cytoplasmic antibodies (ANCA). They are detected in 65%-95% of patients with this disease[43,101]. ANCA are classified into two types, perinuclear (pANCA) and cytoplasmic (cANCA), depending on their staining pattern in immunohistochemical assays. In PSC, pANCA often react not with classical antigens but with antigens of the neutrophil nuclear envelope. Because of this, they are termed atypical pANCA. Serum levels of these antibodies are detected in: (1) 26%-94% of PSC patients; (2) 33%-83% of UC patients; and (3) 0%-27% of CD patients[102,103].

ANCA-associated vasculitis in PSC

Atypical pANCA, detected in approximately two-thirds of PSC patients[104,105], serve as markers of ANCA-associated vasculitis (small-vessel vasculitis)[106,107]. Detection of atypical pANCA in PSC patients indicates involvement of the neuro-vascular plexus in progressive fibrotic changes which happen in both the periductal regions of bile ducts and the mucosal stroma of the colon. The resulting blood flow impairment leads to local hypoxia, which damages PBGs cells and colonic crypt glands, and thereby exacerbates local fibrotic processes.

PBGs, which actively contribute to fibrogenesis and biliary stricture development in classical PSC, have a close anatomical and pathophysiological association with the periductal neurovascular plexus. This plexus - including branches of the hepatic artery and vein, lymphatic vessels, and nerve fibres - is concurrently involved in the underlying fibrotic pathological process. The involvement of the peribiliary neurovascular plexus in the pathological fibrotic process leads to the development of ANCA-associated small-vessel vasculitis, accompanied by the appearance of atypical pANCA. A correlation exists between the level of atypical pANCA and the degree of bile duct narrowing caused by the strictures[103]. According to various authors, the median prevalence of atypical pANCA in PSC is 63%, with values ranging from 26% to 94%[43,101,103]. This wide variation can be explained by the differences in patient groups across studies. These groups included individuals with different levels of periductal fibrosis progression and different numbers of strictures. This led to varying degrees of involvement of the periductal neurovascular plexus in the pathological process.

Atypical pANCA are also detected in small-duct PSC, where "onion-skin" type periductular fibrosis develops, which also involves the periductal neurovascular plexus[108]. Therefore, the presence of pANCA should be considered not only as an indicator of the development of periductular vasculitis of small vessels[106,107], but also as a marker of the progression of periductular fibrosis in PBGs with the development of strictures and/or ductulopenia in PSC. Since all ANCA immunoglobulin subtypes in the serum and bile of PSC patients reflect the periductal fibrotic process, their levels do not correlate with any laboratory parameters associated with cholestasis and hepatocyte damage[105]. However, a positive immunoglobulin G (IgG) ANCA level in bile correlates with disease activity and the presence of dominant strictures, and thus may serve as a prognostic marker of disease activity[105]. Fibrosis formation in the stroma of the colonic mucosa is also associated with neurovascular plexus involvement in the pathological process. This is supported by positive atypical pANCA test results in patients with IBD concurrent with PSC[109].

Involvement of the neurovascular plexus in the pathological process in PSC plays an important role in the spread of metastases during the development of CCC and CRC[22,110]. The involvement of nerve fibers in the pathological process may be accompanied by unpleasant sensations in the right upper quadrant in PSC and abdominal discomfort in PSC associated with IBD.

IgA antibodies to glycoprotein 2 in PSC

According to various authors, IgA antibodies to glycoprotein 2 (GP2) are detected in the blood plasma of 31%-72% of patients with PSC. These antibodies are either absent or present in less than 3% of healthy individuals[102,111,112]. A study aimed at detecting IgG antibodies to various GP2 isoforms showed that they have lower sensitivity and specificity compared to IgA antibodies to the same GP2 isoforms[113].

GP2 was previously thought to be a protein secreted by pancreatic acinar cells and stored in granules on their membrane. Studies have also shown that it can be found in M cells of the intestinal lymphoid tissue[8]. Lopens *et al*[114] demonstrated that GP2 is also expressed by PBGs in PSC and acts as a specific antigen that binds to IgA autoantibodies. In their study, the authors used three methods: Enzyme-linked immunosorbent assays, immunoblotting, and mass spectrometry to confirm the presence of GP2 in the bile of patients with PSC. Biliary GP2 is detected in the bile of all patients with PSC, whereas it is absent in the bile of healthy individuals[114]. Another important finding of this study was the identification of a correlation between the morphological changes in the PBGs in patients with PSC and the presence of GP2 in the bile[114].

Glycoproteins constitute a large group of proteins with diverse functions: They serve as structural molecules, act as protective agents, participate in substance transport, facilitate cell-cell recognition, and more. One of these functions, the structural one, may determine the role of GP2 in the development of PSC. The presence of GP2 in the bile of PSC patients may indicate its potential role in fibrogenesis, which is actively occurring in PBGs in this disease.

Myofibroblasts, which form from stem cells of PBGs and colon crypt glands, actively synthesize collagen. There is a high probability that during fibrogenesis and collagen synthesis, there is an unusual (immunoreactive) GP2. Notably, immunoblotting of bile samples from PSC patients predominantly reveals a high-molecular-weight isoform of GP2[114]. These data suggest a potential role for this specific GP2 variant in the pathophysiology of PSC. Perhaps this protein contains autoantigenic epitopes that have immunoreactive antigenic properties capable of eliciting an immune response [113]. A "healthy" immune system may recognize this immunoreactive antigen as a foreign protein and start to produce autoantibodies in response.

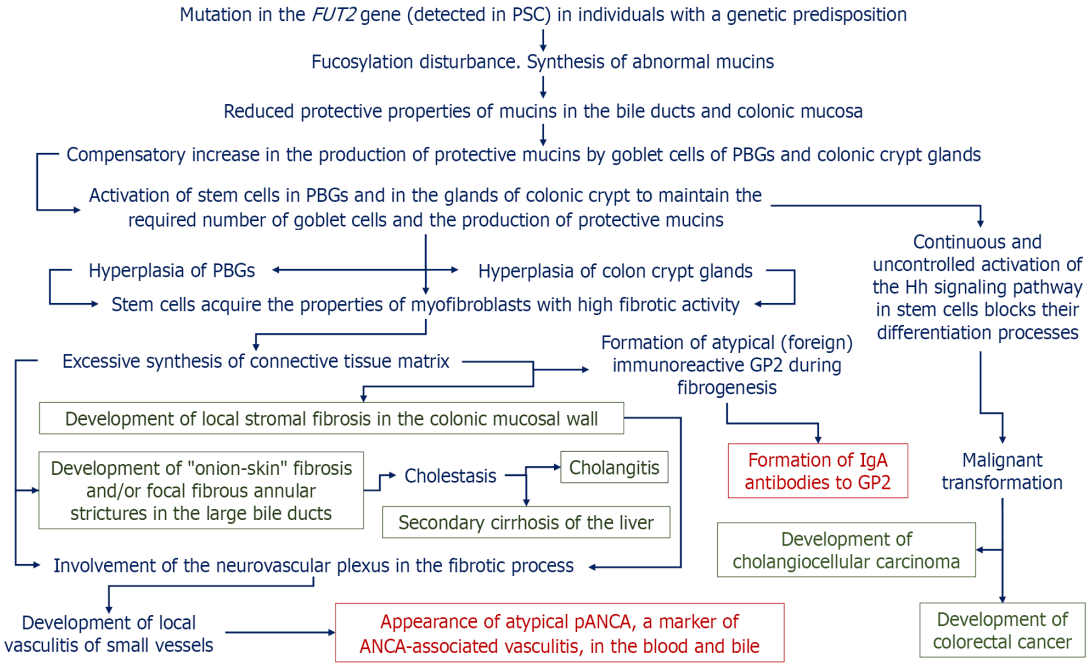


Figure 4 Schematic illustration of the pathogenesis underlying the development of the main clinical and laboratory manifestations in primary sclerosing cholangitis. PSC: Primary sclerosing cholangitis; PBGs: Peribiliary glands; Hh: Hedgehog; GP2: Glycoprotein 2; IgA: Immunoglobulin A; pANCA: Perinuclear antineutrophil cytoplasmic antibodies; ANCA: Antineutrophil cytoplasmic antibodies.

If this is the case, the presence of IgA antibodies to GP2 in PSC may serve as a serological marker for fibrotic changes in both the PBGs of the large bile ducts and the glands of the crypts in the colon mucosa. This hypothesis is supported by the following observations: (1) IgA antibodies to GP2 are detected in both isolated PSC and in cases of PSC combined with IBD[43]; (2) The wide variation in antibody levels reported by different authors indirectly supports this hypothesis: Different degrees of fibrosis correspond to different antibody levels[43,113,114]; and (3) IgA antibodies to GP2 are significantly less common in patients with PSC combined with CD[113].

The presence of IgA antibodies to GP2 in PSC is associated with an increased risk of CCC and higher mortality[115, 116]. Studies by Tornai *et al*[8] demonstrate that patients with IgA antibodies to GP2 have a more severe disease phenotype and lower survival rates, which is thought to be due to increased fibrogenesis or the development of CCC. Similar results regarding the association between anti-GP2 IgA and CCC were obtained by Jendrek *et al*[116] and Wunsch *et al*[117].

The presence of atypical pANCA and IgA antibodies to GP2 in the blood of patients with PSC is a significant indicator of the risk of fibrosis development and its progression in the PBGs of the bile ducts and colon crypt glands, as well as the possibility of developing CCC[102]. The hypothesis suggesting that a mutation in the *FUT2* gene may potentially play a pathogenic role in both isolated PSC and PSC concurrent with IBD is consistent with the proposition that PSC is not a true classical autoimmune disease[118].

CONCLUSION

PSC is described as an idiopathic chronic cholangiopathy characterized by inflammation and fibrosis of the bile ducts. It remains unclear why the pathological process only affects extrahepatic and large intrahepatic bile ducts, while sparing small intralobular and interlobular ducts. Moreover, in the classic form of PSC, the large bile ducts show not diffuse but only local damage, leading to the formation of discretely located concentric fibrotic strictures. Proximal to these fibrotic strictures, dilation of the bile ducts is observed. At the same time, fibrous processes are not observed in these areas. The strong association between PSC and IBD also remains unexplained. The authors of this review, after analyzing the scientific data available in the literature, have formulated a hypothesis that explains the mechanisms underlying the development of major clinical and laboratory signs observed in PSC. According to this hypothesis, there is a common morphofunctional structure in the liver and colon, the simultaneous dysfunction of which may potentially trigger pathological changes in both the liver and the colon among genetically predisposed individuals. In the liver, this structure is the PBGs, and in the large intestine, the crypt glands of the colonic mucosa.

Figure 4 shows a schematic presentation of the mechanism responsible for the development of the main clinical and laboratory signs of PSC, based on the hypothesis that in this disease the pathological process originates in the PBGs of the bile ducts and in the colon crypt glands. The triggering factor of structural and functional changes occurring in the PBGs and crypt glands of the colon is likely to be mutations in genes responsible for the synthesis of enzymes involved in the production of protective mucins.

The hypothesis that in PSC the pathological process may simultaneously emerge in the PBGs of the liver and the colon crypt glands helps explain the close association between PSC and IBD. PBGs and colon crypt glands play an important role not only in mucin production but also in shaping the qualitative and quantitative composition of the microbiota. A mutation in the *FUT2* gene in PSC contributes to the formation of an aggressive biliary microbiota consisting of fungi and bacteria. In the colon, the identified mutation influences the development of gut dysbiosis. This hypothesis of the concurrent involvement of PBGs in the liver and crypt glands of the colon also explains why PSC can develop *de novo* several years after a total colectomy and why IBD may first manifest several years later in patients who have undergone liver transplantation for PSC.

Although the proposed hypothesis sheds light on many aspects of clinical manifestations of PSC and associated complications, further confirmatory studies are undoubtedly needed. Nevertheless, the new perspective on PSC pathogenesis presented in this review opens avenues for the development of early diagnostic methods and the identification of novel therapeutic approaches for this disease.

Limitations of the hypothesis

The proposed hypothesis has several significant limitations that require further investigation and validation: (1) Although the hypothesis effectively explains the relationship between PSC and IBD through shared morphofunctional structures, it does not elucidate the primary etiological trigger that initiates the pathological process in genetically predisposed individuals; (2) The role of *FUT2* gene mutations in the development of PSC requires further in-depth study and confirmation. The scientific literature provides insufficient data on the prevalence of this genetic mutation in isolated PSC, PSC concurrent with IBD, and isolated IBD without PSC; and (3) While the proposed pathogenetic scheme explains the mechanism of development of certain clinical and laboratory manifestations of PSC and associated IBD, the causal relationships outlined in the hypothesis require additional experimental and clinical validation. Despite these limitations, the hypothesis provides a novel perspective on the pathogenesis of PSC and opens new avenues for research in the following areas: (1) Development of early diagnostic methods; (2) Identification of innovative therapeutic approaches; and (3) Conducting further research studies.

REFERENCES

1 **Worthington J**, Chapman R. Primary sclerosing cholangitis. *Orphanet J Rare Dis* 2006; **1**: 41 [*RCA*] [PMID: 17062136 DOI: 10.1186/1750-1172-1-41] [FullText] [Full Text(PDF)]

2 **Vinnitskaya EV**, Abdulkhakov SR, Abdurakhmanov DT, Alikhanov RB, Bakulin IG, Belousova EA, Bueverov AO, Burnevitch EZ, Efanov MG, Eremina EY, Ignatova TM, Ilchenko LY, Karmazanovsky GG, Knyazev OV, Kulezneva YV, Lopatkina TN, Nekrasova TP, Nikitin IG, Pavlenko VV, Parfenov AI, Podymova SD, Raichelson KL, Reisis AR, Sayfutdinov RG, Skazyvaeva EV, Syutkin VE, Khomeriki SG, Haimenova TY, Sandler YG. Important problems in the diagnosis and treatment of primary sclerosing cholangitis (based on the Russian consensus on diagnosis and treatment autoimmune hepatitis. Moscow, 2018). *Ter Arkh* 2019; **91**: 9-15 [*RCA*] [PMID: 31094167 DOI: 10.26442/00403660.2019.02.000075] [FullText]

3 **Raikhelson KL**, Pazenko EV, Prashnova MK, Gomonova VP, Kondrashina EA, Maslyansky AL, Baranovsky AYu. Primary sclerosing cholangitis. 3rd ed. St. Petersburg: Svoe izdatel'stvo, 2025: 48

4 **Manns MP**, Bergquist A, Karlsen TH, Levy C, Muir AJ, Ponsioen C, Trauner M, Wong G, Younossi ZM. Primary sclerosing cholangitis. *Nat Rev Dis Primers* 2025; **11**: 17 [*RCA*] [PMID: 40082445 DOI: 10.1038/s41572-025-00600-x] [FullText]

5 **Karlsen TH**, Schrumpf E, Boberg KM. Primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2010; **24**: 655-666 [*RCA*] [PMID: 20955968 DOI: 10.1016/j.bpg.2010.07.005] [FullText]

6 **Zecher BF**, Schramm C. [Immunopathogenesis and therapy of primary sclerosing cholangitis]. *Z Gastroenterol* 2026; **64**: 56-66 [*RCA*] [PMID: 41587553 DOI: 10.1055/a-2730-4719] [FullText]

7 **van Munster KN**, Bergquist A, Ponsioen CY. Inflammatory bowel disease and primary sclerosing cholangitis: One disease or two? *J Hepatol* 2024; **80**: 155-168 [*RCA*] [PMID: 37940453 DOI: 10.1016/j.jhep.2023.09.031] [FullText]

8 **Tornai D**, Ven PL, Lakatos PL, Papp M. Serological biomarkers for management of primary sclerosing cholangitis. *World J Gastroenterol* 2022; **28**: 2291-2301 [*RCA*] [PMID: 35800183 DOI: 10.3748/wjg.v28.i21.2291] [FullText] [Full Text(PDF)]

9 **Klochkova SV**, Nikityuk DB, Alexeeva NT. Macro-microscopic Anatomy of the Colonic Glands of an Adult. *J Anat Histopathol* 2016; **5**: 31-34 [DOI: 10.18499/2225-7357-2016-5-3-31-34] [FullText]

10 **Kiseleva Y**, Maslennikov R, Poluektova E, Zolnikova O, Sigidaev A, Zharikov Y, Shirokova E, Ivashkin V. Microbiome-immune interactions in autoimmune liver diseases. *World J Gastroenterol* 2026; **32**: 115853 [*RCA*] [DOI: 10.3748/wjg.v32.i12.115853] [FullText] [Full Text(PDF)]

11 **Gerlovin ES**. Histogenesis and differentiation of digestive glands. St. Petersburg: Nauka, 1978: 225

12 **Sapin MR**, Nikityuk DB, Shadlinsky WB, Movsumov NT. Small glands of the digestive and respiratory systems. Moscow: Elista, APP "Dzhangar", 2001: 130

13 **Akhrieva KhM**, Tertychnyy AS, Pachuashvili NV, Urusova LS. Characteristics of the colonic crypts in inflammatory bowel diseases. *vmirvz* 2024; **14**: 42-50 [DOI: 10.20340/vmi-rvz.2024.3.MORPH.4] [FullText]

14 **Magro F**, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, Villanacci V, Becheanu G, Borralho Nunes P, Cathomas G, Fries W, Jouret-Mourin A, Mescoli C, de Petris G, Rubio CA, Shepherd NA, Vieth M, Eliakim R; European Society of Pathology (ESP); European Crohn's and Colitis Organisation (ECCO). European consensus on the histopathology of inflammatory bowel disease. *J Crohns Colitis* 2013; **7**: 827-851 [*RCA*] [PMID: 23870728 DOI: 10.1016/j.crohns.2013.06.001] [FullText]

15 **Hohenester S**, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, Beuers U. A biliary HCO3- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. *Hepatology* 2012; **55**: 173-183 [*RCA*] [PMID: 21932391 DOI: 10.1002/hep.24691] [FullText]

16 **Hofmann AF**. The enterohepatic circulation of bile acids in mammals: form and functions. *Front Biosci (Landmark Ed)* 2009; **14**: 2584-2598

[*RCA*] [PMID: 19273221 DOI: 10.2741/3399] [FullText]
17 **Matsubara T**, Kozaka K, Matsui O, Nakanuma Y, Uesaka K, Inoue D, Yoneda N, Yoshida K, Kitao A, Yokka A, Koda W, Gabata T, Kobayashi S. Peribiliary glands: development, dysfunction, related conditions and imaging findings. *Abdom Radiol (NY)* 2020; **45**: 416-436 [*RCA*] [PMID: 31707436 DOI: 10.1007/s00261-019-02298-4] [FullText]
18 **Banales JM**, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. *World J Gastroenterol* 2006; **12**: 3496-3511 [*RCA*] [PMID: 16773707 DOI: 10.3748/wjg.v12.i22.3496] [FullText] [Full Text(PDF)]
19 **Carpino G**, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, Wang Y, Semeraro R, Anceschi M, Brunelli R, Alvaro D, Reid LM, Gaudio E. Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. *J Anat* 2012; **220**: 186-199 [*RCA*] [PMID: 22136171 DOI: 10.1111/j.1469-7580.2011.01462.x] [FullText] [Full Text (PDF)]
20 **Sharipova EI**, Gazizov IM, Gumerova AA, Kiassov AP. Peribiliary glands of biliary tree as a niche of multipotent stem cells. *Genes Cells* 2014; **9**: 29-33 [DOI: 10.23868/gc120279] [FullText]
21 **Cardinale V**, Wang Y, Carpino G, Alvaro D, Reid L, Gaudio E. Multipotent stem cells in the biliary tree. *Ital J Anat Embryol* 2010; **115**: 85-90 [*RCA*] [PMID: 21072995] [FullText]
22 **Nakanuma Y**, Katayanagi K, Terada T, Saito K. Intrahepatic peribiliary glands of humans. I. Anatomy, development and presumed functions. *J Gastroenterol Hepatol* 1994; **9**: 75-79 [*RCA*] [PMID: 8155872 DOI: 10.1111/j.1440-1746.1994.tb01220.x] [FullText]
23 **Nakanuma Y**. A novel approach to biliary tract pathology based on similarities to pancreatic counterparts: is the biliary tract an incomplete pancreas? *Pathol Int* 2010; **60**: 419-429 [*RCA*] [PMID: 20518896 DOI: 10.1111/j.1440-1827.2010.02543.x] [FullText]
24 **Carpino G**, Cardinale V, Renzi A, Hov JR, Berloco PB, Rossi M, Karlsen TH, Alvaro D, Gaudio E. Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis. *J Hepatol* 2015; **63**: 1220-1228 [*RCA*] [PMID: 26119688 DOI: 10.1016/j.jhep.2015.06.018] [FullText]
25 **DiPaola F**, Shivakumar P, Pfister J, Walters S, Sabla G, Bezerra JA. Identification of intramural epithelial networks linked to peribiliary glands that express progenitor cell markers and proliferate after injury in mice. *Hepatology* 2013; **58**: 1486-1496 [*RCA*] [PMID: 23703727 DOI: 10.1002/hep.26485] [FullText]
26 **Cardinale V**, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology* 2011; **54**: 2159-2172 [*RCA*] [PMID: 21809358 DOI: 10.1002/hep.24590] [FullText]
27 **Lanzoni G**, Oikawa T, Wang Y, Cui CB, Carpino G, Cardinale V, Gerber D, Gabriel M, Dominguez-Bendala J, Furth ME, Gaudio E, Alvaro D, Inverardi L, Reid LM. Concise review: clinical programs of stem cell therapies for liver and pancreas. *Stem Cells* 2013; **31**: 2047-2060 [*RCA*] [PMID: 23873634 DOI: 10.1002/stem.1457] [FullText]
28 **Oikawa T**. Cancer Stem cells and their cellular origins in primary liver and biliary tract cancers. *Hepatology* 2016; **64**: 645-651 [*RCA*] [PMID: 26849406 DOI: 10.1002/hep.28485] [FullText]
29 **Zolotova NA**, Makarova OV. Barrier function of colonic mucins in health and ulcerative colitis. *Clin Exp Morphology* 2016; **3**: 69-74
30 **Johansson ME**, Sjövall H, Hansson GC. The gastrointestinal mucus system in health and disease. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 352-361 [*RCA*] [PMID: 23478383 DOI: 10.1038/nrgastro.2013.35] [FullText] [Full Text(PDF)]
31 **Maroni L**, van de Graaf SF, Hohenester SD, Oude Elferink RP, Beuers U. Fucosyltransferase 2: a genetic risk factor for primary sclerosing cholangitis and Crohn's disease--a comprehensive review. *Clin Rev Allergy Immunol* 2015; **48**: 182-191 [*RCA*] [PMID: 24828903 DOI: 10.1007/s12016-014-8423-1] [FullText]
32 **Pelaseyed T**, Bergström JH, Gustafsson JK, Ermund A, Birchenough GM, Schütte A, van der Post S, Svensson F, Rodríguez-Piñeiro AM, Nyström EE, Wising C, Johansson ME, Hansson GC. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol Rev* 2014; **260**: 8-20 [*RCA*] [PMID: 24942678 DOI: 10.1111/imr.12182] [FullText] [Full Text(PDF)]
33 **Hansson GC**. Mucins and the Microbiome. *Annu Rev Biochem* 2020; **89**: 769-793 [*RCA*] [PMID: 32243763 DOI: 10.1146/annurev-biochem-011520-105053] [FullText]
34 **Maillette de Buy Wenniger LJ**, Hohenester S, Maroni L, van Vliet SJ, Oude Elferink RP, Beuers U. The Cholangiocyte Glycocalyx Stabilizes the 'Biliary HCO3 Umbrella': An Integrated Line of Defense against Toxic Bile Acids. *Dig Dis* 2015; **33**: 397-407 [*RCA*] [PMID: 26045275 DOI: 10.1159/000371864] [FullText]
35 **Reshetnyak VI**, Maev IV. Mechanism of formation and significance of antimitochondrial autoantibodies in the pathogenesis of primary biliary cholangitis. *Explor Immunol* 2024; **4**: 624-639 [*RCA*] [DOI: 10.37349/ei.2024.00163] [FullText]
36 **Reshetnyak VI**, Maev IV. New insights into the pathogenesis of primary biliary cholangitis asymptomatic stage. *World J Gastroenterol* 2023; **29**: 5292-5304 [PMID: 37899787 DOI: 10.3748/wjg.v29.i37.5292] [FullText]
37 **Rupp C**, Friedrich K, Folseraas T, Wannhoff A, Bode KA, Weiss KH, Schirmacher P, Sauer P, Stremmel W, Gotthardt DN. Fut2 genotype is a risk factor for dominant stenosis and biliary candida infections in primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2014; **39**: 873-882 [*RCA*] [PMID: 24612312 DOI: 10.1111/apt.12663] [FullText]
38 **Lindesmith L**, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R. Human susceptibility and resistance to Norwalk virus infection. *Nat Med* 2003; **9**: 548-553 [*RCA*] [PMID: 12692541 DOI: 10.1038/nm860] [FullText]
39 **Cheng S**, Hu J, Wu X, Pan JA, Jiao N, Li Y, Huang Y, Lin X, Zou Y, Chen Y, Zhu L, Zhi M, Lan P. Altered gut microbiome in FUT2 loss-of-function mutants in support of personalized medicine for inflammatory bowel diseases. *J Genet Genomics* 2021; **48**: 771-780 [*RCA*] [PMID: 34419617 DOI: 10.1016/j.jgg.2021.08.003] [FullText]
40 **Hu M**, Zhang X, Li J, Chen L, He X, Sui T. Fucosyltransferase 2: A Genetic Risk Factor for Intestinal Diseases. *Front Microbiol* 2022; **13**: 940196 [*RCA*] [PMID: 35923409 DOI: 10.3389/fmicb.2022.940196] [FullText] [Full Text(PDF)]
41 **Hoskins LC**. The ABO blood group antigens and their secretion by healthy and diseased gastric mucosa. *Ann N Y Acad Sci* 1967; **140**: 848-865 [*RCA*] [PMID: 5339665 DOI: 10.1111/j.1749-6632.1967.tb51007.x] [FullText]
42 **Kelly RJ**, Ernst LK, Larsen RD, Bryant JG, Robinson JS, Lowe JB. Molecular basis for H blood group deficiency in Bombay (Oh) and para-Bombay individuals. *Proc Natl Acad Sci U S A* 1994; **91**: 5843-5847 [*RCA*] [PMID: 7912436 DOI: 10.1073/pnas.91.13.5843] [FullText]
43 **Rolfes PS**, de Zoeten EF, Forman L, Mack CL. Primary sclerosing cholangitis: a narrative review of diagnostic and prognostic biomarkers. *Transl Gastroenterol Hepatol* 2025; **10**: 72 [*RCA*] [PMID: 41221136 DOI: 10.21037/tgh-25-13] [FullText] [Full Text(PDF)]
44 **Ji SG**, Juran BD, Mucha S, Folseraas T, Jostins L, Melum E, Kumasaka N, Atkinson EJ, Schlicht EM, Liu JZ, Shah T, Gutierrez-Achury J,

Boberg KM, Bergquist A, Vermeire S, Eksteen B, Durie PR, Farkkila M, Müller T, Schramm C, Sterneck M, Weismüller TJ, Gotthardt DN, Ellinghaus D, Braun F, Teufel A, Laudes M, Lieb W, Jacobs G, Beuers U, Weersma RK, Wijmenga C, Marschall HU, Milkiewicz P, Pares A, Kontula K, Chazouillères O, Invernizzi P, Goode E, Spiess K, Moore C, Sambrook J, Ouwehand WH, Roberts DJ, Danesh J, Floreani A, Gulamhusein AF, Eaton JE, Schreiber S, Coltescu C, Bowlus CL, Luketic VA, Odin JA, Chopra KB, Kowdley KV, Chalasani N, Manns MP, Srivastava B, Mells G, Sandford RN, Alexander G, Gaffney DJ, Chapman RW, Hirschfield GM, de Andrade M; UK-PSC Consortium; International IBD Genetics Consortium; International PSC Study Group, Rushbrook SM, Franke A, Karlsen TH, Lazaridis KN, Anderson CA. Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease. *Nat Genet* 2017; **49**: 269-273 [*RCA*] [PMID: 27992413 DOI: 10.1038/ng.3745] [FullText] [Full Text(PDF)]

45 **Chapman MH**, Thorburn D, Hirschfield GM, Webster GGJ, Rushbrook SM, Alexander G, Collier J, Dyson JK, Jones DE, Patanwala I, Thain C, Walmsley M, Pereira SP. British Society of Gastroenterology and UK-PSC guidelines for the diagnosis and management of primary sclerosing cholangitis. *Gut* 2019; **68**: 1356-1378 [*RCA*] [PMID: 31154395 DOI: 10.1136/gutjnl-2018-317993] [FullText] [Full Text(PDF)]

46 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**: 237-267 [*RCA*] [PMID: 19501929 DOI: 10.1016/j.jhep.2009.04.009] [FullText]

47 **European Society of Gastrointestinal Endoscopy**; European Association for the Study of the Liver. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. *J Hepatol* 2017; **66**: 1265-1281 [*RCA*] [PMID: 28427764 DOI: 10.1016/j.jhep.2017.02.013] [FullText]

48 **Overi D**, Carpino G, Cardinale V, Franchitto A, Safarikia S, Onori P, Alvaro D, Gaudio E. Contribution of Resident Stem Cells to Liver and Biliary Tree Regeneration in Human Diseases. *Int J Mol Sci* 2018; **19**: 2917 [*RCA*] [PMID: 30257529 DOI: 10.3390/ijms19102917] [FullText] [Full Text(PDF)]

49 **Nakanuma Y**, Sasaki M, Harada K. Autophagy and senescence in fibrosing cholangiopathies. *J Hepatol* 2015; **62**: 934-945 [*RCA*] [PMID: 25435435 DOI: 10.1016/j.jhep.2014.11.027] [FullText]

50 **Aabakken L**, Karlsen TH, Albert J, Arvanitakis M, Chazouilleres O, Dumonceau JM, Färkkilä M, Fickert P, Hirschfield GM, Laghi A, Marzioni M, Fernandez M, Pereira SP, Pohl J, Poley JW, Ponsioen CY, Schramm C, Swahn F, Tringali A, Hassan C. Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline. *Endoscopy* 2017; **49**: 588-608 [*RCA*] [PMID: 28420030 DOI: 10.1055/s-0043-107029] [FullText]

51 **Dyson JK**, Beuers U, Jones DEJ, Lohse AW, Hudson M. Primary sclerosing cholangitis. *Lancet* 2018; **391**: 2547-2559 [*RCA*] [PMID: 29452711 DOI: 10.1016/S0140-6736(18)30300-3] [FullText]

52 **Kaplan GG**, Laupland KB, Butzner D, Urbanski SJ, Lee SS. The burden of large and small duct primary sclerosing cholangitis in adults and children: a population-based analysis. *Am J Gastroenterol* 2007; **102**: 1042-1049 [*RCA*] [PMID: 17313496 DOI: 10.1111/j.1572-0241.2007.01103.x] [FullText]

53 **Boberg KM**, Schrumpf E, Fausa O, Elgjo K, Kolmannskog F, Haaland T, Holter E. Hepatobiliary disease in ulcerative colitis. An analysis of 18 patients with hepatobiliary lesions classified as small-duct primary sclerosing cholangitis. *Scand J Gastroenterol* 1994; **29**: 744-752 [*RCA*] [PMID: 7973436 DOI: 10.3109/00365529409092504] [FullText]

54 **Angulo P**, Maor-Kendler Y, Lindor KD. Small-duct primary sclerosing cholangitis: a long-term follow-up study. *Hepatology* 2002; **35**: 1494-1500 [*RCA*] [PMID: 12029635 DOI: 10.1053/jhep.2002.33202] [FullText]

55 **Björnsson E**, Boberg KM, Cullen S, Fleming K, Clausen OP, Fausa O, Schrumpf E, Chapman RW. Patients with small duct primary sclerosing cholangitis have a favourable long term prognosis. *Gut* 2002; **51**: 731-735 [*RCA*] [PMID: 12377815 DOI: 10.1136/gut.51.5.731] [FullText] [Full Text(PDF)]

56 **Ludwig J**, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. *Hepatology* 1981; **1**: 632-640 [*RCA*] [PMID: 7308996 DOI: 10.1002/hep.1840010612] [FullText]

57 **Kulaksiz H**, Rudolph G, Kloeters-Plachky P, Sauer P, Geiss H, Stiehl A. Biliary candida infections in primary sclerosing cholangitis. *J Hepatol* 2006; **45**: 711-716 [*RCA*] [PMID: 16979779 DOI: 10.1016/j.jhep.2006.07.022] [FullText]

58 **Liwinski T**, Heinemann M, Schramm C. The intestinal and biliary microbiome in autoimmune liver disease-current evidence and concepts. *Semin Immunopathol* 2022; **44**: 485-507 [*RCA*] [PMID: 35536431 DOI: 10.1007/s00281-022-00936-6] [FullText] [Full Text(PDF)]

59 **Broomé U**, Olsson R, Lööf L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzén H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610-615 [*RCA*] [PMID: 8707097 DOI: 10.1136/gut.38.4.610] [FullText] [Full Text(PDF)]

60 **Ricciuto A**, Kamath BM, Griffiths AM. The IBD and PSC Phenotypes of PSC-IBD. *Curr Gastroenterol Rep* 2018; **20**: 16 [*RCA*] [PMID: 29594739 DOI: 10.1007/s11894-018-0620-2] [FullText]

61 **Tischendorf JJ**, Hecker H, Krüger M, Manns MP, Meier PN. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. *Am J Gastroenterol* 2007; **102**: 107-114 [*RCA*] [PMID: 17037993 DOI: 10.1111/j.1572-0241.2006.00872.x] [FullText]

62 **de Vries AB**, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. *World J Gastroenterol* 2015; **21**: 1956-1971 [*RCA*] [PMID: 25684965 DOI: 10.3748/wjg.v21.i6.1956] [FullText] [Full Text(PDF)]

63 **Joo M**, Abreu-e-Lima P, Farraye F, Smith T, Swaroop P, Gardner L, Lauwers GY, Odze RD. Pathologic features of ulcerative colitis in patients with primary sclerosing cholangitis: a case-control study. *Am J Surg Pathol* 2009; **33**: 854-862 [*RCA*] [PMID: 19295408 DOI: 10.1097/PAS.0b013e318196d018] [FullText]

64 **Schaeffer DF**, Win LL, Hafezi-Bakhtiari S, Cino M, Hirschfield GM, El-Zimaity H. The phenotypic expression of inflammatory bowel disease in patients with primary sclerosing cholangitis differs in the distribution of colitis. *Dig Dis Sci* 2013; **58**: 2608-2614 [*RCA*] [PMID: 23670229 DOI: 10.1007/s10620-013-2697-7] [FullText]

65 **Jørgensen KK**, Grzyb K, Lundin KE, Clausen OP, Aamodt G, Schrumpf E, Vatn MH, Boberg KM. Inflammatory bowel disease in patients with primary sclerosing cholangitis: clinical characterization in liver transplanted and nontransplanted patients. *Inflamm Bowel Dis* 2012; **18**: 536-545 [*RCA*] [PMID: 21456044 DOI: 10.1002/ibd.21699] [FullText]

66 **Sano H**, Nakazawa T, Ando T, Hayashi K, Naitoh I, Okumura F, Miyabe K, Yoshida M, Takahashi S, Ohara H, Joh T. Clinical characteristics of inflammatory bowel disease associated with primary sclerosing cholangitis. *J Hepatobiliary Pancreat Sci* 2011; **18**: 154-161 [*RCA*] [PMID: 20740366 DOI: 10.1007/s00534-010-0319-8] [FullText]

67 **Krugliak Cleveland N**, Rubin D, Meckel K, Tran A, Aelvoet A, Gonsalves A, Gaetano JN, Williams K, Jabri B, Wroblewski K, Pekow J.

P196 Patients with ulcerative colitis (UC) and concomitant primary sclerosing cholangitis (PSC) have more subclinical endoscopic and histologic disease activity in the right colon compared to UC patients without PSC. *J Crohns Colitis* 2017; **11**: S175-S176 [DOI: 10.1093/ecco-jcc/jjx002.321] [FullText]

68 **Hirasawa K**, Sato Y, Hosoda Y, Yamamoto T, Hanai H. Immunohistochemical localization of angiotensin II receptor and local renin-angiotensin system in human colonic mucosa. *J Histochem Cytochem* 2002; **50**: 275-282 [*RCA*] [PMID: 11799146 DOI: 10.1177/002215540205000215] [FullText]

69 **Rojas A**, Schneider I, Lindner C, Gonzàlez I, Morales MA. Receptor for advanced glycation end-products axis and coronavirus disease 2019 in inflammatory bowel diseases: A dangerous liaison? *World J Gastroenterol* 2021; **27**: 2270-2280 [*RCA*] [PMID: 34040321 DOI: 10.3748/wjg.v27.i19.2270] [FullText] [Full Text(PDF)]

70 **Rojas A**, Lindner C, Schneider I, Gonzalez I, Uribarri J. The RAGE Axis: A Relevant Inflammatory Hub in Human Diseases. *Biomolecules* 2024; **14**: 412 [*RCA*] [PMID: 38672429 DOI: 10.3390/biom14040412] [FullText] [Full Text(PDF)]

71 **Garg M**, Royce SG, Tikellis C, Shallue C, Batu D, Velkoska E, Burrell LM, Patel SK, Beswick L, Jackson A, Britto K, Lukies M, Sluka P, Wardan H, Hirokawa Y, Tan CW, Faux M, Burgess AW, Hosking P, Monagle S, Thomas M, Gibson PR, Lubel J. Imbalance of the renin-angiotensin system may contribute to inflammation and fibrosis in IBD: a novel therapeutic target? *Gut* 2020; **69**: 841-851 [*RCA*] [PMID: 31409604 DOI: 10.1136/gutjnl-2019-318512] [FullText]

72 **Fujitani N**, Liu Y, Toda S, Shirouzu K, Okamura T, Kimura H. Expression of H type 1 antigen of ABO histo-blood group in normal colon and aberrant expressions of H type 2 and H type 3/4 antigens in colon cancer. *Glycoconj J* 2000; **17**: 331-338 [*RCA*] [PMID: 11261842 DOI: 10.1023/a:1007173722426] [FullText]

73 **Pazenco EV**, Karev VE, Raikhelson KL, Shchukina OB, Botina AV, Kharitonov AG, Semenov NV. [Clinical and Morphological Characteristics of Intestinal Lesions in Patients with Primary Sclerosing Cholangitis]. *Eksp Klin Gastroenterol* 2016; 18-24 [*RCA*] [PMID: 30284417] [FullText]

74 **Barrie A**, Mourabet ME, Weyant K, Clarke K, Gajendran M, Rivers C, Park SY, Hartman D, Saul M, Regueiro M, Yadav D, Binion DG. Recurrent blood eosinophilia in ulcerative colitis is associated with severe disease and primary sclerosing cholangitis. *Dig Dis Sci* 2013; **58**: 222-228 [*RCA*] [PMID: 22855293 DOI: 10.1007/s10620-012-2329-7] [FullText] [Full Text(PDF)]

75 **Loftus EV Jr**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [*RCA*] [PMID: 15591511 DOI: 10.1136/gut.2004.046615] [FullText] [Full Text(PDF)]

76 **Lundqvist K**, Broomé U. Differences in colonic disease activity in patients with ulcerative colitis with and without primary sclerosing cholangitis: a case control study. *Dis Colon Rectum* 1997; **40**: 451-456 [*RCA*] [PMID: 9106695 DOI: 10.1007/BF02258391] [FullText]

77 **Moayyeri A**, Daryani NE, Bahrami H, Haghpanah B, Nayyer-Habibi A, Sadatsafavi M. Clinical course of ulcerative colitis in patients with and without primary sclerosing cholangitis. *J Gastroenterol Hepatol* 2005; **20**: 366-370 [*RCA*] [PMID: 15740478 DOI: 10.1111/j.1440-1746.2005.03727.x] [FullText]

78 **Nielsen MJ**, Thorburn D, Leeming DJ, Hov JR, Nygård S, Moum B, Saffioti F, Gilja OH, Boberg KM, Mazza G, Røsjø H, Pinzani M, Karlsen TH, Karsdal MA, Vesterhus M. Serological markers of extracellular matrix remodeling predict transplant-free survival in primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2018; **48**: 179-189 [*RCA*] [PMID: 29851098 DOI: 10.1111/apt.14806] [FullText]

79 **Ingham PW**. Hedgehog signaling. *Curr Top Dev Biol* 2022; **149**: 1-58 [*RCA*] [PMID: 35606054 DOI: 10.1016/bs.ctdb.2022.04.003] [Full Text]

80 **Liebner S**. Sonic hedgehog causes mural cells to jump 'n' run. *Blood* 2014; **123**: 2285-2286 [*RCA*] [PMID: 24723676 DOI: 10.1182/blood-2014-02-554782] [FullText]

81 **Jiang J**. Hedgehog signaling mechanism and role in cancer. *Semin Cancer Biol* 2022; **85**: 107-122 [*RCA*] [PMID: 33836254 DOI: 10.1016/j.semcancer.2021.04.003] [FullText]

82 **Cardinale V**, Paradiso S, Alvaro D. Biliary stem cells in health and cholangiopathies and cholangiocarcinoma. *Curr Opin Gastroenterol* 2024; **40**: 92-98 [*RCA*] [PMID: 38320197 DOI: 10.1097/MOG.0000000000001005] [FullText]

83 **Carpino G**, Cardinale V, Folseraas T, Overi D, Grzyb K, Costantini D, Berloco PB, Di Matteo S, Karlsen TH, Alvaro D, Gaudio E. Neoplastic Transformation of the Peribiliary Stem Cell Niche in Cholangiocarcinoma Arisen in Primary Sclerosing Cholangitis. *Hepatology* 2019; **69**: 622-638 [*RCA*] [PMID: 30102768 DOI: 10.1002/hep.30210] [FullText]

84 **Terasaki S**, Nakanuma Y, Unoura M, Kaneko S, Kobayashi K. Involvement of peribiliary glands in primary sclerosing cholangitis: a histopathologic study. *Intern Med* 1997; **36**: 766-770 [*RCA*] [PMID: 9392346 DOI: 10.2169/internalmedicine.36.766] [FullText]

85 **Brivio S**, Cadamuro M, Fabris L, Strazzabosco M. Epithelial-to-Mesenchymal Transition and Cancer Invasiveness: What Can We Learn from Cholangiocarcinoma? *J Clin Med* 2015; **4**: 2028-2041 [*RCA*] [PMID: 26703747 DOI: 10.3390/jcm4121958] [FullText] [Full Text(PDF)]

86 **Souza M**, Lima LCV, Al-Sharif L, Huang DQ. Incidence of Hepatobiliary Malignancies in Primary Sclerosing Cholangitis: Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2025; **23**: 1695-1709.e13 [*RCA*] [PMID: 39709139 DOI: 10.1016/j.cgh.2024.09.037] [FullText]

87 **Björnsson E**. Small-duct primary sclerosing cholangitis. *Curr Gastroenterol Rep* 2009; **11**: 37-41 [*RCA*] [PMID: 19166657 DOI: 10.1007/s11894-009-0006-6] [FullText]

88 **Uluçeçen SG**. Inflammatory bowel disease and primary sclerosing cholangitis. *J Enterocolitis* 2023; **2**: 19-24 [DOI: 10.14744/Jenterocolitis.2023.23051] [FullText]

89 **Annese V**, Beaugerie L, Egan L, Biancone L, Bolling C, Brandts C, Dierickx D, Dummer R, Fiorino G, Gornet JM, Higgins P, Katsanos KH, Nissen L, Pellino G, Rogler G, Scaldaferri F, Szymanska E, Eliakim R; ECCO. European Evidence-based Consensus: Inflammatory Bowel Disease and Malignancies. *J Crohns Colitis* 2015; **9**: 945-965 [*RCA*] [PMID: 26294789 DOI: 10.1093/ecco-jcc/jjv141] [FullText]

90 **Gurbuz AK**, Giardiello FM, Bayless TM. Colorectal neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. *Dis Colon Rectum* 1995; **38**: 37-41 [*RCA*] [PMID: 7813342 DOI: 10.1007/BF02053855] [FullText]

91 **Imam MH**, Thackeray EW, Lindor KD. Colonic neoplasia in young patients with inflammatory bowel disease and primary sclerosing cholangitis. *Colorectal Dis* 2013; **15**: 198-203 [*RCA*] [PMID: 22712545 DOI: 10.1111/j.1463-1318.2012.03133.x] [FullText]

92 **Wang R**, Leong RW. Primary sclerosing cholangitis as an independent risk factor for colorectal cancer in the context of inflammatory bowel disease: a review of the literature. *World J Gastroenterol* 2014; **20**: 8783-8789 [*RCA*] [PMID: 25083052 DOI: 10.3748/wjg.v20.i27.8783] [Full Text] [Full Text(PDF)]

93 **Choi WT**, Salomao M, Zhao L, Alpert L, Setia N, Liao X, Drage MG, Westerhoff M, Cheng J, Lauwers GY, Ko HM. Hypermucinous, Goblet Cell-Deficient and Crypt Cell Dysplasias in Inflammatory Bowel Disease are Often Associated with Flat/Invisible Endoscopic Appearance and

Advanced Neoplasia on Follow-Up. *J Crohns Colitis* 2022; **16**: 98-108 [*RCA*] [PMID: 34232295 DOI: 10.1093/ecco-jcc/jjab120] [FullText]
94 **Brackmann S**, Andersen SN, Aamodt G, Langmark F, Clausen OP, Aadland E, Fausa O, Rydning A, Vatn MH. Relationship between clinical parameters and the colitis-colorectal cancer interval in a cohort of patients with colorectal cancer in inflammatory bowel disease. *Scand J Gastroenterol* 2009; **44**: 46-55 [*RCA*] [PMID: 18609187 DOI: 10.1080/00365520801977568] [FullText]
95 **Aranake-Chrisinger J**, Dassopoulos T, Yan Y, Nalbantoglu I. Primary sclerosing cholangitis associated colitis: Characterization of clinical, histologic features, and their associations with liver transplantation. *World J Gastroenterol* 2020; **26**: 4126-4139 [*RCA*] [PMID: 32821074 DOI: 10.3748/wjg.v26.i28.4126] [FullText] [Full Text(PDF)]
96 **Zhang R**, Rabinovitch PS, Mattis AN, Lauwers GY, Choi WT. DNA content abnormality frequently develops in the right/proximal colon in patients with primary sclerosing cholangitis and inflammatory bowel disease and is highly predictive of subsequent detection of dysplasia. *Histopathology* 2023; **83**: 116-125 [*RCA*] [PMID: 37012656 DOI: 10.1111/his.14913] [FullText]
97 **Cheng H**, Bjerknes M, Amar J, Gardiner G. Crypt production in normal and diseased human colonic epithelium. *Anat Rec* 1986; **216**: 44-48 [*RCA*] [PMID: 3094402 DOI: 10.1002/ar.1092160108] [FullText]
98 **Rubio CA**, Schmidt PT. Crypts in Asymmetric Fission in Endoscopic Biopsies from Swedish Patients With Inflammatory Bowel Disease. *Anticancer Res* 2021; **41**: 3511-3517 [*RCA*] [PMID: 34230146 DOI: 10.21873/anticanres.15138] [FullText]
99 **Grant AJ**, Lalor PF, Salmi M, Jalkanen S, Adams DH. Homing of mucosal lymphocytes to the liver in the pathogenesis of hepatic complications of inflammatory bowel disease. *Lancet* 2002; **359**: 150-157 [*RCA*] [PMID: 11809275 DOI: 10.1016/S0140-6736(02)07374-9] [FullText]
100 **Alipour Z**, Stashek K. Recently described types of dysplasia associated with IBD: tips and clues for the practising pathologist. *J Clin Pathol* 2024; **77**: 77-81 [*RCA*] [PMID: 37918911 DOI: 10.1136/jcp-2023-209141] [FullText]
101 **Deniziaut G**, Ballot E, Johanet C. Antineutrophil cytoplasmic auto-antibodies (ANCA) in autoimmune hepatitis and primary sclerosing cholangitis. *Clin Res Hepatol Gastroenterol* 2013; **37**: 105-107 [*RCA*] [PMID: 22998811 DOI: 10.1016/j.clinre.2012.07.008] [FullText]
102 **Aleksandrova EN**, Dorofeev AS, Novikov AA, Sandler YG. Autoantibodies in autoimmune liver diseases (review of literature). *Russ Clin Lab Diagn* 2023; **68**: 464-474 [DOI: 10.51620/0869-2084-2023-68-8-464-474] [FullText]
103 **Hov JR**, Boberg KM, Karlsen TH. Autoantibodies in primary sclerosing cholangitis. *World J Gastroenterol* 2008; **14**: 3781-3791 [*RCA*] [PMID: 18609700 DOI: 10.3748/wjg.14.3781] [FullText] [Full Text(PDF)]
104 **Hov JR**, Boberg KM, Taraldsrud E, Vesterhus M, Boyadzhieva M, Solberg IC, Schrumpf E, Vatn MH, Lie BA, Molberg Ø, Karlsen TH. Antineutrophil antibodies define clinical and genetic subgroups in primary sclerosing cholangitis. *Liver Int* 2017; **37**: 458-465 [*RCA*] [PMID: 27558072 DOI: 10.1111/liv.13238] [FullText]
105 **Lenzen H**, Weismüller TJ, Negm AA, Wlecke J, Loges S, Strassburg CP, Manns MP, Lankisch TO. Antineutrophil cytoplasmic antibodies in bile are associated with disease activity in primary sclerosing cholangitis. *Scand J Gastroenterol* 2013; **48**: 1205-1212 [*RCA*] [PMID: 23957616 DOI: 10.3109/00365521.2013.825313] [FullText]
106 **Moiseev S**, Cohen Tervaert JW, Arimura Y, Bogdanos DP, Csernok E, Damoiseaux J, Ferrante M, Flores-Suárez LF, Fritzler MJ, Invernizzi P, Jayne D, Jennette JC, Little MA, McAdoo SP, Novikov P, Pusey CD, Radice A, Salama AD, Savige JA, Segelmark M, Shoenfeld Y, Sinico RA, Sousa MJ, Specks U, Terrier B, Tzioufas AG, Vermeire S, Zhao MH, Bossuyt X. 2020 international consensus on ANCA testing beyond systemic vasculitis. *Autoimmun Rev* 2020; **19**: 102618 [*RCA*] [PMID: 32663621 DOI: 10.1016/j.autrev.2020.102618] [FullText]
107 **Reshetnyak TM**, Vorkel EN, Nurbayeva KS, Lila AM. Granulomatosis with polyangiitis and microscopic polyangiitis: pathogenesis and clinical manifestations. *Sovrem Revmatol/Mod Rheumatol* 2025; **19**: 86-95 [DOI: 10.14412/1996-7012-2025-4-86-95] [FullText]
108 **Tervaert JW**, van Hoek B, Koek G. Antineutrophil cytoplasmic antibodies in small-duct primary sclerosing cholangitis. *Gastroenterology* 2009; **136**: 364; author reply 364-364; author reply 365 [*RCA*] [PMID: 19056386 DOI: 10.1053/j.gastro.2008.11.033] [FullText]
109 **Prideaux L**, De Cruz P, Ng SC, Kamm MA. Serological antibodies in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2012; **18**: 1340-1355 [*RCA*] [PMID: 22069240 DOI: 10.1002/ibd.21903] [FullText]
110 **Sato H**, Nakanuma Y, Kozaka K, Sato Y, Ikeda H. Spread of hilar cholangiocarcinomas *via* peribiliary gland network: a hither-to-unrecognized route of periductal infiltration. *Int J Clin Exp Pathol* 2013; **6**: 318-322 [*RCA*] [PMID: 23330019] [FullText]
111 **Tornai D**, Papp M. Editorial: serologic antibodies in primary sclerosing cholangitis - a tell-tale sign of compromised gut-liver immunity? *Aliment Pharmacol Ther* 2021; **53**: 350-351 [*RCA*] [PMID: 33368511 DOI: 10.1111/apt.16201] [FullText]
112 **Tornai T**, Tornai D, Sipeki N, Tornai I, Alsulaimani R, Fechner K, Roggenbuck D, Norman GL, Veres G, Par G, Par A, Szalay F, Lakatos PL, Antal-Szalmas P, Papp M. Loss of tolerance to gut immunity protein, glycoprotein 2 (GP2) is associated with progressive disease course in primary sclerosing cholangitis. *Sci Rep* 2018; **8**: 399 [*RCA*] [PMID: 29321484 DOI: 10.1038/s41598-017-18622-1] [FullText] [Full Text (PDF)]
113 **Sowa M**, Kolenda R, Baumgart DC, Pratschke J, Papp M, Tornai T, Suchanski J, Bogdanos DP, Mytilinaiou MG, Hammermann J, Laass MW, Conrad K, Schramm C, Franke A, Roggenbuck D, Schierack P. Mucosal Autoimmunity to Cell-Bound GP2 Isoforms Is a Sensitive Marker in PSC and Associated With the Clinical Phenotype. *Front Immunol* 2018; **9**: 1959 [*RCA*] [PMID: 30233574 DOI: 10.3389/fimmu.2018.01959] [FullText] [Full Text(PDF)]
114 **Lopens S**, Schierack P, Krause J, Piaszczyński M, Król R, Staroń R, Krupa Ł, Gutkowski K, Kruk B, Grąt M, Krawczyk M, Patkowski W, Glaser F, Rödiger S, Grossmann K, Pająk J, Milkiewicz P, Lammert F, Zieniewicz K, Schramm C, Roggenbuck D, Krawczyk M. Antimicrobial glycoprotein 2 (GP2) in gallstones, bile fluid and peribiliary glands of patients with primary sclerosing cholangitis. *Clin Chim Acta* 2024; **562**: 119841 [*RCA*] [PMID: 38964568 DOI: 10.1016/j.cca.2024.119841] [FullText]
115 **Sebode M**, Weiler-Normann C, Liwinski T, Schramm C. Autoantibodies in Autoimmune Liver Disease-Clinical and Diagnostic Relevance. *Front Immunol* 2018; **9**: 609 [*RCA*] [PMID: 29636752 DOI: 10.3389/fimmu.2018.00609] [FullText] [Full Text(PDF)]
116 **Jendrek ST**, Gotthardt D, Nitzsche T, Widmann L, Korf T, Michaels MA, Weiss KH, Liaskou E, Vesterhus M, Karlsen TH, Mindorf S, Schemmer P, Bär F, Teegen B, Schröder T, Ehlers M, Hammers CM, Komorowski L, Lehnert H, Fellermann K, Derer S, Hov JR, Sina C. Anti-GP2 IgA autoantibodies are associated with poor survival and cholangiocarcinoma in primary sclerosing cholangitis. *Gut* 2017; **66**: 137-144 [*RCA*] [PMID: 27406039 DOI: 10.1136/gutjnl-2016-311739] [FullText]
117 **Wunsch E**, Norman GL, Milkiewicz M, Krawczyk M, Bentow C, Shums Z, Mahler M, Lopens S, Reinhold D, Franke A, Schramm C, Roggenbuck D, Milkiewicz P. Anti-glycoprotein 2 (anti-GP2) IgA and anti-neutrophil cytoplasmic antibodies to serine proteinase 3 (PR3-ANCA): antibodies to predict severe disease, poor survival and cholangiocarcinoma in primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2021; **53**: 302-313 [*RCA*] [PMID: 33159471 DOI: 10.1111/apt.16153] [FullText] [Full Text(PDF)]
118 **Karlsen TH**, Folseraas T, Thorburn D, Vesterhus M. Primary sclerosing cholangitis - a comprehensive review. *J Hepatol* 2017; **67**: 1298-1323

[*RCA*] [PMID: 28802875 DOI: 10.1016/j.jhep.2017.07.022] [FullText]

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MINIREVIEWS

Hepatic hydatid cyst disease in low- and middle-income countries: Spectrum of imaging findings and complications

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Abstract

Hepatic hydatid cyst disease is a significant public health issue in endemic low- and middle-income countries, and imaging plays a pivotal role in diagnosis, classification, management, and follow-up. In resource-limited settings, ultrasound (US) is often the imaging modality of choice, with computed tomography (CT) and magnetic resonance imaging (MRI) usually reserved for complex or atypical cases. This pictorial review illustrates the characteristic and atypical imaging features of hepatic hydatid cysts across US, CT, and MRI, emphasizing practical imaging approaches applicable in low- and middle-income countries. US features include daughter cysts, hydatid sand, and detached membranes, whereas CT provides a more detailed assessment of cyst morphology, calcifications, and complications. MRI offers superior soft-tissue contrast and improved evaluation of biliary involvement and complex disease. Important complications include exophytic growth, rupture, transdiaphragmatic thoracic involvement, perforation into hollow viscera, peritoneal seeding, biliary communication and abdominal wall invasion. The US based World Health Organization-Informal Working Group on Echinococcosis classification is also illustrated, highlighting its role in guiding treatment and follow-up. A structured, image-based approach using available modalities enables accurate diagnosis and effective management, whereas pictorial recognition of key imaging features facilitates early detection and improved outcomes in resource-limited settings.

Key Words: Hydatid cyst; Liver; Imaging; Ultrasound; Computed tomography; Magnetic resonance imaging; Echinococcosis; Complications

Core Tip: Hepatic hydatid disease is highly prevalent in low- and middle-income countries, where imaging is central to diagnosis, staging, and management. This pictorial review demonstrates the spectrum of imaging findings across ultrasound, computed tomography, and magnetic resonance imaging, emphasizing practical, resource-adapted interpretation. Key typical and atypical features, complications, and characteristic radiologic signs are illustrated alongside the World Health Organization-Informal Working Group on Echinococcosis classification to reinforce standardized staging. By focusing on commonly available modalities and real-world diagnostic challenges, this review aims to improve recognition, guide treatment decisions, and enhance diagnostic accuracy, particularly in low- and middle-income countries.

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INTRODUCTION

Hydatid disease (HD), or cystic echinococcosis (CE), is a parasitic infection caused by the larval stage of *Echinococcus* tapeworm. The two primary forms of hepatic HD are CE caused by *Echinococcus granulosus*, which is characterized by well-defined hepatic cystic lesions (CLs); and the less common but more aggressive alveolar echinococcosis caused by *Echinococcus multilocularis* which presents as an infiltrative, "tumor-like" mass[1]. HD remains endemic in many low- and middle-income countries (LMICs), particularly in regions where livestock farming and close contact with dogs facilitate transmission, including the Mediterranean Basin, the Middle East, Africa, South America, and parts of Asia[1,2].

Humans are usually infected by ingesting parasite eggs through contaminated food or direct contact with infected dogs. After ingestion, embryos penetrate the intestinal mucosa, enter the portal circulation, and lodge in the liver, where they develop into hydatid cysts. Cysts grow slowly, reaching approximately 1 cm in the first six months and enlarging by approximately 2-3 cm annually; however, in some cases rapid growth rates of up to 4-5 cm per year have been documented[2,3]. The liver is the most frequently involved organ, representing the first filter of the portal circulation but the involvement of other locations in virtually all parts of the body has been described[4-7].

In this pictorial review, we illustrate the spectrum of imaging findings and local complications of hepatic HD based on surgically and laboratory confirmed cases encountered at our institution over the past decade.

IMAGING APPROACH TO HEPATIC HD

Radiologic imaging is the cornerstone for the diagnosis of hepatic CE, whereas serology plays a supportive role. Imaging not only confirms the presence of hydatid cysts but also characterizes morphology, viability, and complications, which directly guide management[8,9].

Ultrasound (US) is generally the first-line modality for screening, staging, and follow-up due to its availability, cost-effectiveness, and suitability for resource-limited settings such as Pakistan. The rise of affordable, portable US devices now allows these examinations to be performed at the point of care, even in isolated rural communities[10]. It enables the identification of characteristic features including daughter cysts, membranes, and calcifications, which form the basis of the World Health Organization-Informal Working Group on Echinococcosis (WHO-IWGE) and Gharbi classification systems that reflect cyst activity and guide treatment[11].

Although US remains the cornerstone of diagnosis and staging in LMICs due to its accessibility and cost-effectiveness, computed tomography (CT) and magnetic resonance imaging (MRI) play complementary roles in evaluating complex disease, complications, extrahepatic extension, and preoperative planning. Accordingly, this review emphasizes US for routine diagnosis while illustrating the additional value of cross-sectional imaging when available. CT is superior for defining anatomy, detecting calcification, and evaluating complications such as rupture, infection, and extrahepatic spread, and is particularly useful for surgical planning[12,13]. However, its performance in precise staging is limited compared with that of US or MRI due to lower soft tissue contrast leading to misclassification. MRI provides excellent soft tissue detail and is especially useful for assessing biliary or neural involvement and differentiating hydatid cysts from simple cysts, although it is less sensitive for detecting calcification[12]. Additionally, diffusion-weighted imaging and fluid attenuation inversion recovery sequences can also reliably differentiate hydatid cysts from simple hepatic cysts [14].

IMAGING FEATURES OF HEPATIC HD

Hepatic hydatid cysts typically appear as well-defined CLs with a fibrous capsule and may contain internal daughter cysts or detached membranes, which are characteristic imaging features[12]. The cyst wall is composed of three layers: The outermost pericyst, which is a product of the host's inflammatory response and contains inflammatory cells; the exocyst, which is the outer, acellular, protective layer of the parasite itself; and the innermost endocyst, which contains

proliferating cells that produce daughter cysts and cyst fluid[15].

On US, hydatid lesions appear as well-defined anechoic avascular cysts, which may be unilocular or multivesicular, with a double-line sign (visualized as two echogenic lines with an intervening hypoechoic layer)[16], internal hydatid sand, daughter cysts, or detached membranes ("water lily sign"), and possible rim calcification[17]. If wall calcifications are present, they can present as hyperechoic rims with posterior acoustic shadowing.

CT reveals well-defined CLs with fluid-to-complex attenuation, internal septations, daughter cysts, membranes, and calcification, and is particularly useful for detecting complications[18,19]. CT further adds value by demonstrating distinct morphological patterns that correlate with pathological stages, including unilocular cystic, multivesicular, collapsed inner wall, partially solidified, solidified, and calcified types, as well as complicated forms[20]. Additionally, hydatid cysts may present atypical patterns, such as exophytic growth; multicystic confluent lesions; or complicated cysts that rupture into the biliary tree, peritoneum, or thoracic cavity[21,22].

MRI usually shows variable T1/T2 signals depending on the cyst content, a hypointense T2 rim representing the pericyst, and characteristic appearances of daughter cysts (attached to the periphery of the endocyst with relative T1 hypointensity to the mother cyst matrix) and low-signal detached membranes ("snake sign")[18].

The exact morphology varies depending upon the stage of evolution of the cyst. Typically, these cysts start off as simple, fluid-filled unilocular cysts, producing multiple daughter cysts as they mature. Once the parasite dies, the cyst membranes detach and the cyst eventually solidifies or calcifies[23]. This morphological variation is the basis for classifying hydatid cysts into distinct categories, each of which is defined by unique imaging characteristics.

Classification and stage-specific radiologic features of hepatic hydatid cysts

Several imaging-based classification systems have been proposed for hepatic CE. For routine clinical practice, particularly in LMICs, the WHO-IWGE classification is the preferred system because it classifies cysts according to their US morphology, reflects biological activity, and directly guides treatment selection and follow-up[24]. The Gharbi classification, introduced in 1981, was the first widely adopted US-based staging system and remains useful for interpreting older literature[25]. Accordingly, the WHO-IWGE classification forms the basis of current clinical management, while the Gharbi classification is usually used for historical context and comparison.

Historical US classification: Gharbi system

Before the WHO-IWGE classification, Gharbi *et al*[25] classified hepatic hydatid cysts according to their US morphology. The system comprises five types: Type I - purely fluid-filled unilocular cysts; type II - cysts with detached membranes ("water-lily sign"); type III - multivesicular cysts containing daughter cysts ("honeycomb" appearance); type IV - heterogeneous pseudo-solid cysts; and type V - cysts with thick calcified walls producing posterior acoustic shadowing. Although largely superseded by the WHO-IWGE classification, the Gharbi system remains valuable for interpreting historical literature and in educational pictorial reviews. The WHO-IWGE stages correlate approximately with the Gharbi types, with CE1 corresponding to Gharbi I, CE2 to Gharbi III, CE3a to Gharbi II, CE4 to Gharbi IV, and CE5 to Gharbi V, while additionally providing information on cyst activity and management, including suitability for interventions such as percutaneous aspiration, injection and reaspiration (PAIR)[3].

WHO-IWGE classification

The WHO-IWGE classification categorizes hepatic hydatid cysts into five stages (CE1-CE5) based primarily on US morphology, reflecting the biological activity and natural evolution of the parasite (Figure 1)[24]. CE1 and CE2 represent active cysts with viable protoscolices, CE3 cysts are transitional, and CE4 and CE5 are considered inactive stages. Owing to its close correlation with cyst viability and treatment strategy, the WHO-IWGE classification is the current standard for staging, therapeutic decision-making, and follow-up.

The CL stage represents the earliest, undifferentiated stage in this classification. These are anechoic uniloculated cysts- with no echoes or internal septations- and are difficult to differentiate from simple hepatic cysts by imaging.

CE1 cysts (Gharbi I) represent the early active stage. US typically reveals an anechoic, well-defined cyst containing fine echogenic particles known as "hydatid sand", which shift with patient position and settle rapidly within the cyst fluid ("falling snowflakes" sign) (Figure 1A). This "sand" basically represents freed scolices and brood capsules of the parasite, which can be confirmed by microscopic evaluation of hydatid fluid aspirate to provide a definitive diagnosis[26]. On CT, these cysts appear as unilocular homogeneous water-attenuating, thick-walled lesions with smooth margins without any internal septations or calcifications. Larger cysts occasionally demonstrate pseudo-wall enhancement due to compression of adjacent hepatic tissue (Figure 2). In contrast, true wall enhancement, typically observed in superinfections, tends to be thicker or more irregular and is often accompanied by surrounding inflammatory changes such as adjacent hepatic edema, fat stranding, or intracystic gas. On MRI, CE1 cysts typically present homogeneous low signals on T1-weighted sequences and high signals on T2-weighted sequences. A defining characteristic is the "rim sign", which is a thin, peripheral, T1 and T2 hypointense rim that represents the host's fibrotic reaction[27].

CE2 cysts (Gharbi III) correspond to the multivesicular active phase. During this stage, the germinal layer actively proliferates to produce daughter cysts that contain viable protoscoleces[28]. US usually reveals a multiloculated CL with internal septa and multiple daughter cysts, producing a "honeycomb" appearance (Figure 1B). On CT, daughter cysts appear embedded within a higher-density maternal matrix. Subtypes include type IIA (peripheral daughter cysts), which have a "spoke wheel appearance"; type IIB (large daughter cysts occupying most of the mother cyst which have a "rosette" appearance); and type IIC (with a relatively high-density maternal matrix). These subtypes reflect the progressive maturity of the cyst[10]. MRI reveals daughter cysts as hypo- or isointense relative to the maternal matrix on T1- and T2-weighted images.

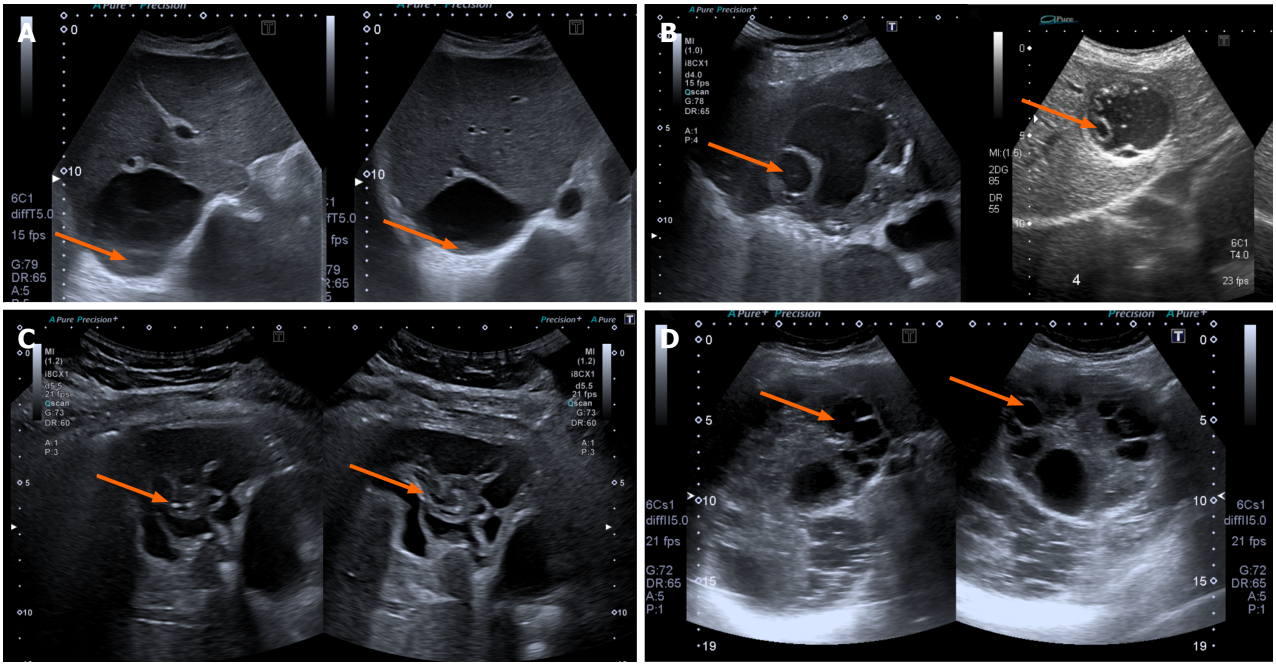


Figure 1 Ultrasound images showing the World Health Organization–Informal Working Group on Echinococcosis classification of hydatid cysts (cystic echinococcosis 1-3). A: Cystic echinococcosis (CE) 1 hydatid cyst with the presence of echogenic fine "hydatid sand" (arrows); B: CE2 hydatid cysts with the presence of peripheral daughter cysts (arrows); C: Internal detached membranes within a CE3a-type cyst (arrows); D: CE3b hydatid cysts with internal membranes, daughter cysts and a central solid maternal matrix (arrows).

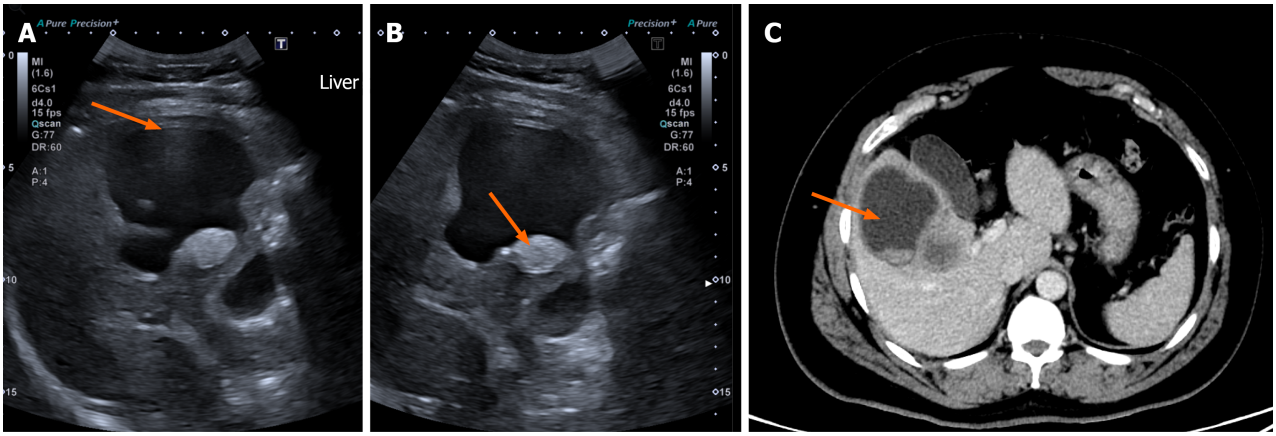


Figure 2 Cystic echinococcosis 1 hydatid cyst. A and B: Ultrasound and images showing a lobulated cystic lesion in the right lobe of the liver with a well-defined wall (arrow in A) and an echogenic nodule at its base (arrow in B), most likely corresponding to cystic echinococcosis 1 with hydatid sand; C: Axial computed tomography image depicts a non-specific low-attenuation cyst (arrow) with subtle pseudo-enhancement, highlighting the superior sensitivity of ultrasound for characterization of early-stage disease.

CE3 cysts represent transitional cysts, showing early degeneration. CE3a (Gharbi II) features detached endocyst membranes, producing the classic "water-lily sign" also known as the "camalote sign" on US and CT (Figure 1C and Figure 3)[12]. CE3b is characterized by daughter cysts within a partially solid or collapsed maternal matrix (Figure 1D). The solidified matrix appears echogenic on US and demonstrates increased attenuation on CT. MRI reveals mixed signal intensity depending on the amount of proteinaceous debris and daughter cyst composition. The "snake sign" or "double line sign" has been used to describe detached, floating membranes with low signals on all sequences (Figure 4)[18]. These cysts are at risk for rupture or superinfection and require careful follow-up.

CE4 cysts (Gharbi IV) are degenerative cysts that undergo fibrosis and early calcification[9]. US shows heterogeneous echogenicity without daughter cysts (Figure 5A and B). Calcifications are visualized as hyperechoic foci with posterior acoustic shadowing. CT reveals a heterogeneous, solid lesion with mixed attenuation and no definite cystic areas. The cyst wall is often irregular with the development of calcification (Figure 5C and D). MRI shows variable signal intensity. Subtle calcifications can be identified as drop-out signals on susceptibility-weighted imaging. CE4 cysts are generally inactive and uncommonly develop acute complications. When present, complications are usually related to mass effect or secondary infection rather than intrinsic cyst activity. Rarely, inactive CE4 cysts may demonstrate reactivation, with

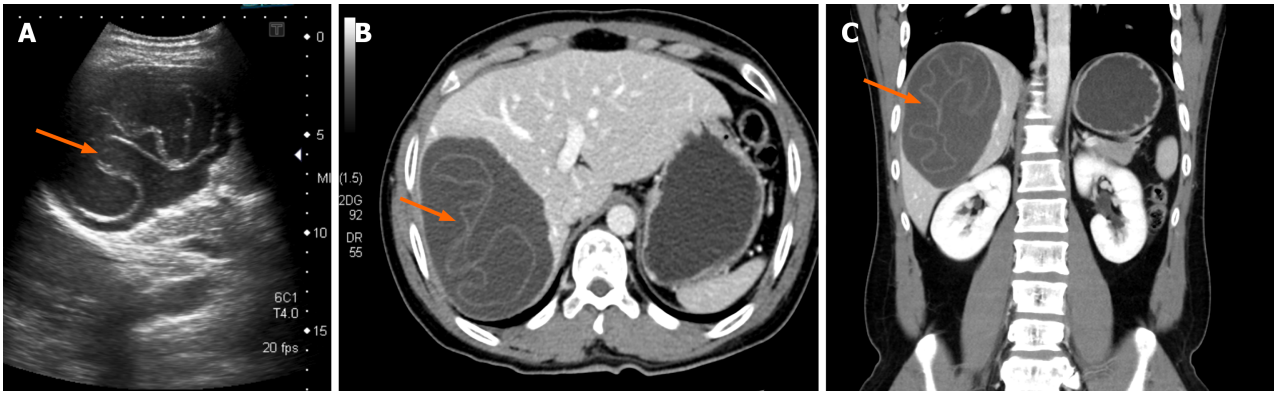


Figure 3 Cystic echinococcosis 3a hydatid cyst. A-C: Ultrasound image, and axial and coronal computed tomography images revealing the characteristic water lily sign indicative of detached membranes of a cystic echinococcosis 3a hydatid cyst (arrows).

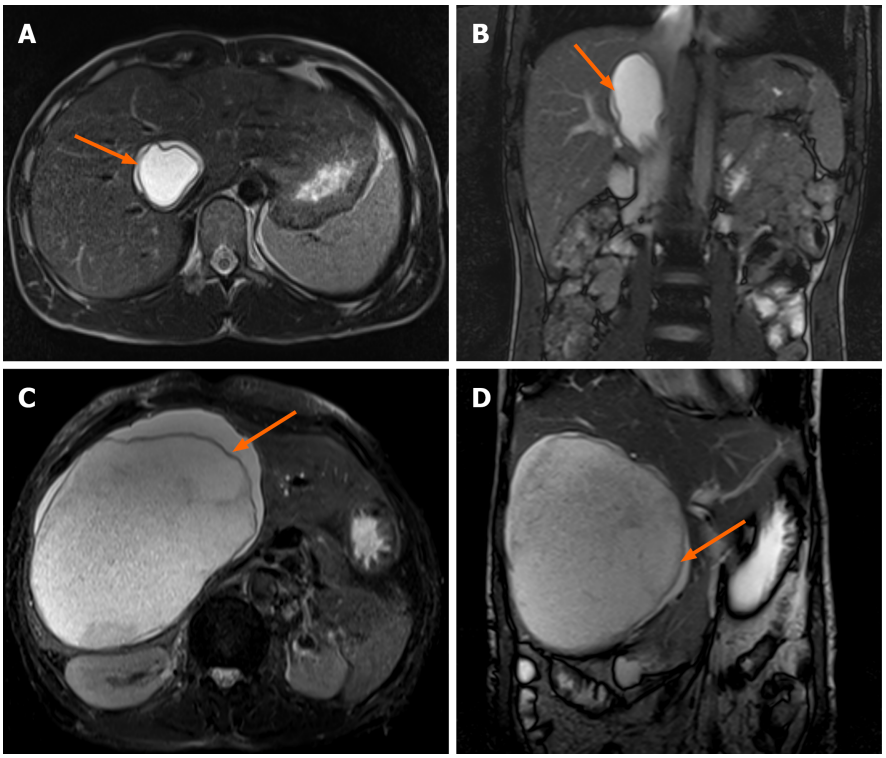


Figure 4 Magnetic resonance images of two patients with hepatic hydatid cysts. A and B: Axial T2-weighted (A) and coronal T2-weighted (B) images showing the T2 hypointense endocyst (arrows) giving a "double line" sign; C and D: Axial T2-weighted (C) and coronal T2-weighted image (D) from another patient with a large hydatid cyst in the right lobe of the liver showing the double line sign (arrows) and heterogeneous central T2 signal intensity.

progression to an active or transitional stage, most commonly resembling CE3b morphology[29].

CE5 cysts (Gharbi V) are inactive cysts with extensive calcification. US shows calcified, hyperechoic walls with posterior acoustic shadowing. CT usually demonstrates total ring-like wall calcification, matrix calcification, or curvilinear calcifications of internal membranes (Figure 6). On MRI, these heavily calcified cysts demonstrate low signal intensity on all sequences[27]. These cysts are considered dead and rarely require intervention.

LOCAL COMPLICATIONS OF HEPATIC HYDATID CYSTS

Mass effect

Hepatic hydatid cysts can lead to a range of local complications, which are particularly important in active type I and II cysts because of their growth potential. Mass effect is a common consequence of large cysts compressing adjacent structures and has been reported to be the most common radiological complication in up to 38% of cases[19]. Compression of vascular structures, particularly the portal vein (Figure 7), may result in atrophy of the affected lobe and

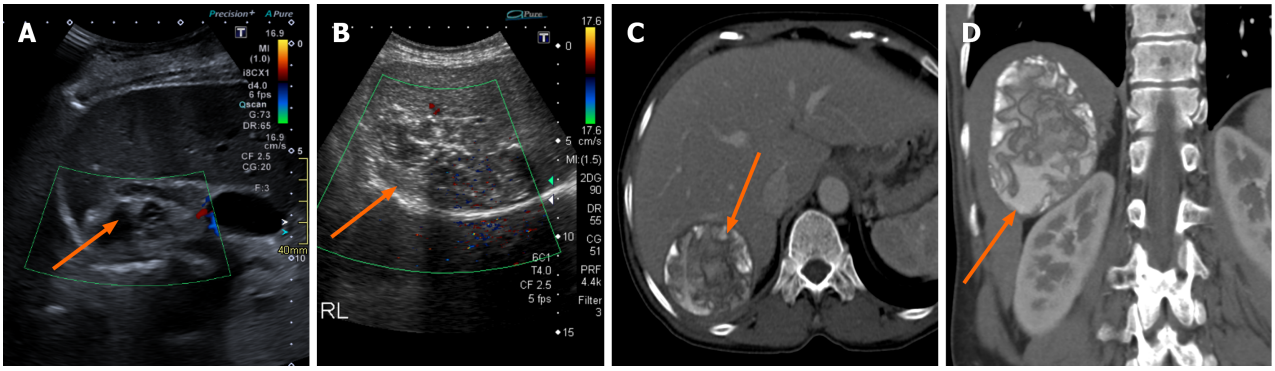


Figure 5 Cystic echinococcosis 4 hydatid cysts. A and B: Ultrasound images from two different patients showing a cystic echinococcosis 4 degenerative hydatid cyst in the right lobe of the liver (arrows) with a heterogeneous appearance on ultrasound with mixed echoes and no definite daughter vesicles; C and D: Axial (C) and coronal (D) images reveal the characteristic cystic echinococcosis 4 hydatid cyst with crumpled membranes and calcifications (arrows).

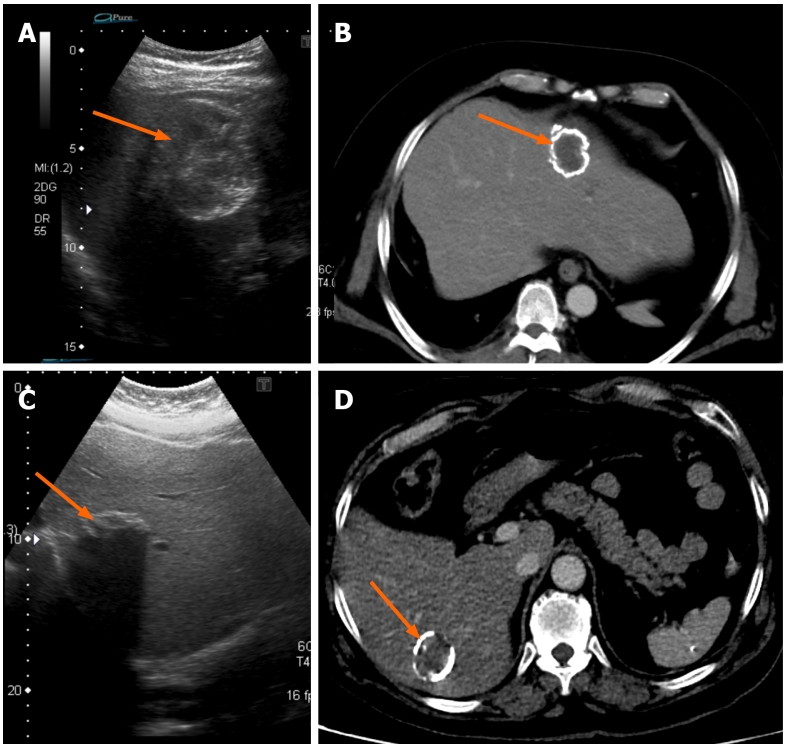


Figure 6 Cystic echinococcosis 5 hydatid cyst disease. A: Ultrasound image showing a cystic echinococcosis 5 hydatid cyst with dense peripheral calcification revealing posterior acoustic shadowing (arrow); B: Computed tomography axial image showing a densely peripherally calcified cyst in the liver (arrow); C: Ultrasound image of another patient with a type V hydatid cyst in the right lobe of liver showing marked posterior acoustic shadowing (arrow); D: Computed tomography axial image shows a densely calcified lesion in the right lobe of the liver (arrow) corresponding to a calcified inactive hydatid cyst.

compensatory hypertrophy of the uninvolved liver. Similarly, large cysts can cause biliary duct dilatation either by extrinsic compression or by perforation into the ducts[21].

Biliary complications

Biliary involvement is one of the most common and important complications of hepatic hydatid cysts. Large cysts can compress nearby bile ducts, causing obstruction, dilatation, or cholestasis, whereas cyst rupture into the biliary system can lead to direct communication (Figure 8). The rupture risk increases with size, and 80% of cysts more than 7.5 cm in size rupture into the bile ducts[30]. Microerosion from pressure may lead to the formation of small fistulas, whereas wide perforations allow the contents of the cyst - including hydatid sand, daughter cysts, or germinal membranes - to enter the biliary tree, causing obstruction and jaundice. Small biliary communications may be difficult to detect on imaging (Figure 9), but MR cholangiography can demonstrate ducts entering the cyst, and US or CT may reveal dilated ducts with echogenic or high-density intraluminal material[22,31,32].

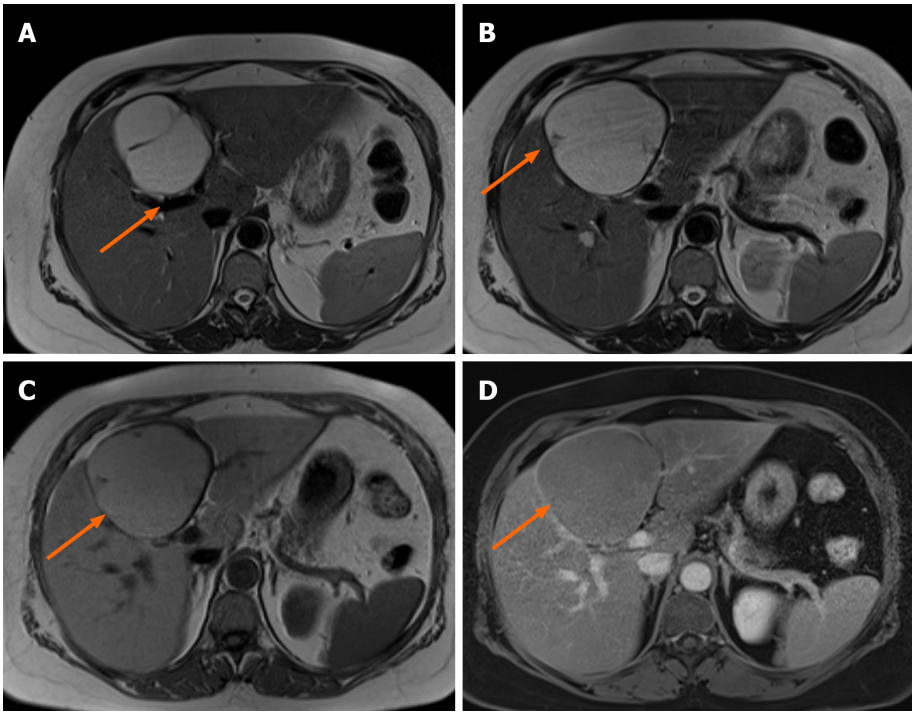


Figure 7 Magnetic resonance images showing mass effect of a hydatid cyst on the portal vein. A and B: Axial T2-weighted images showing a cystic lesion in the liver compressing the bifurcation of the intrahepatic portal vein (arrow in A). The cyst has a T2 hypointense rim (arrow in B); C and D: Axial T1 pre-contrast (C) and axial T1 post-contrast (D) images show some T1 hyperintensity within the cyst (arrow in C) with mild pericystic enhancement (arrow in D).

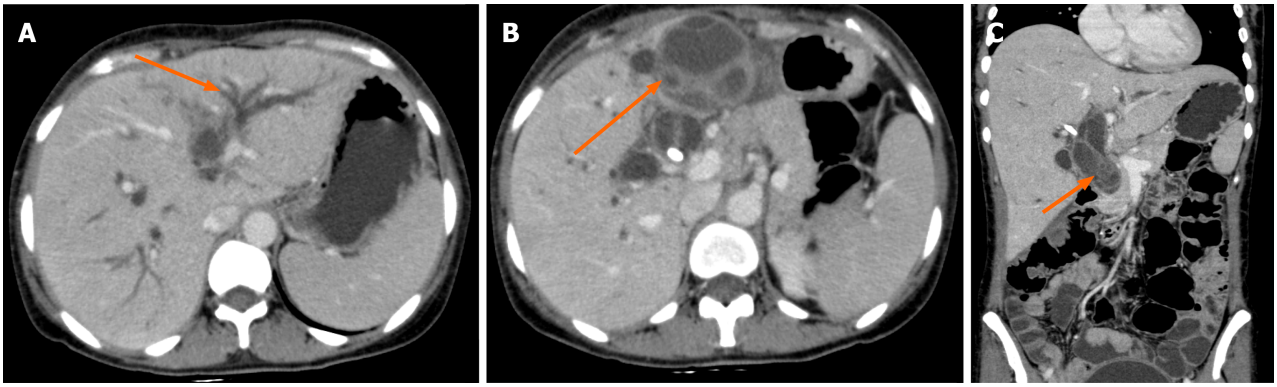


Figure 8 Computed tomography images of a patient with biliary hydatid disease. A: Axial computed tomography (CT) image showing moderate intrahepatic biliary dilatation (arrow); B: Axial CT image shows at least three well-defined cystic lesions present in the liver and porta hepatis (arrow), consistent with hydatid cysts; C: Coronal CT image shows extension of hydatid disease into the common bile duct, likely due to biliary rupture with extrahepatic biliary dilatation (arrow).

Rupture and peritoneal seeding

Rupture of hydatid cysts can be internal (contained), communicating, or direct. Internal rupture occurs when the endocyst detaches from the pericyst but the outer wall remains intact; this rupture is often observed in degenerating or treated cysts- and signals parasite death. Communicating rupture involves the passage of cyst contents into the biliary tract and is observed in type I, II, and IV cysts. Direct rupture, where both the endocyst and pericyst breach, can cause peritoneal dissemination, anaphylaxis, or secondary disease[21,22]. Rupture is more likely in subcapsular cysts due to the minimal surrounding tissue (Figure 10).

Imaging may reveal floating membranes (water-lily sign) on US or CT, and serpentine or tubular structures in the biliary tree on US or MR. Finally, peritoneal seeding (Figure 11) is usually secondary to hepatic cyst rupture, surgery, or trauma. Peritoneal HD is often multiple and asymptomatic until large, with an overall incidence of approximately 13% in patients with abdominal HD. CT and MRI are valuable for assessing the entire peritoneal cavity and identifying cysts of varying stages[33,34].

Presence of fat and gas

Although uncommon, some hepatic hydatid cysts may contain macroscopic fat or fat-fluid levels. Two main mechanisms

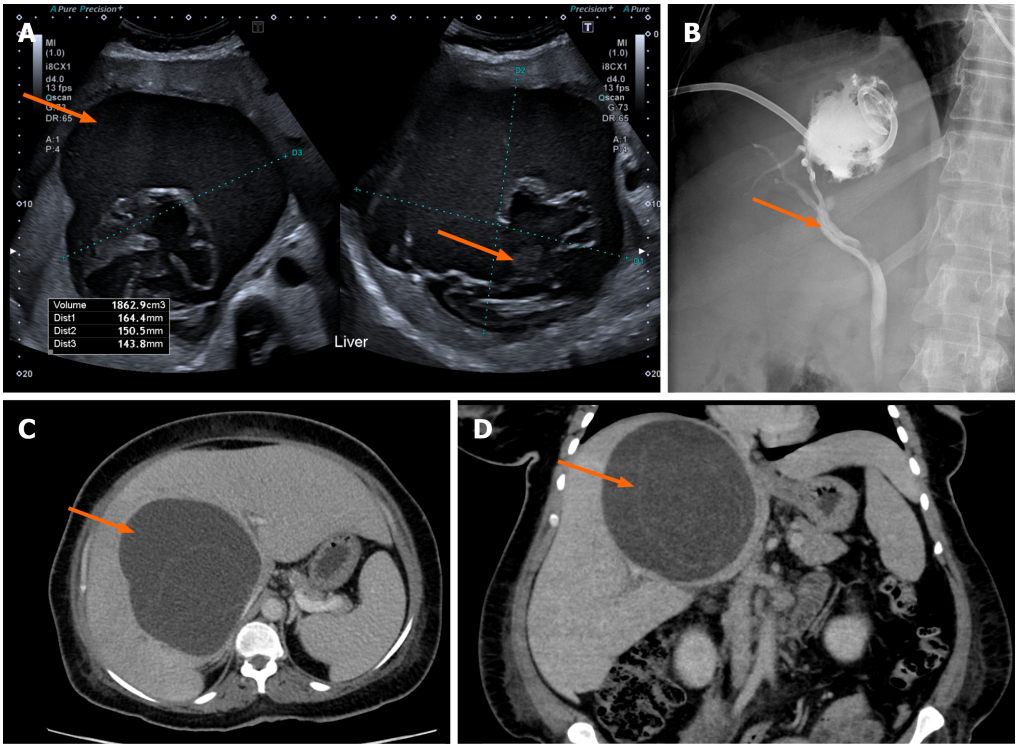


Figure 9 Biliary complications of hydatid cyst. A: Ultrasound images show a large characteristic case of cystic echinococcosis 3a hydatid cyst disease with internal floating membranes (arrows); B: A fluoroscopic exam performed after catheter placement and the percutaneous aspiration injection and reaspiration procedure shows opacification of the biliary tree, suggesting a fistula with biliary communication (arrow); C: Axial computed tomography image shows a large cyst in the right lobe of the liver (arrow); D: Coronal computed tomography image showing floating internal membranes within the cyst (arrow).

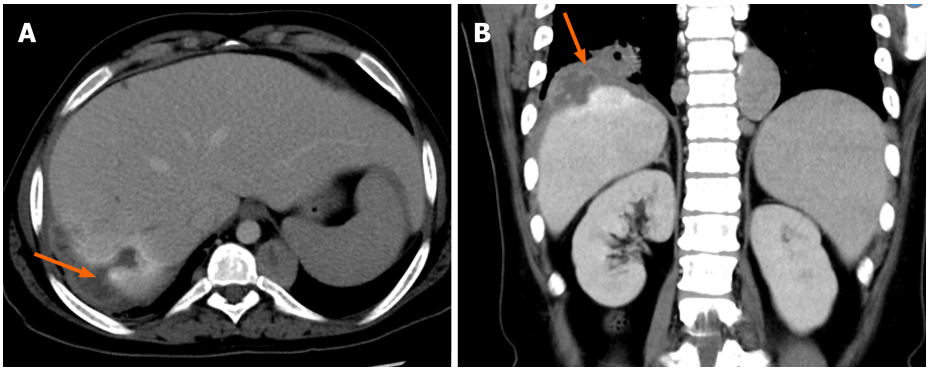


Figure 10 Hydatid cyst disease with rupture and transdiaphragmatic spread. A and B: Axial computed tomography (A) and coronal computed tomography (B) images showing subcapsular rupture of hydatid disease in the right lobe of the liver involving segment VII (arrow in A) with transdiaphragmatic extension into the ipsilateral right thoracic cavity (arrow in B).

have been suggested: Degeneration of hydatid membranes as part of the natural aging process - or as an indirect sign of cyst - biliary communication. On CT, fat within the cyst may appear as low-attenuation globules or as a fat-fluid level[21,35].

Secondary (superinfection) infection of hydatid cysts is often secondary to rupture. Infected cysts display thickened, enhancing walls on CT and MRI, with patchy enhancement of adjacent hepatic parenchyma. Gas or air–fluid levels within the cavity are best detected *via* CT (Figure 12). Importantly, intracystic gas is not specific for infection and may also be seen following prior interventions such as surgery, PAIR, or aspiration procedures[36,37]. On MRI, the presence of diffusion restriction within the cyst contents may suggest superimposed purulent infection; however, diffusion-weighted imaging findings in infected hydatid cysts have not been systematically validated, and restricted diffusion may also be encountered in degenerative (CE4) hydatid cysts[38].

Exophytic growth

Exophytic growth occurs *via* the bare area of the liver or gastrohepatic ligament, allowing the extension of the cyst into the thoracic or peritoneal cavities. Transdiaphragmatic spread into the lung, mediastinum, or even heart occurs in 0.6%-16% of cases, most commonly from cysts in the posterior right lobe[39]. Imaging may reveal diaphragmatic elevation,

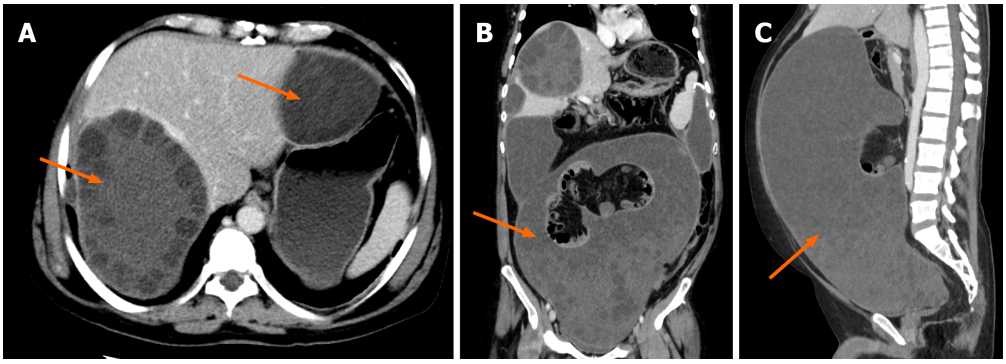


Figure 11 Hydatid cyst disease with intraperitoneal dissemination. A-C: Axial, coronal and sagittal computed tomography images showing at least two hydatid cysts with daughter cysts with a typical rosette appearance in the left and right lobes of the liver (arrows in A). Coronal and sagittal sections show diffuse dissemination of hydatid disease into the peritoneum (B and C).

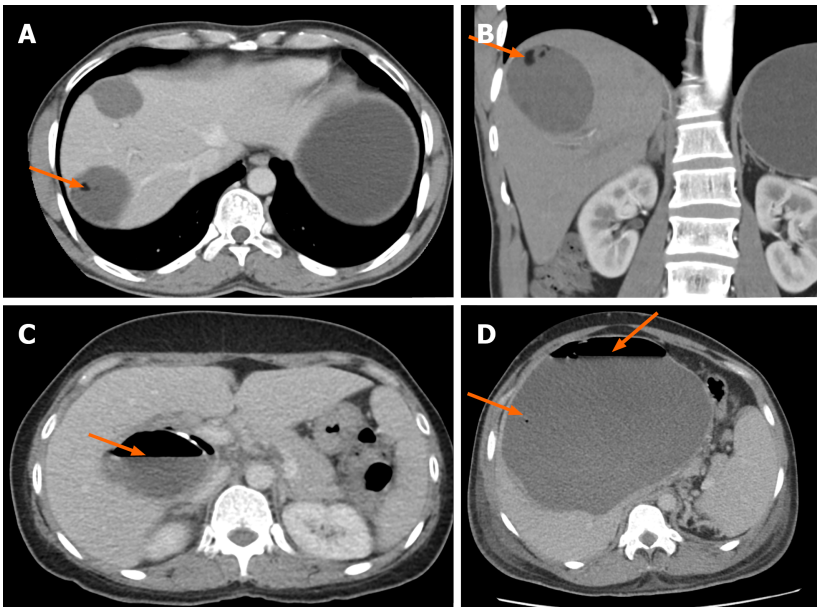


Figure 12 Fat and gas within hydatid cyst disease. A and B: Computed tomography (CT) axial and coronal CT images showing a focal fat density lobule within the hydatid cyst in the right lobe of the liver (arrows); C and D: Axial CT sections from two different patients showing air specks and air-fluid levels within the hydatid cyst (arrows). This was iatrogenic due to surgical deroofing of the cyst in C and due to superinfection of the cyst in D.

pleural effusion, atelectasis, or consolidation. Direct migration produces an hourglass-shaped cyst crossing the diaphragm, best appreciated on coronal or sagittal MRI. Cyst perforation into the lung can cause parenchymal consolidation, bronchial fistula, or expectoration of cyst membranes, whereas pleural involvement can lead to empyema or multiple pleural cysts[36]. Additionally, there may be exophytic extension and involvement of the abdominal wall (Figure 13)[40].

POSITRON EMISSION TOMOGRAPHY/CT IN HEPATIC HD

Positron emission tomography/CT is not routinely used for the diagnosis of hepatic HD but may be useful in selected cases with suspected superinfection, atypical imaging features, or equivocal disease activity. Active or infected cysts may demonstrate mild-to-moderate fluorodeoxyglucose uptake, whereas inactive or calcified cysts typically show no significant uptake (Figure 14); a characteristic "doughnut sign" has also been described in the literature[41]. Positron emission tomography/CT may additionally aid in detecting extrahepatic disease. However, its role in LMICs is limited by cost and availability, and US, CT, and MRI remain the primary imaging modalities.

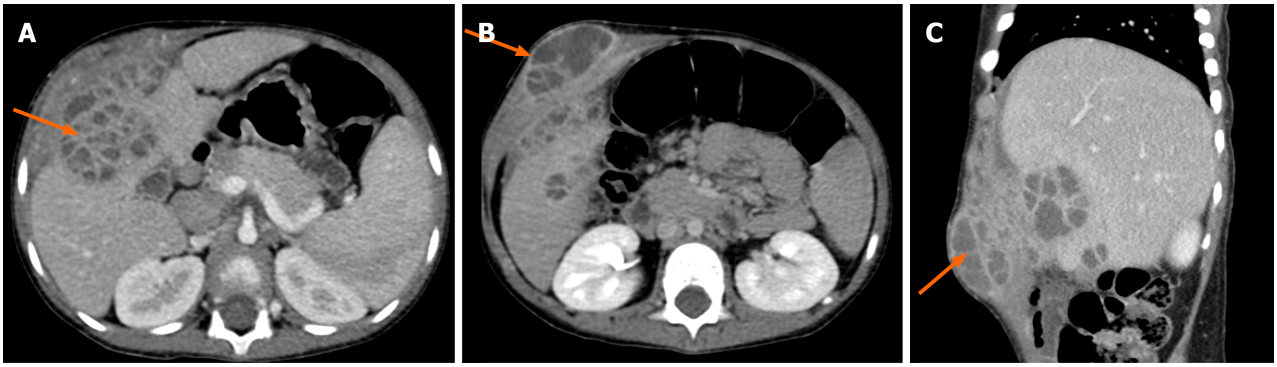


Figure 13 Hepatic hydatid disease with exophytic involvement of the abdominal wall. A: Computed tomography axial image showing a multicystic hydatid cyst in the right lobe of the liver involving segments IV and V (arrow); B and C: Axial and sagittal computed tomography images showing extension of the lesion from the subcapsular location of the liver and into the anterior abdominal wall (arrows).

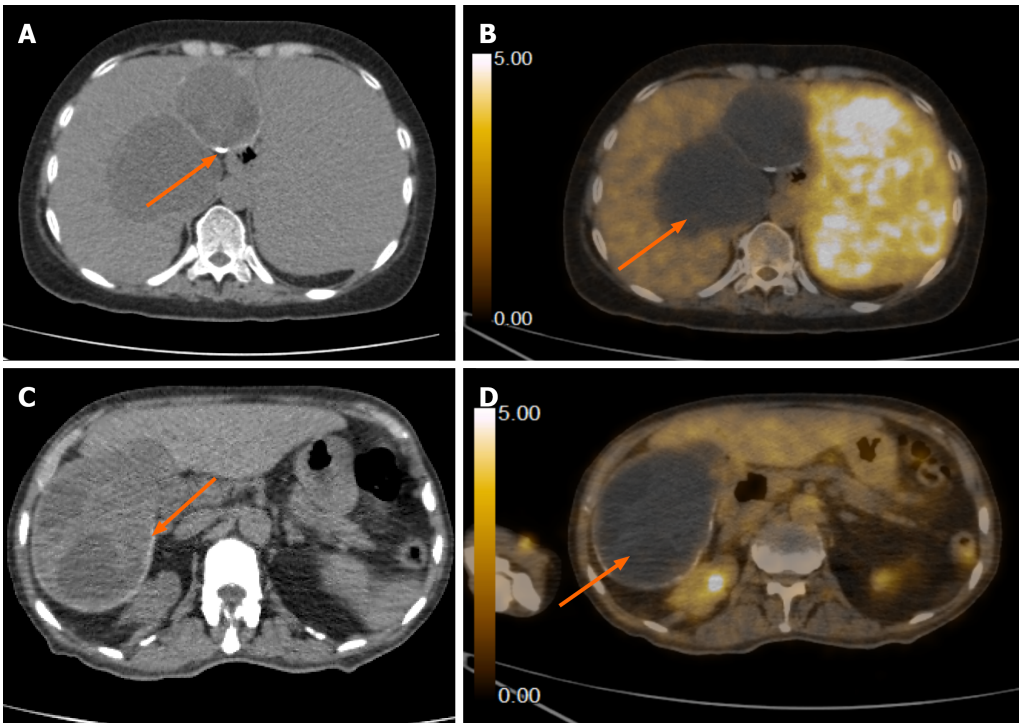


Figure 14 Positron emission tomography/computed tomography images of hydatid cysts in two patients. A: Plain computed tomography (CT) axial image showing a cystic lesion with wall calcification (arrow); B: Fused positron emission tomography/CT axial image shows no evidence of fluorodeoxyglucose activity within the lesion (arrow); C: Plain CT axial image shows another hydatid cyst in segment VI of the liver with peripheral calcification (arrow); D: Fused positron emission tomography/CT axial image shows no corresponding fluorodeoxyglucose activity (arrow).

RADIOLOGICAL MANAGEMENT OF HEPATIC HYDATID CYSTS

Radiological intervention plays a key role in the management of hepatic hydatid cysts, particularly in patients who are poor surgical candidates or in resource-limited settings. Generally, cysts less than 5 cm in size are considered for medical management with albendazole, whereas larger cysts are often considered for intervention[42,43]. Percutaneous drainage under US or CT guidance allows controlled evacuation of the contents of the cyst, minimizing the risk of rupture and anaphylaxis[44]. The PAIR technique - puncture, aspiration, injection, and re-aspiration - involves cyst puncture, aspiration of fluid, instillation of a scolicidal agent (most commonly 95% ethanol or 20% hypertonic saline), and removal of residual contents (Figure 15)[45]. PAIR is most effective in treating CE1 and selected CE2 cysts, whereas calcified or completely inactive cysts (CE4-CE5) are generally not suitable. Scolicidal agents should be used with caution, and cystobiliary communication should be excluded (*e.g.*, by aspiration of bile or contrast injection) before instillation to avoid severe biliary complications such as chemical or sclerosing cholangitis[45-47].

Imaging guidance is crucial for accurate needle placement, avoiding vascular or biliary structures, and for monitoring complications such as infection, bleeding, or biliary communication. Follow-up imaging with US or CT is used to assess cyst resolution, recurrence, or residual daughter cysts. In select cases, radiological interventions may serve as adjuncts to surgery, reduce cyst size preoperatively, or provide definitive therapy in high-risk patients, emphasizing the critical role

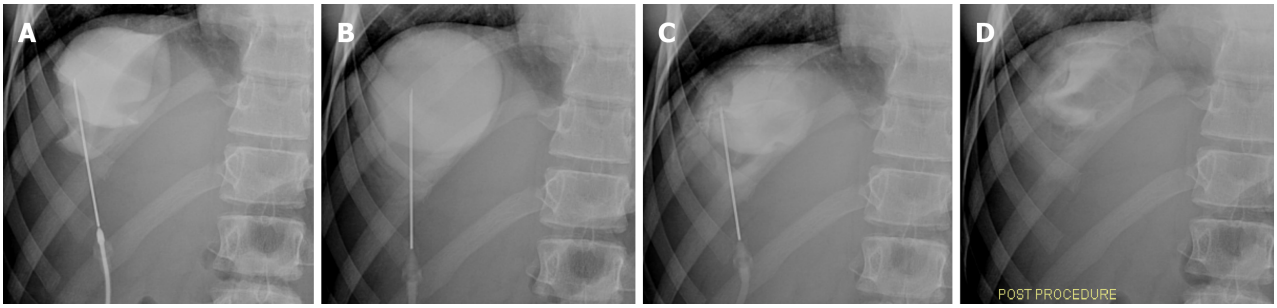


Figure 15 Percutaneous aspiration injection and reaspiration procedure fluoroscopic images. A 15-year-old girl presented with cystic echinococcosis 2 hydatid cysts in the right lobe of the liver and was diagnosed *via* ultrasound. Fluoroscopic images from percutaneous puncture, aspiration, injection and reaspiration performed for the management of hydatid cysts with post-procedure film. The cavity was rinsed with 100 mL of 20% hypertonic saline multiple times, and 20 mL of absolute alcohol was also injected into the cyst after aspiration. A: Aspiration; B: Injection; C: Reaspiration; D: Post-procedure film.

of imaging in both diagnosis and minimally invasive management[48].

IMAGING DIFFERENTIALS OF HEPATIC HYDATID CYSTS

The imaging appearance of hepatic HD may overlap with several cystic hepatic lesions, particularly in early or complicated stages (Table 1). CE1 cysts may closely mimic simple hepatic cysts on CT because of similar fluid attenuation; however, simple cysts lack any internal architectural evolution or complexity and show no associated stage-related changes at follow-up, whereas hydatid cysts may demonstrate progressive structural changes over time. US may be particularly valuable in differentiating the two[49].

Hepatic abscesses typically show thick, irregular rim enhancement with diffusion restriction, perilesional inflammatory edema, and possible intralesional gas, reflecting active infection, features not observed in uncomplicated HD. Unlike hydatid cysts, they lack internal daughter cysts and membranes and typically have ill-defined, shaggy, irregular walls. Abscesses also show surrounding parenchymal inflammatory changes, such as a hypoechoic halo on US or low-attenuation edema on CT[50,51].

Cystic or necrotic hepatic neoplasms such as metastases and biliary cystadenoma/cystadenocarcinoma are suggested by the presence of enhancing solid components, mural nodules, multiloculations and internal septations, which are uncommon in HD and favor a neoplastic etiology[52,53].

Alveolar echinococcosis (AE), caused by *Echinococcus multilocularis*, is an important, although less common, differential diagnosis. Unlike CE, AE typically presents as an ill-defined, infiltrative hepatic mass with numerous small cystic spaces within a heterogeneous solid matrix, often mimicking a slow-growing malignancy. Distinguishing features include the absence of a well-defined capsule, irregular central necrosis, coarse or punctate calcifications, biliary or vascular invasion, and adjacent organ extension. On CT and MRI, AE usually demonstrates minimal or peripheral enhancement, with a low-signal-intensity peripheral fibrotic rim on T2-weighted MRI and, in some cases, delayed peripheral fibroinflammatory enhancement[54,55].

CHALLENGES IN LMICs

LMICs face a unique set of challenges that significantly influence the imaging-based diagnosis and characterization of hepatic HD. These regions often have a relatively higher disease burden due to increased exposure to livestock and close contact with dogs, which serve as definitive hosts in the life cycle of *Echinococcus granulosus*[56,57]. Limited access to advanced imaging modalities, such as CT and MRI, results in a heavy reliance on US, which, although cost-effective and widely available, is inherently operator-dependent and subject to variability in interpretation[19,58]. This is further compounded by the absence of standardized imaging protocols, including inconsistent application of classification systems such as those proposed by the World Health Organization[59].

Additionally, the lack of structured screening programs and poor healthcare accessibility, particularly in remote areas, contribute to delayed clinical presentation, with patients often presenting at advanced stages characterized by large, complex, or complicated cysts. Financial constraints also limit access to follow-up imaging, hindering adequate monitoring of disease progression and treatment response[57,59]. Collectively, these factors not only complicate accurate diagnosis but also shape the spectrum of imaging findings observed in LMICs and endemic regions, with a higher prevalence of advanced and atypical presentations than in high-income settings.

Table 1 Imaging differentials of hepatic hydatid disease with key distinguishing imaging features

Differential diagnosis	Key differentiating imaging features
Simple hepatic cyst	Homogeneous water attenuation, thin smooth wall, no enhancement, no calcification, no septations or evolution on follow-up
Pyogenic liver abscess	Thick irregular rim enhancement, diffusion restriction, perilesional inflammatory edema, possible intralesional gas, clinical fever/right upper quadrant pain
Amoebic liver abscess	Solitary lesion with shaggy/ill-defined wall, peripheral enhancement, surrounding inflammatory changes, clinical fever/right upper quadrant pain
Cystic metastases	Irregular wall thickening with enhancing solid components, heterogeneous internal architecture, often known primary malignancy
Biliary cystic neoplasm	Multiloculated cystic lesion with enhancing septations and mural nodules or papillary projections
Hydatid disease	Cystic lesion with variable internal architecture depending on stage, typically without dominant solid enhancing components or aggressive inflammatory features

CONCLUSION

Hepatic hydatid cysts exhibit a wide spectrum of imaging appearances, reflecting their stage, activity, and potential complications. A combined understanding of WHO-IWGE and Gharbi classification, along with descriptive CT/MRI morphology enables accurate diagnosis, staging, and management. US remains the primary modality for detection and follow-up, particularly in LMICs, while CT and MRI provide complementary information on cyst morphology, calcification, daughter cysts, biliary involvement, and local or distant complications. Recognition of mass effect, biliary communication, rupture, infection, exophytic growth, transdiaphragmatic spread, and peritoneal seeding is essential for guiding treatment and preventing morbidity. Familiarity with these imaging patterns allows radiologists to provide comprehensive assessment, optimize patient management, and improve outcomes in endemic regions.

REFERENCES

1 **Bhalla VP**, Paul S, Klar E. Hydatid Disease of the Liver. *Visc Med* 2023; **39**: 112-120 [*RCA*] [PMID: 37899792 DOI: 10.1159/000533807] [FullText]

2 **Aydın Y**, Özgökçe M, Bilal Ulas A, Durmaz F, Kaşali K, Eren S, Aksungur N, Eroğlu A. Doubling time in pulmonary and hepatic hydatid cysts. *Turk Gogus Kalp Damar Cerrahisi Derg* 2024; **32**: 185-194 [*RCA*] [PMID: 38933308 DOI: 10.5606/tgkdc.dergisi.2024.25844] [Full Text] [Full Text(PDF)]

3 **Rinaldi F**, Brunetti E, Neumayr A, Maestri M, Goblirsch S, Tamarozzi F. Cystic echinococcosis of the liver: A primer for hepatologists. *World J Hepatol* 2014; **6**: 293-305 [*RCA*] [PMID: 24868323 DOI: 10.4254/wjh.v6.i5.293] [FullText] [Full Text(PDF)]

4 **Pedrosa I**, Saíz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid disease: radiologic and pathologic features and complications. *Radiographics* 2000; **20**: 795-817 [*RCA*] [PMID: 10835129 DOI: 10.1148/radiographics.20.3.g00ma06795] [FullText]

5 **Khalili N**, Iranpour P, Khalili N, Haseli S. Hydatid Disease: A Pictorial Review of Uncommon Locations. *Iran J Med Sci* 2023; **48**: 118-129 [*RCA*] [PMID: 36895460 DOI: 10.30476/IJMS.2022.93123.2442] [FullText] [Full Text(PDF)]

6 **Abbasi B**, Akhavan R, Ghamari Khameneh A, Darban Hosseini Amirkhiz G, Rezaei-Dalouei H, Tayebi S, Hashemi J, Aminizadeh B, Darban Hosseini Amirkhiz S. Computed tomography and magnetic resonance imaging of hydatid disease: A pictorial review of uncommon imaging presentations. *Heliyon* 2021; **7**: e07086 [*RCA*] [PMID: 34095581 DOI: 10.1016/j.heliyon.2021.e07086] [FullText] [Full Text(PDF)]

7 **Zalaquett E**, Menias C, Garrido F, Vargas M, Olivares JF, Campos D, Pinochet N, Luna A, Dahiya N, Huete Á. Imaging of Hydatid Disease with a Focus on Extrahepatic Involvement. *Radiographics* 2017; **37**: 901-923 [*RCA*] [PMID: 28493801 DOI: 10.1148/rg.2017160172] [Full Text]

8 **Brunetti E**, Kern P, Vuitton DA; Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010; **114**: 1-16 [*RCA*] [PMID: 19931502 DOI: 10.1016/j.actatropica.2009.11.001] [FullText]

9 **Stojkovic M**, Rosenberger K, Kauczor HU, Junghanss T, Hosch W. Diagnosing and staging of cystic echinococcosis: how do CT and MRI perform in comparison to ultrasound? *PLoS Negl Trop Dis* 2012; **6**: e1880 [*RCA*] [PMID: 23145199 DOI: 10.1371/journal.pntd.0001880] [Full Text] [Full Text(PDF)]

10 **Siles-Lucas M**, Uchiumi L, Tamarozzi F. 'No cyst, no echinococcosis': a scoping review update on the diagnosis of cystic echinococcosis after the issue of the WHO-IWGE Expert Consensus and current perspectives. *Curr Opin Infect Dis* 2023; **36**: 333-340 [*RCA*] [PMID: 37466103 DOI: 10.1097/QCO.0000000000000941] [FullText] [Full Text(PDF)]

11 **Rogan MT**, Hai WY, Richardson R, Zeyhle E, Craig PS. Hydatid cysts: does every picture tell a story? *Trends Parasitol* 2006; **22**: 431-438 [*RCA*] [PMID: 16843726 DOI: 10.1016/j.pt.2006.07.003] [FullText]

12 **Marrone G**, Crino' F, Caruso S, Mamone G, Carollo V, Milazzo M, Gruttadauria S, Luca A, Gridelli B. Multidisciplinary imaging of liver hydatidosis. *World J Gastroenterol* 2012; **18**: 1438-1447 [*RCA*] [PMID: 22509075 DOI: 10.3748/wjg.v18.i13.1438] [FullText] [Full Text (PDF)]

13 **Suwan Z**. Sonographic findings in hydatid disease of the liver: comparison with other imaging methods. *Ann Trop Med Parasitol* 1995; **89**: 261-269 [*RCA*] [PMID: 7668917 DOI: 10.1080/00034983.1995.11812951] [FullText]

14 **Yalcinoz K**, Ikizceli T, Kahveci S, Karahan OI. Diffusion-weighted MRI and FLAIR sequence for differentiation of hydatid cysts and simple cysts in the liver. *Eur J Radiol Open* 2021; **8**: 100355 [*RCA*] [PMID: 34136590 DOI: 10.1016/j.ejro.2021.100355] [FullText] [Full Text(PDF)]

15 **Díaz Á**, Fernández C, Pittini Á, Seoane PI, Allen JE, Casaravilla C. The laminated layer: Recent advances and insights into Echinococcus biology and evolution. *Exp Parasitol* 2015; **158**: 23-30 [*RCA*] [PMID: 25816974 DOI: 10.1016/j.exppara.2015.03.019] [FullText]

16 **Caremani M**, Lapini L, Caremani D, Occhini U. Sonographic diagnosis of hydatidosis: the sign of the cyst wall. *Eur J Ultrasound* 2003; **16**: 217-223 [*RCA*] [PMID: 12573791 DOI: 10.1016/s0929-8266(02)00076-9] [FullText]

17 **Czermak BV**, Akhan O, Hiemetzberger R, Zelger B, Vogel W, Jaschke W, Rieger M, Kim SY, Lim JH. Echinococcosis of the liver. *Abdom Imaging* 2008; **33**: 133-143 [*RCA*] [PMID: 17912581 DOI: 10.1007/s00261-007-9331-0] [FullText]

18 **Mortelé KJ**, Ros PR. Cystic focal liver lesions in the adult: differential CT and MR imaging features. *Radiographics* 2001; **21**: 895-910 [*RCA*] [PMID: 11452064 DOI: 10.1148/radiographics.21.4.g01jl16895] [FullText]

19 **Al-Shehari M**, Obadiel YA, Saryah LA, Al-Hamli M, Al-Absi M, Jowah HM. Computed Tomography (CT) Patterns of Hepatic Cystic Echinococcosis (CE) Cysts: A 19-Year Retrospective Study at a Tertiary Center in Sana'a, Yemen. *J Epidemiol Glob Health* 2025; **15**: 85 [*RCA*] [PMID: 40512449 DOI: 10.1007/s44197-025-00429-3] [FullText] [Full Text(PDF)]

20 **Zhang H**, Zhang L, Zhang C, Zhu YH, Hong YE, Li L, Lai L. CT imaging features and diagnostic algorithm for hepatic cystic echinococcosis. *Sci Rep* 2025; **15**: 10671 [*RCA*] [PMID: 40148408 DOI: 10.1038/s41598-025-94860-y] [FullText] [Full Text(PDF)]

21 **Greco S**, Cannella R, Giambelluca D, Pecoraro G, Battaglia E, Midiri M, Brancatelli G, Vernuccio F. Complications of hepatic echinococcosis: multimodality imaging approach. *Insights Imaging* 2019; **10**: 113 [*RCA*] [PMID: 31792750 DOI: 10.1186/s13244-019-0805-8] [FullText] [Full Text(PDF)]

22 **Alexiou K**, Mitsos S, Fotopoulos A, Karanikas I, Tavernaraki K, Konstantinidis F, Antonopoulos P, Ekonomou N. Complications of Hydatid Cysts of the Liver: Spiral Computed Tomography Findings. *Gastroenterology Res* 2012; **5**: 139-143 [*RCA*] [PMID: 27785194 DOI: 10.4021/gr460e] [FullText] [Full Text(PDF)]

23 **Lewall DB**. Hydatid disease: biology, pathology, imaging and classification. *Clin Radiol* 1998; **53**: 863-874 [*RCA*] [PMID: 9867269 DOI: 10.1016/s0009-9260(98)80212-2] [FullText]

24 **WHO Informal Working Group**. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003; **85**: 253-261 [*RCA*] [PMID: 12606104 DOI: 10.1016/s0001-706x(02)00223-1] [FullText]

25 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. *Radiology* 1981; **139**: 459-463 [*RCA*] [PMID: 7220891 DOI: 10.1148/radiology.139.2.7220891] [FullText]

26 **Saint Martin G**, Chiesa JC. "Falling snowflakes," an ultrasound sign of hydatid sand. *J Ultrasound Med* 1984; **3**: 257-260 [*RCA*] [PMID: 6748138 DOI: 10.7863/jum.1984.3.6.257] [FullText]

27 **Zubčić M**, Bienenfeld FS. Application of WHO-IWGE-Classification of the hydatid disease of liver in MRI. [cited 1 June 2026]. Available from: https://epos.myesr.org/poster/esr/ecr2025/C-27868

28 **Zhang T**, Li B, Liu Y, Liu S. Risk Factors Associated With Echinococcosis in the General Chinese Population: A Meta-Analysis and Systematic Review. *Front Public Health* 2022; **10**: 821265 [*RCA*] [PMID: 35655451 DOI: 10.3389/fpubh.2022.821265] [FullText] [Full Text (PDF)]

29 **Brunetti E**, Tamarozzi F, Macpherson C, Filice C, Piontek MS, Kabaalioglu A, Dong Y, Atkinson N, Richter J, Schreiber-Dietrich D, Dietrich CF. Ultrasound and Cystic Echinococcosis. *Ultrasound Int Open* 2018; **4**: E70-E78 [*RCA*] [PMID: 30364890 DOI: 10.1055/a-0650-3807] [Full Text] [Full Text(PDF)]

30 **Baykan AH**, Aydin E, Koc M, Sahin H, Karul A, Baykan ME, Ikizceli T, Erturk SM. Hydatid disease: imaging, treatment, and beyond. *Clin Radiol* 2025; **80**: 106748 [*RCA*] [PMID: 39631363 DOI: 10.1016/j.crad.2024.106748] [FullText]

31 **Al-Anbagi U**, Mostafa A, Mohammed IH, Nashwan AJ. Hepatic Hydatid Cyst Complicated by Biliary Obstruction: A Case Report. *Cureus* 2025; **17**: e85319 [*RCA*] [PMID: 40621278 DOI: 10.7759/cureus.85319] [FullText] [Full Text(PDF)]

32 **Ufuk F**, Duran M. Intrabiliary Rupture of Hepatic Hydatid Cyst Leading to Biliary Obstruction, Cholangitis, and Septicemia. *J Emerg Med* 2018; **54**: e15-e17 [*RCA*] [PMID: 29107478 DOI: 10.1016/j.jemermed.2017.09.009] [FullText]

33 **El Bakouri A**, Fatine A, Eddaoudi Y, Bouali M, El Hattabi K, Bensardi F, Fadil A. Peritoneal hydatidosis: An exceptional case report. *Ann Med Surg (Lond)* 2022; **83**: 104606 [*RCA*] [PMID: 36389191 DOI: 10.1016/j.amsu.2022.104606] [FullText]

34 **Kosmidis C**, Efthimiadis C, Anthimidis G, Vasileiadou K, Koimtzis G, Tzeveleki I, Koletsa T, Prousalidis J, Fahantidis E, Basdanis G, Michalopoulos A, Kesisoglou I. Management of peritoneal hydatid cysts: A fourty-year experience. *Heliyon* 2018; **4**: e00994 [*RCA*] [PMID: 30555954 DOI: 10.1016/j.heliyon.2018.e00994] [FullText] [Full Text(PDF)]

35 **Gulhane A**, Kotkar R, Ghate M. Fat within hepatic hydatid cyst - a case report. *Eurorad* 2017 [DOI: 10.1594/EURORAD/CASE.15046] [Full Text]

36 **Polat P**, Atamanalp SS. Hepatic hydatid disease: radiographics findings. *Eurasian J Med* 2009; **41**: 49-55 [*RCA*] [PMID: 25610064] [FullText]

37 **Mehta P**, Prakash M, Khandelwal N. Radiological manifestations of hydatid disease and its complications. *Trop Parasitol* 2016; **6**: 103-112 [*RCA*] [PMID: 27722098 DOI: 10.4103/2229-5070.190812] [FullText]

38 **Oruç E**, Yıldırım N, Topal NB, Kılıçturgay S, Akgöz S, Savcı G. The role of diffusion-weighted MRI in the classification of liver hydatid cysts and differentiation of simple cysts and abscesses from hydatid cysts. *Diagn Interv Radiol* 2010; **16**: 279-287 [*RCA*] [PMID: 20658444 DOI: 10.4261/1305-3825.DIR.2807-09.2] [FullText]

39 **Rodríguez-Laiz G**, Melgar P, Bolufer S, Navarro Martínez J, Ramia JM. Liver and lung hydatid cysts with transdiaphragmatic rupture treated by radical surgery. *Ann R Coll Surg Engl* 2022; **104**: e125-e127 [*RCA*] [PMID: 34931529 DOI: 10.1308/rcsann.2021.0183] [FullText]

40 **Alghofaily KA**, Saeedan MB, Aljohani IM, Alrasheed M, McWilliams S, Aldosary A, Neimatallah M. Hepatic hydatid disease complications: review of imaging findings and clinical implications. *Abdom Radiol (NY)* 2017; **42**: 199-210 [*RCA*] [PMID: 27487777 DOI: 10.1007/s00261-016-0860-2] [FullText]

41 **Demir H**, Halac M, Yilmaz S, Isgoren S, Sonmezoglu K, Uslu I. A doughnut sign of a hydatid cyst of the liver on F-18 FDG PET/CT. *Clin Nucl Med* 2008; **33**: 876-877 [*RCA*] [PMID: 19033795 DOI: 10.1097/RLU.0b013e31818bf2df] [FullText]

42 **Botezatu C**, Mastalier B, Patrascu T. Hepatic hydatid cyst - diagnose and treatment algorithm. *J Med Life* 2018; **11**: 203-209 [*RCA*] [PMID: 30364592 DOI: 10.25122/jml-2018-0045] [FullText] [Full Text(PDF)]

43 **Pakala T**, Molina M, Wu GY. Hepatic Echinococcal Cysts: A Review. *J Clin Transl Hepatol* 2016; **4**: 39-46 [*RCA*] [PMID: 27047771 DOI: 10.14218/JCTH.2015.00036] [FullText]

44 **Battyány I**, Herbert Z, Rostás T, Vincze A, Fülöp A, Harmat Z, Gasztonyi B. Successful percutaneous drainage of a giant hydatid cyst in the liver. *World J Gastroenterol* 2006; **12**: 812-814 [*RCA*] [PMID: 16521202 DOI: 10.3748/wjg.v12.i5.812] [FullText] [Full Text(PDF)]

45 **Shahid M**, Hilal K, Khan M, Ejaz ZH, Altaf S, Islam S, Khandwala K. Imaging insights into pediatric liver masses: A comprehensive

minireview for hepatology practice. *World J Hepatol* 2025; **17**: 107041 [*RCA*] [PMID: 40901586 DOI: 10.4254/wjh.v17.i8.107041] [FullText] [Full Text(PDF)]

46 **Khuroo MS**. Percutaneous Drainage in Hepatic Hydatidosis-The PAIR Technique: Concept, Technique, and Results. *J Clin Exp Hepatol* 2021; **11**: 592-602 [*RCA*] [PMID: 34511821 DOI: 10.1016/j.jceh.2021.05.005] [FullText] [Full Text(PDF)]

47 **Giorgio A**, Di Sarno A, de Stefano G, Liorre G, Farella N, Scognamiglio U, Giorgio V. Sonography and clinical outcome of viable hydatid liver cysts treated with double percutaneous aspiration and ethanol injection as first-line therapy: efficacy and long-term follow-up. *AJR Am J Roentgenol* 2009; **193**: W186-W192 [*RCA*] [PMID: 19696257 DOI: 10.2214/AJR.08.1518] [FullText]

48 **Pavlidis ET**, Galanis IN, Pavlidis TE. Current considerations for the management of liver echinococcosis. *World J Gastroenterol* 2025; **31**: 103973 [*RCA*] [PMID: 40093668 DOI: 10.3748/wjg.v31.i10.103973] [FullText] [Full Text(PDF)]

49 **Ran B**, Aji T, Jiang T, Zhang R, Guo Q, Abulizi A, Yimiti Y, Wen H, Shao Y. Differentiation between hepatic cystic echinococcosis types 1 and simple hepatic cysts: A retrospective analysis. *Medicine (Baltimore)* 2019; **98**: e13731 [*RCA*] [PMID: 30608385 DOI: 10.1097/MD.0000000000013731] [FullText] [Full Text(PDF)]

50 **Mortelé KJ**, Segatto E, Ros PR. The infected liver: radiologic-pathologic correlation. *Radiographics* 2004; **24**: 937-955 [*RCA*] [PMID: 15256619 DOI: 10.1148/rg.244035719] [FullText]

51 **Magacha HM**, Vedantam V, Vedantam N, Jagadish A. Liver Hydatid Cyst Masquerading as a Liver Abscess. *Cureus* 2023; **15**: e34334 [*RCA*] [PMID: 36865972 DOI: 10.7759/cureus.34334] [FullText] [Full Text(PDF)]

52 **Godkhindi VM**, Gupta N, Pai A, M A AA, Saravu K. Hydatid Cyst in Liver Masquerading As Metastatic Deposits From Ovarian Carcinoma. *Cureus* 2021; **13**: e17503 [*RCA*] [PMID: 34595075 DOI: 10.7759/cureus.17503] [FullText] [Full Text(PDF)]

53 **Tholomier C**, Wang Y, Aleynikova O, Vanounou T, Pelletier JS. Biliary mucinous cystic neoplasm mimicking a hydatid cyst: a case report and literature review. *BMC Gastroenterol* 2019; **19**: 103 [*RCA*] [PMID: 31234803 DOI: 10.1186/s12876-019-1001-5] [FullText] [Full Text (PDF)]

54 **Kantarci M**, Bayraktutan U, Karabulut N, Aydinli B, Ogul H, Yuce I, Calik M, Eren S, Atamanalp SS, Oto A. Alveolar echinococcosis: spectrum of findings at cross-sectional imaging. *Radiographics* 2012; **32**: 2053-2070 [*RCA*] [PMID: 23150858 DOI: 10.1148/rg.327125708] [FullText]

55 **Bulakçı M**, Kartal MG, Yılmaz S, Yılmaz E, Yılmaz R, Şahin D, Aşık M, Erol OB. Multimodality imaging in diagnosis and management of alveolar echinococcosis: an update. *Diagn Interv Radiol* 2016; **22**: 247-256 [*RCA*] [PMID: 27082120 DOI: 10.5152/dir.2015.15456] [FullText] [Full Text(PDF)]

56 **Muqaddas H**, Mehmood N, Ahmed F, Fatima M, Rasool M, Zafar S, Riaz A and Nauman M. Problems and Perspectives Related to Cystic Echinococcosis in Pakistan: Solutions in One Health Context. In: Aguilar-Marcelino L, Younus M,Khan A, Saeed NM and Abbas RZ, editors. Pakistan: Unique Scientific Publishers, 2023: 172-179

57 **Tamarozzi F**, Legnardi M, Fittipaldo A, Drigo M, Cassini R. Epidemiological distribution of Echinococcus granulosus s.l. infection in human and domestic animal hosts in European Mediterranean and Balkan countries: A systematic review. *PLoS Negl Trop Dis* 2020; **14**: e0008519 [*RCA*] [PMID: 32776936 DOI: 10.1371/journal.pntd.0008519] [FullText] [Full Text(PDF)]

58 **Frija G**, Blažić I, Frush DP, Hierath M, Kawooya M, Donoso-Bach L, Brkljačić B. How to improve access to medical imaging in low- and middle-income countries ? *EClinicalMedicine* 2021; **38**: 101034 [*RCA*] [PMID: 34337368 DOI: 10.1016/j.eclinm.2021.101034] [FullText] [Full Text(PDF)]

59 **Bold B**, Schindler C, Narankhuu U, Shagj A, Bavuujav E, Sodov S, Nyamdorj T, Zinsstag J. The Diagnostic Challenge of Cystic Echinococcosis in Humans: First Assessment of Underreporting Rates in Mongolia. *Trop Med Infect Dis* 2024; **9**: 163 [*RCA*] [PMID: 39058205 DOI: 10.3390/tropicalmed9070163] [FullText]

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MINIREVIEWS

Metabolic dysfunction-associated steatotic liver disease and the consequence of extra-hepatic malignancy

Benjamin Blaske, Michael Ward, Ragesh Babu Thandassery

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as the most prevalent chronic liver condition worldwide, affecting an estimated one-quarter to one-third of the global adult population. Once conceptualized primarily as a hepatic disorder, MASLD is now recognized as a systemic metabolic disease with broad extra-hepatic consequences. Among the most clinically significant of these is a rising body of evidence linking MASLD to an increased risk of malignancies outside the liver. This review synthesizes current evidence on extra-hepatic cancers in MASLD, organized into two categories: Gastrointestinal (GI)-related malignancies and non-GI malignancies. We discuss the shared oncogenic mechanisms underlying these associations, including insulin resistance, chronic inflammation, lipotoxicity, gut microbiome dysbiosis, and adipokine dysregulation. Emerging data demonstrates rising trends in extra-hepatic cancer burden in MASLD populations, with particular concern for young-onset disease and advanced hepatic fibrosis as risk-amplifying factors. We also address current diagnostic gaps and the clinical imperative for developing MASLD-specific cancer surveillance strategies.

Key Words: Metabolic dysfunction-associated steatotic liver disease; Extra-hepatic malignancy; Colorectal cancer; Pancreatic cancer; Breast cancer; Insulin resistance; Obesity; Cancer surveillance; Gut microbiome; Inflammation

Core Tip: Metabolic dysfunction-associated steatotic liver disease (MASLD) confers increased risk of extra-hepatic malignancies spanning multiple organ systems, beyond its well-recognized association with hepatocellular carcinoma. Shared oncogenic pathways which include insulin resistance, chronic systemic inflammation, lipotoxicity, and gut dysbiosis, link MASLD to both gastrointestinal (GI) cancers (colorectal, pancreatic, esophagogastric, biliary) and non-GI cancers (breast, gynecologic, thyroid, urinary, lung). Globally, the burden and prevalence of MASLD is rising, with young-onset disease and advanced fibrosis as key risk amplifiers. Dedicated cancer surveillance protocols for MASLD populations are urgently needed.

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) represents the most common chronic liver condition globally[1,2]. It is defined by the presence of hepatic steatosis with at least one trait of metabolic syndrome in the absence of significant alcohol consumption or other identifiable causes of hepatic steatosis. Initially the disease was described as non-alcoholic fatty liver disease (NAFLD) with nomenclature shifting toward the name metabolic associated fatty liver disease (MAFLD) as prevalence and research proved its systemic complexity[1]. In 2023, the multisociety Delphi consensus concluded a definition of MASLD to provide a non-stigmatizing and accurate representation of the disease[2]. Worldwide the prevalence of MASLD continues to rise with a prevalence of approximately 25%-38% driven by parallel obesity and type 2 diabetes epidemics[3]. There are notable geographic discrepancies, however it remains evident the overall healthcare burden is substantial[4]. MASLD is not a disease restricted to hepatic manifestations; rather clinical evidence continues to show its systemic effect. Cardiovascular disease (CVD), chronic kidney disease, type 2 diabetes mellitus (T2DM), and colorectal cancers are the most significant complications of MASLD and causes for patient mortality [5]. Important liver physiological mechanisms are impacted in MASLD believed to be associated with "systemic inflammatory milieu initiated partly by liver-secreted cytokines and molecules"[6]. Consequently, this inflammatory state likely contributes toward systemic insulin resistance and related metabolic dysfunction which play a pathogenic role in development of liver-related morbidities and extrahepatic complications[7]. The leading cause of death is predominantly CVD followed by malignancies, liver-related mortality, and diabetes-related mortality[8]. Extrahepatic malignancy related deaths have outpaced those from hepatocellular carcinoma (HCC) and given the rapidly increasing MASLD population burden this poses a critical health and economic issue[9]. The 2022 meta-analysis completed by Thomas *et al* [10] demonstrated a pooled extrahepatic cancer incidence rate in MASLD was 10.58 per 1000 person-years, over eight-fold more frequent than HCC. Additional studies confirm this rising trend and identify that the malignancy burden applies across multiple organ systems including gastrointestinal (GI), pulmonary, breast, gynecological, and urinary[11,12]. Although CVD remains the leading cause-specific mortality in patients with MASLD, malignancies consistently represent the second leading cause of death, frequently outpacing liver-related mortality[13,14]. This review provides a comprehensive synthesis of the current evidence linking MASLD to extra-hepatic malignancies, organized according to a dual framework: GI-related cancers and non-GI cancers. For each cancer type, we examine epidemiological associations, proposed oncogenic mechanisms, and emerging trends. We also discuss the broader implications for cancer surveillance and risk reduction in MASLD populations. While several narrative reviews have addressed individual MASLD-cancer associations, the present synthesis integrates GI and non-GI malignancies within a mechanistic framework while explicitly incorporating young-onset disease, fibrosis-stage stratification, sex-specific dimorphism, and their surveillance implications, which we frame as a research and practice agenda rather than a claim of comprehensiveness.

MASLD PATHOPHYSIOLOGY AND ONCOGENESIS: SHARED MECHANISMS

Before examining individual cancer types, it is essential to establish the mechanistic framework connecting MASLD to extra-hepatic malignancy. Several interrelated pathophysiological pathways common to MASLD create a systemic pro-oncogenic milieu predisposing to cancer development across multiple organ systems.

Insulin resistance and hyperinsulinemia

Evidence suggests that insulin resistance and increased insulin levels are key hormonal mechanisms linked to elevated risk of several cancer types[15]. In patients with MASLD there is a notable predisposition for developing T2DM[16]. Hyperinsulinemia is thought to promote oncogenesis through upregulation of insulin-like growth factor-1 (IGF-1) and subsequent activation of intracellular signaling pathways involved in mitogenesis, inhibition of apoptosis, and angiogenesis[15]. Insulin resistance therefore represents a central driver of both MASLD progression and carcinogenesis.

Chronic low-grade inflammation and cytokine milieu

Chronic low-grade inflammation is a defining biological feature of MASLD and a highly suspected mediator of its extrahepatic oncogenic burden. In a pro-inflammatory state, oncogenic mechanisms are constitutively activated. Targher and colleagues argue that this chronic inflammatory state is "crucially involved in MASLD progression" and additionally promotes extrahepatic cancers, with nuclear factor kappaB (NF-κB) centrally implicated in the generation of hepatic insulin resistance[7]. There is evidence that fat deposition induces dynamic changes fostering inflammation and immune responses that enhance cancer susceptibility[17]. With obesity coexisting in 80%-90% of patients with MASLD, the so-called "metainflammation" of adipose tissue compounds the hepatic inflammatory signal, producing a sustained systemic milieu of elevated tumor necrosis factor alpha, interleukin-6, high-sensitivity C-reactive protein, and interleukin-1RA[7]. Direct quantitative support for this pathway has recently emerged from a United Kingdom Biobank analysis in which 11.2%-31.0% of MASLD-cancer was mediated by inflammation[18].

Lipotoxicity and oxidative stress

In MASLD, hepatic accumulation of saturated free fatty acids (FFAs) and their lipotoxic intermediates, including diacylglycerols, ceramides, and lysophosphatidylcholine, exceeds the buffering capacity of mitochondrial β-oxidation. This drives a cascade of injurious intracellular events with oncogenic consequences. The resulting mitochondrial structural and functional dysfunction is central to this process: Impaired electron transport chain activity generates excess reactive oxygen species (ROS), which in turn inflict oxidative damage to DNA, proteins, and membrane lipids[19]. Lipid peroxidation byproducts, most notably malondialdehyde and 4-hydroxynonenal, form mutagenic DNA adducts, induce strand breaks, and promote epigenetic dysregulation including aberrant CpG island methylation that silences tumor suppressor genes[19]. Mitochondrial dysfunction further disrupts cellular bioenergetics and amplifies the pro-inflammatory signaling initiated by upstream insulin resistance, creating a self-reinforcing cycle of lipotoxic and oxidative injury[19]. In the broader metabolic context of altered lipid substrate utilization and FFA signaling, impaired ketogenic flux, which normally acts as a mitochondria-protective pathway, further illustrates how dysregulated lipid metabolism in MASLD generates systemic oncogenic pressure beyond the liver[20].

Gut microbiome dysbiosis and the gut-liver axis

MASLD is both a cause and consequence of gut microbiome dysbiosis, and the bidirectional gut-liver axis represents one of the most mechanistically detailed pathways linking MASLD to extrahepatic malignancy. In healthy physiology, the intestinal microbiota generate short-chain fatty acids (SCFAs), principally butyrate, propionate, and acetate, through fermentation of dietary fiber[21]. These metabolites exert anti-inflammatory, barrier-protective, and regulatory T-cell-inducing effects on the colonic mucosa[21]. In MASLD-associated dysbiosis, SCFA-producing taxa (principally *Faecalibacterium prausnitzii* and *Roseburia spp.*) are depleted and replaced by dysbiotic, gram-negative organisms, shifting the hepatic portal influx toward lipopolysaccharide and other pathogen-associated molecular patterns that activate hepatic toll-like receptor-4 and NF-κB signaling, sustaining the systemic inflammatory milieu described in the subsection on chronic low-grade inflammation and cytokine milieu[5,22].

Simultaneously, dysbiotic microbiota alter the conversion of primary to secondary bile acids: Reduced 7α-dehydroxylation activity by *Clostridium* cluster XIVa and IV species depresses the ratio of primary-to-secondary bile acids, elevating deoxycholic acid (DCA) and lithocholic acid concentrations in the enterohepatic and systemic circulation[21]. These secondary bile acids are genotoxic and pro-proliferative at concentrations found in the colon, biliary tree, and liver, and experimental blockade of DCA production suppresses hepatic carcinogenesis in murine models[5]. Beyond bile acid metabolism, dysbiosis-associated increases in intestinal permeability allow microbial translocation products to reach distant organs *via* the systemic circulation, a mechanism relevant to extrahepatic carcinogenesis at multiple sites[22,23]. Collectively, the gut-liver axis serves as a shared mechanistic conduit for MASLD-associated oncogenesis, most prominently within the GI tract (Figure 1). This provides a biological rationale for the especially robust MASLD-colorectal cancer (CRC) associations described in the discussion of colorectal cancer.

Hepatocyte-derived extracellular vesicles and systemic crosstalk

Beyond the traditional pathways of cytokine signaling and metabolite exchange, hepatocyte-derived extracellular vesicles (EVs), including exosomes and microvesicles, have emerged as a distinct mechanism of inter-organ communication relevant to MASLD-associated extrahepatic oncogenesis. Steatotic and stressed hepatocytes release EVs whose composition reflects the altered lipidomic, transcriptomic, and proteomic state of the injured liver. These vesicles transport bioactive cargo, including microRNAs, mRNAs, lipid mediators, and damage-associated molecular patterns, through the systemic circulation to distant tissues, where they modulate recipient cell phenotype and function[24]. In MASLD, this EV-mediated signaling contributes to progression of hepatic injury but also extends the pathophysiology of the disease beyond the liver, providing a candidate mechanism by which a "local" hepatic disorder produces "global" oncogenic effects in distant organs such as the breast, lung, and colon[23,24]. The broader framework of inter-organ crosstalk linking MAFLD to multi-organ disease, mediated by insulin resistance, visceral adiposity, and now EV signaling, reinforces this conceptual shift from viewing MASLD as a hepatic condition to viewing it as a systemic metabolic disorder with measurable downstream oncologic consequences[23].

Adipose tissue and hormonal dysregulation

MASLD exists within a broader context of metabolic-endocrine dysregulation in which adipose tissue is a major contributor to systemic oncogenic signaling. The disease has been reciprocally linked to multiple endocrinopathies, including hypothyroidism, polycystic ovary syndrome, growth hormone deficiency, hypogonadism, and hyper-

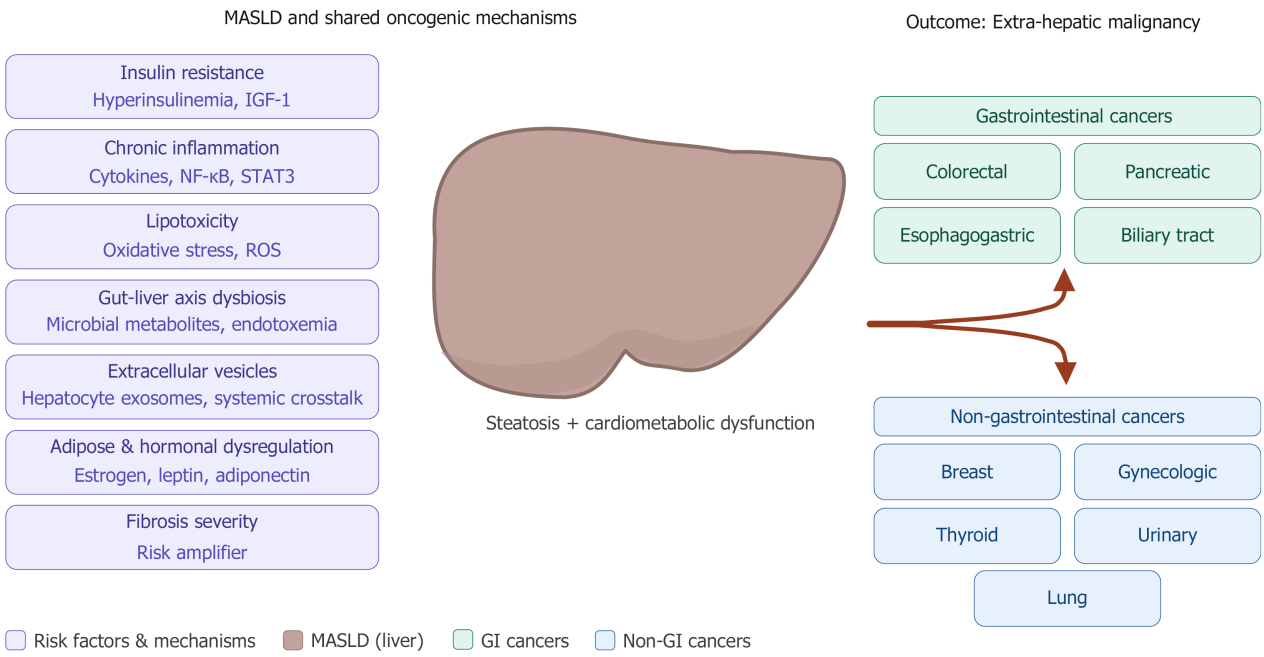


Figure 1 Shared oncogenic mechanisms of metabolic dysfunction-associated steatotic liver disease and extrahepatic malignancy as the downstream outcome. Metabolic dysfunction-associated steatotic liver disease and its shared oncogenic mechanisms, which include insulin resistance, chronic inflammation, lipotoxicity, gut-liver axis dysbiosis, extracellular vesicle signaling, adipose and hormonal dysregulation, and fibrosis severity, predispose to gastrointestinal and non-gastrointestinal extrahepatic malignancies. IGF-1: Insulin-like growth factor 1; NF-κB: Nuclear factor kappa B; STAT3: Signal transducer and activator of transcription 3; ROS: Reactive oxygen species; GI: Gastrointestinal; MASLD: Metabolic dysfunction-associated steatotic liver disease.

cortisolism, each of which can independently modify cancer risk[25]. MASLD is now increasingly understood as a sexually dimorphic disease where circulating estrogen and androgen levels, and the estrogen-to-androgen ratio, influence hepatic disease trajectory and the spectrum of associated hormone-sensitive cancers, particularly breast and gynecologic malignancies[26].

Multiple mechanisms originating from adipose tissue are thought to mediate this hormonal contribution to cancer risk. Adipose tissue is a principal site of aromatase activity, and peripheral aromatization of androgens to estrogens generates an estrogen excess that, when unopposed by progesterone, sustains proliferative signaling in estrogen-receptor-positive breast and endometrial tissue[26]. Adipokine balance also becomes disturbed. Leptin, which rises in the setting of MASLD and obesity, activates JAK/STAT, MAPK, and PI3K/Akt pathways implicated in tumor proliferation and angiogenesis, while adiponectin, an anti-proliferative and insulin-sensitizing adipokine, is paradoxically reduced[26]. In addition, dysregulation of hepatokines in MASLD including fetuin-A, fibroblast growth factor 21, and selenoprotein P, further contributes to peripheral inflammation and altered tissue homeostasis with downstream oncologic implications [26]. Together, these endocrine and adipokine disturbances offer an explanation for the disproportionate burden of breast, endometrial, and ovarian cancer observed in women with MASLD, as detailed in the discussions of breast cancer, gynecologic cancers, and sex differences and dimorphic cancer risk.

Fibrosis severity as a cancer risk modifier

While hepatic fibrosis is the dominant determinant of liver-related mortality in MASLD, its relationship with extrahepatic cancer risk is more nuanced. The Vilar-Gomez *et al*[27] multinational cohort of 458 patients with biopsy-confirmed advanced NAFLD demonstrated a critical clinical distinction: Patients with bridging fibrosis (F3) developed predominantly nonhepatic cancers and vascular events, whereas those with cirrhosis (F4) developed predominantly liver-related events. This finding identifies advanced non-cirrhotic fibrosis as the key clinical stage at which extrahepatic oncologic risk is concentrated. The NASH Clinical Research Network prospective study by Sanyal *et al*[28] confirmed fibrosis stage as the dominant predictor of all-cause mortality, with patients harboring advanced fibrosis at baseline experiencing markedly elevated cumulative mortality over a median 4-year follow-up. Simon *et al*[29], in a Swedish population-based cohort of 8892 adults with biopsy-confirmed NAFLD, demonstrated that overall cancer incidence rose monotonically across categories of simple steatosis, non-fibrotic NASH, non-cirrhotic fibrosis, and cirrhosis, with diabetes further amplifying this gradient. However, the dose-response was driven primarily by HCC and only modestly by extrahepatic cancers (pancreatic, kidney/bladder, and melanoma). Importantly, the Swedish Björkström *et al*[30] cohort study reported that liver cancer risk in NAFLD was elevated independent of cirrhosis, and that the extrahepatic cancer signal, though slightly elevated, was modest in magnitude. Similarly, the Thomas 2022 meta-analysis found that extrahepatic cancer incidence was not further amplified in patients with advanced fibrosis or cirrhosis[10]. These findings are consistent with a model in which extrahepatic carcinogenesis in MASLD is driven by systemic metabolic exposure rather than the hepatic fibrosis pathway itself. In addition, a multicenter cross-sectional study of the Chinese MASLD population found that cardiometabolic burden, including hypertension and hyperglycemia, together with higher Fibrosis-4 index (FIB-4), was

associated with extrahepatic cancers. This indicated that metabolic exposure and fibrosis stage are jointly related to extrahepatic risk[31]. Genetic data further support a systemic rather than a liver-specific driver in a meta-analysis of 232033 individuals with MASLD which found that the PNPLA3 I148M variant conferred dose-dependent increases in liver-related events and HCC but showed no association with extrahepatic cancers among homozygous risk-allele carriers compared with wild-type homozygotes [hazard ratio (HR) 0.96, 95%CI: 0.67-1.37], implying that the extrahepatic cancer excess in MASLD is mediated by shared metabolic dysfunction rather than by the hepatic genetic risk allele[32]. Collectively, these data support the use of fibrosis stage as a clinical stratifier for liver-related outcomes, while extra-hepatic cancer risk in MASLD is best understood as dependent on cumulative metabolic burden and disease duration rather than fibrosis stage alone.

GASTROINTESTINAL EXTRAHEPATIC MALIGNANCIES IN MASLD

The GI tract shares key anatomical and physiological connections with the liver through the portal circulation and gut-liver axis, which may partly explain the particularly strong associations between MASLD and GI tract malignancies. The key features of GI-related extra-hepatic cancers in MASLD are summarized in Table 1.

CRC

The association between CRC and MASLD is the most extensively characterized of the GI extrahepatic malignancies. The 2026 international Delphi consensus statement affirmed that MASLD independently increases CRC risk and that greater metabolic burden and disease severity amplify this risk[33]. An updated 2025 meta-analysis of 48 studies encompassing more than 56 million participants reported pooled odds ratios of 1.37 for CRC, 1.81 for colorectal adenomas, and 1.86 for colorectal polyps among individuals with NAFLD/MASLD[34]. A meta-analysis by Mantovani *et al*[12] similarly described an approximately 1.5- to 2-fold elevation in CRC risk, and a 21-year community-based cohort demonstrated a near 2-fold increase in incident malignancies, with GI tumors among the predominant sites[12,35].

The mechanistic basis is best framed through the gut-liver axis. MASLD-associated intestinal dysbiosis alters microbial conversion of primary to secondary bile acids, particularly DCA, which has been implicated in colonic genotoxicity and tumor promotion. Concurrent depletion of SCFA-producing taxa attenuates mucosal anti-inflammatory and regulatory T-cell signaling[21,22]. Increased intestinal permeability and lipopolysaccharide translocation drive NF-κB-mediated chronic low-grade inflammation, while systemic hyperinsulinemia and IGF-1 axis activation provide additional mitogenic stimuli along the adenoma-carcinoma sequence[36].

Importantly, the MASLD-CRC association persists after adjustment for obesity, T2DM, and other components of metabolic syndrome, supporting an independent oncogenic contribution from hepatic steatosis and hepatic fibrosis itself [12,36]. This association extends to lean MASLD, where the magnitude of the link with colorectal adenoma may exceed that observed in obese individuals[34]. Such findings underscore the need for MASLD-specific CRC risk stratification tools beyond conventional metabolic markers.

Pancreatic cancer

Pancreatic ductal adenocarcinoma represents a biologically plausible target of MASLD-driven metabolic stress. The available epidemiological data support a positive, though more modest, association than observed for CRC. In the largest meta-analysis on this question, encompassing 18 cohort studies and approximately 16.7 million participants, MASLD was associated with a pooled hazard ratio of 1.41 (95%CI: 1.11-1.79) for incident pancreatic cancer[37]. The Mantovani *et al*[12] meta-analysis in Gut (10 cohorts, 182202 participants) similarly demonstrated a 1.5- to 2-fold increase in GI cancer risk, including pancreatic cancer, among individuals with NAFLD, with effect estimates persisting after adjustment for age, sex, smoking, obesity, and diabetes. The pooled-incidence meta-analysis by Thomas *et al*[10] further reinforced pancreatic cancer as one of the malignancies with elevated incidence in NAFLD. Notably, in that analysis extrahepatic cancer incidence was not further amplified in patients with advanced fibrosis or cirrhosis. This pattern highlights the importance of systemic metabolic, rather than purely fibrosis-driven mechanisms, in pathways of pancreatic carcinogenesis. Earlier systematic reviews and contemporary narrative syntheses have reached convergent conclusions[9,11,36].

Mechanistically, MASLD and pancreatic cancer share several oncogenic axes. Hepatic insulin resistance with compensatory hyperinsulinemia activates insulin/IGF-1 signaling, exerting mitogenic, anti-apoptotic, and pro-angiogenic effects on pancreatic acinar and ductal epithelium[15]. Visceral adiposity and ectopic intra-pancreatic fat deposition generate a local pro-inflammatory milieu, with adipokine dysregulation, FFA lipotoxicity, and macrophage-mediated stromal remodeling implicated in acinar-to-ductal metaplasia, a recognized precursor lesion[16,36]. T2DM, which develops in a substantial proportion of MASLD patients, is itself an established pancreatic cancer risk factor and likely functions as both co-driver and effect modifier. This overlap is acknowledged in the 2026 American Diabetes Association (ADA) Standards of Care and the 2025 ADA-Endocrine Society consensus, both of which cite GI cancers, including pancreatic cancer, among the extrahepatic manifestations of MASLD warranting integrated cardiometabolic-oncologic risk assessment[38,39].

Overall, the MASLD-pancreatic cancer signal is consistent across independent meta-analyses but of moderate magnitude and disentangling MASLD's contribution from that of T2DM and central adiposity remains a methodological challenge. Dedicated cohorts with detailed adjustment for diabetes status, glycemic control, and pancreatic imaging are needed to refine risk estimates and inform any future surveillance strategy.

Table 1 Gastrointestinal extrahepatic malignancies in metabolic dysfunction-associated steatotic liver disease: Cancer type, key studies, risk estimates, proposed mechanisms, and trends

Cancer type	Ref.	Risk estimate (HR or OR)	Proposed mechanisms	Trend/notes
Colorectal cancer	Wu *et al*[33], 2026 (international Delphi consensus, *Gut*); Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis, $n \approx$ 16.7M); Allen *et al*[35], 2019 (community cohort, *J Hepatol*); Azimi *et al*[34], 2025 (meta-analysis, $n >$ 56M)	HR 1.33 (Zhou *et al*[37] meta-analysis); approximately 1.5-2-fold (Mantovani *et al*[12], 2022); OR 1.37 (Azimi *et al*[34], 2025)	Gut dysbiosis; secondary bile acid alterations; hyperinsulinemia; mucosal inflammation; IGF-1 axis activation	Rising; strongest GI cancer association; independent of obesity and T2DM
Pancreatic cancer	Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis, *Eur J Clin Invest*); Thomas *et al*[9], 2024 (review, *Lancet GE&H*); Thomas *et al*[10], 2022 (meta-analysis, *Eur J Cancer*); Mantovani *et al*[36], 2024	HR 1.41 (Zhou *et al*[37] meta-analysis); approximately 1.5-2-fold (Mantovani *et al*[12], 2022)	Insulin resistance; visceral adiposity; acinar inflammation; shared T2DM pathway	Rising; evidence strengthening with larger population cohorts
Esophageal/gastric cancer	Zou *et al*[40], 2023 (meta-analysis); Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis); Thomas *et al*[9], 2024 (*Lancet GE & H*); Mantovani *et al*[36], 2024	Gastric: HR 1.47 (Zhou *et al*[37]), RR 1.49 (Zou *et al*[40]); esophageal: RR 1.76 (Zou *et al*[40]), null in Zhou *et al*[37] meta-analysis	GERD; Barrett's esophagus; visceral adiposity; reflux-related mucosal injury	Moderate; gastric cancer signal more consistent than esophageal across meta-analyses
Biliary tract cancer (gallbladder/cholangiocarcinoma)	Park *et al*[41], 2021 (nationwide Korean cohort, $n \approx$ 8.1M, *Eur J Cancer*); Zhou *et al*[37], 2024 (meta-analysis); Mantovani *et al*[36], 2024	BTC overall: AHR 1.28 (Park *et al*[41], 2021), HR 1.27 (Zhou *et al*[37]); cholangiocarcinoma aHR 1.33; gallbladder aHR 1.14	Biliary stasis; cholelithiasis; chronic biliary inflammation	Emerging; cholangiocarcinoma shows the stronger signal; gallbladder signal weaker; Western validation limited

HR: Hazard ratio; OR: Odds ratio; aHR: Adjusted hazard ratio; RR: Relative risk; IGF-1: Insulin-like growth factor-1; T2DM: Type 2 diabetes mellitus; GERD: Gastroesophageal reflux disease; BTC: Biliary tract cancer; GI: Gastrointestinal.

Esophageal and gastric cancer

The association between MASLD and upper GI malignancies is biologically plausible, mediated through visceral adiposity, gastroesophageal reflux disease (GERD), Barrett's esophagus, and shared metabolic-inflammatory pathways. However, the supporting epidemiological data are less consistent than for CRC. The most directly relevant evidence comes from the systematic review and meta-analysis by Zou *et al*[40], which pooled data from 8 studies encompassing 8629525 participants and reported significantly increased risks of both gastric cancer [pooled relative risk (RR) 1.49, 95%CI: 1.17-1.91] and esophageal cancer (pooled RR 1.76, 95%CI: 1.34-2.32) among patients with MAFLD/NAFLD. The Mantovani *et al*[12] meta-analysis in Gut similarly grouped esophageal and gastric cancers within the GI cancers showing a 1.5- to 2-fold increase in MASLD, with risk independent of the major confounders. Importantly, however, the larger Zhou *et al*[37] meta-analysis of 18 cohort studies (approximately 16.7 million participants) found a positive signal for gastric cancer (HR 1.47, 95%CI: 1.07-2.01) but a null association for esophageal cancer (HR 1.26, 95%CI: 0.86-1.86). This indicates that, while pooled cohort data support a gastric cancer signal, they do not consistently reproduce the esophageal cancer association observed in earlier studies. The Thomas *et al*[9] review and Mantovani *et al*[36] GI-cancers narrative highlight the same heterogeneity and call for site- and histology-specific stratification.

The proposed mechanisms differ between the two sites. For esophageal adenocarcinoma, central adiposity associated with MASLD promotes GERD and Barrett's esophagus, providing a chronic inflammatory and metaplastic substrate for malignant transformation[9,36]. For gastric cancer, hyperinsulinemia, IGF-1 axis activation, and adipokine dysregulation overlap with mechanisms implicated in colorectal carcinogenesis, while gut-liver axis perturbations may further modulate gastric mucosal homeostasis[36].

The gastric cancer signal in MASLD appears more reproducible than the esophageal cancer signal, with the latter being driven primarily by Zou *et al*[40] and not confirmed in the larger and more recent Zhou *et al*[37] pooled analysis. Histology-stratified analyses (esophageal adenocarcinoma *vs* squamous cell carcinoma; gastric cardia *vs* non-cardia) and adjustment for Helicobacter pylori status, smoking, and alcohol exposure are needed to clarify the magnitude and biological direction of these associations.

Biliary tract cancers (gallbladder and cholangiocarcinoma)

Biliary tract cancers (BTCs) represent an emerging and biologically coherent association with MASLD, mechanistically linked through chronic biliary stasis, cholelithiasis, and low-grade inflammatory injury to the biliary epithelium. The most informative single dataset is the nationwide South Korean cohort study by Park *et al*[41], which followed 8120674

adults and reported a significantly increased risk of BTC overall in NAFLD [(adjusted hazard ratio, aHR) 1.28, 95%CI: 1.20-1.37], with the strongest signal for cholangiocarcinoma (aHR 1.33, 95%CI: 1.23-1.43) and a more modest, borderline-significant signal for gallbladder cancer (aHR 1.14, 95%CI: 1.003-1.29); concomitant NAFLD and diabetes were associated with a 47% increase in BTC risk. This nationwide signal is reinforced by the Zhou *et al*[37] meta-analysis of 18 cohort studies (approximately 16.7 million participants), which reported a pooled HR of 1.27 (95%CI: 1.18-1.37) for BTC in MASLD. The Mantovani *et al*[36] narrative review on GI extrahepatic cancers in MASLD frames these findings within a coherent mechanistic and epidemiologic synthesis.

Mechanistically, MASLD is associated with cholesterol gallstone disease, biliary sludge, and altered bile composition, all of which promote chronic biliary inflammation and a pro-carcinogenic ductal microenvironment. Hepatic insulin resistance and dyslipidemia drive supersaturation of bile with cholesterol, while gut-liver axis perturbations and altered enterohepatic bile acid cycling, particularly elevated secondary bile acids such as DCA, provide additional genotoxic and proliferative stimuli to cholangiocytes[36]. T2DM, which commonly coexists with MASLD[38,39], independently amplifies BTC risk and likely accounts for some of the diabetes-MASLD synergy observed in Park *et al*[41].

A few caveats are worth noting. The cholangiocarcinoma signal is consistently stronger than the gallbladder cancer signal, which contrasts with the broader obesity-cancer literature, where gallbladder cancer is the more commonly reported biliary association. Whether this reflects true biology or differential ascertainment requires further study. A further limitation is that much of the strongest evidence derives from a single Asian nationwide cohort. Validation in Western populations, particularly with differentiation into intrahepatic and extrahepatic cholangiocarcinoma, remains limited. Despite these caveats, BTC, and particularly cholangiocarcinoma, is an emerging MASLD-associated malignancy warranting attention in long-term cancer surveillance discussions.

NON-GASTROINTESTINAL EXTRAHEPATIC MALIGNANCIES IN MASLD

Beyond the GI tract, MASLD has become increasingly associated with malignancies in other organ systems through systemic hormonal, inflammatory, and metabolic mechanisms. Key features of the non-GI extrahepatic malignancies are briefly summarized in Table 2.

Breast cancer

Breast cancer is one of the highest-burden non-GI malignancy associations in MASLD. In fact, in a Korean cohort of 25947 subjects followed for a median of 7.5 years, MASLD was independently associated with breast cancer in females after adjusting for demographic and metabolic factors (HR 1.92; 95%CI: 1.15-3.20)[42]. The findings were corroborated at scale; a meta-analysis of 10 cohorts amassing 182202 individuals reported that MASLD conferred an approximately 1.2 to 1.5-fold increased risk of breast cancer, regardless of age, sex, smoking, diabetes, and obesity[12]. More recently, a larger meta-analysis of 18 cohort studies (about 16.7 million participants) confirmed a statistically significant association between MASLD and breast cancer (HR 1.17; 95%CI: 1.08-1.26)[37]. Zhang and Nguyen[26] in 2025 further characterized MASLD as a sexually dimorphic disease, stating that sex hormones, particularly the levels and ratios of circulating estrogens and androgens, play a huge central role in both the development of MASLD and its association with hormone-sensitive cancers. The hormonal and adipokine mechanisms underlying this association, with their net effect being a sex-specific pro-tumorigenic environment in breast tissue, are detailed in the subsection on adipose tissue and hormonal dysregulation[26]. The risk is dimorphic by menopausal status, with an elevated risk directed towards postmenopausal women with MASLD. This reflects a greater contribution of peripheral aromatization to circulating estrogen levels after ovarian senescence[26]. Data seen in the United Kingdom Biobank supports this claim, showing MASLD was positively associated with postmenopausal breast cancer[43].

Gynecologic cancers (endometrial and ovarian)

Amongst all gynecologic malignancies, it has been found that endometrial cancer demonstrates the most consistent and strongest association with MASLD. This correlation is grounded by a robust hormonal and metabolic rationale. Estrogen excess and unopposed endometrial stimulation, hallmarks of the metabolic setting in MASLD, are linked to endometrial cancer spanning multiple meta-analyses. The Mantovani *et al*[12] meta-analysis (2022) reported that MASLD was associated with approximately 1.2 to 1.5-fold increased risk of gynecological cancers, independent of potential confounders. Interestingly, a JAMA review by Tilg *et al*[44] cited pooled data that showed MASLD was associated with a higher risk of gynecological cancers (HR 1.62; 95%CI: 1.13-2.32). The Zhou *et al*[37] meta-analysis (2024) of 18 cohort studies confirmed an association with female reproductive cancers (HR 1.36; 95%CI: 1.11-1.66). Thomas *et al*[10] cited that the common occurring extrahepatic cancers in MASLD included uterine malignancy, and additionally the incidence rates were not higher in patients with advanced fibrosis or cirrhosis. This suggests that what drives the gynecological risk is the metabolic environment, rather than the liver disease severity. A large nationwide Korean cohort study of roughly 2 million women by Jeong *et al*[45] provided granular data which was stratified by menopausal status. In premenopausal women, MASLD was associated with increased risks of endometrial cancer (HR 1.63; 95%CI: 1.50-1.79) and ovarian cancer (HR 1.22; 95%CI: 1.12-1.33). Similarly, for postmenopausal women, MASLD conferred elevated risks for endometrial (HR 1.42; 95%CI: 1.32-1.54) and ovarian cancer (HR 1.14; 95%CI: 1.08-1.20).

Ovarian cancer has been less extensively studied in its association with MASLD. However, it has garnered attention after data from Park *et al*[46], who conducted a Korean cohort study of over 2 million women aged 20-39 years followed for a median of 11.5 years. Over a course of 27 million person-years of follow-up, 6319 women were diagnosed with young-onset ovarian cancer. MASLD was independently associated with an increased risk of young-onset ovarian cancer

Table 2 Non-gastrointestinal extrahepatic malignancies in metabolic dysfunction-associated steatotic liver disease: Cancer type, key studies, risk estimates, proposed mechanisms, and trends

Cancer type	Ref.	Risk estimate (HR or OR)	Proposed mechanisms	Trend/notes
Breast cancer	Lee *et al*[51], 2026 (nationwide Korean cohort); Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis); Thomas *et al*[9], 2024 (*Lancet GE & H*); Zhang and Nguyen[26], 2025 (review)	HR 1.17 (Zhou *et al*[37] meta-analysis); HR 1.20 in postmenopausal women with BMI 25-30 (Lee *et al*[51], 2026); OR approximately 1.2-1.5 (Mantovani *et al*[12], 2022)	Peripheral estrogen excess; leptin signaling; IR-IGF-1 axis; adipokine dysregulation	Rising; highest-burden non-GI cancer association; postmenopausal women at greatest risk
Endometrial cancer	Allen *et al*[35], 2019 (community cohort, *J Hepatol*); Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Thomas *et al*[10], 2022 (meta-analysis, *Eur J Cancer*); Thomas *et al*[9], 2024 (*Lancet GE & H*); Zhang and Nguyen[26], 2025 (review)	Approximately 2-fold uterine cancer risk in NAFLD (Allen *et al*[35], 2019); pooled OR approximately 1.5-2.1 across studies	Unopposed estrogen stimulation; hyperinsulinemia; adipokine excess	Rising; strong mechanistic plausibility; consistent across populations
Ovarian cancer	Park *et al*[46], 2025 (nationwide Korean cohort, *n* = 2.3M women, ages 20-39); Zhou *et al*[37], 2024 (meta-analysis); Zhang and Nguyen[26], 2025 (review, Metabolism)	HR 1.30 young-onset ovarian cancer (Park *et al*[46], 2025); HR 1.36 female genital organ cancers (Zhou *et al*[37] meta-analysis)	Hormonal dysregulation; PCOS overlap; adipokine signaling	Emerging; young-onset data of concern; limited overall evidence base
Thyroid cancer	Moon *et al*[47], 2025 (Korean cohort, thyroid); Zhou *et al*[37], 2024 (meta-analysis); Chen *et al*[49], 2025 (meta-analysis); Kwon *et al*[48], 2024 (cohort); Liu *et al*[43], 2022 (metabolism)	HR 1.36 (Moon *et al*[47], 2025); HR 1.46 (Zhou *et al*[37] meta-analysis); HR 1.69 in women (Liu *et al*[43], 2022 United Kingdom Biobank)	Insulin resistance; TSH dysregulation; thyroid nodularity in MASLD	Emerging; predominantly Asian cohort data; Western validation needed
Urinary system cancer (renal cell/bladder)	Bril and Elbert[68], 2025 (review); Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis); Thomas *et al*[10], 2022; Thomas *et al*[9], 2024	HR 1.45 (Zhou *et al*[37] meta-analysis); OR approximately 1.2-1.5 (Mantovani *et al*[12], 2022); kidney/bladder cancers documented in pooled NAFLD incidence data	Adiposity-driven ROS; chronic renal inflammation; altered adipokine signaling	Underexplored; mechanistically plausible; dedicated studies lacking
Lung cancer	Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis); Pan *et al*[69], 2025 (review); Thomas *et al*[9], 2024 (*Lancet GE & H*)	OR approximately 1.2-1.5 (Mantovani *et al*[12], 2022); null association in Zhou *et al*[37], 2024 meta-analysis	Systemic inflammation; adipokine-mediated bronchial epithelial effects; shared metabolic exposures	Mixed; signal present in some cohorts but null in pooled analyses; smoking confounding remains a key concern
Prostate cancer	Mantovani *et al*[12], 2022 (meta-analysis, *Gut*); Zhou *et al*[37], 2024 (meta-analysis); Thomas *et al*[10], 2022 (meta-analysis, *Eur J Cancer*); Thomas *et al*[9], 2024 (*Lancet GE & H*)	Conflicting; null in pooled meta-analyses (Mantovani *et al*[12], 2022; Zhou *et al*[37], 2024); inverse association reported in some Korean cohorts	Testosterone deficiency may reduce androgen-driven risk; complex hormonal interplay in MASLD/obesity	Uncertain; inverse/null in most studies; mechanism warrants further investigation

HR: Hazard ratio; OR: Odds ratio; IR: Insulin resistance; IGF-1: Insulin-like growth factor-1; BMI: Body mass index; TSH: Thyroid-stimulating hormone; PCOS: Polycystic ovarian syndrome; ROS: Reactive oxygen species; GI: Gastrointestinal; NAFLD: Non-alcoholic fatty liver disease; MASLD: Metabolic dysfunction-associated steatotic liver disease.

(HR 1.30; 95%CI: 1.16-1.45), and this risk increased with MASLD severity (moderate MASLD: HR 1.26; severe MASLD: HR 1.45, P for trend 0.01)[46]. Mechanistically, gynecological cancers share considerable overlap with breast cancer in the MASLD context, including estrogen excess, polycystic ovary syndrome-related anovulatory cycles, and dysregulated adipokine signaling[39].

Thyroid cancer

An emerging association between MASLD and thyroid cancer has been identified, with the most significant data shown from Asian cohort studies. In a cohort from Moon *et al*[47], analysis of roughly 200000 Korean adults aged ≥ 40 years found that MASLD was independently associated with a higher risk of thyroid cancer (HR 1.36; 95%CI: 1.17-1.58) over an average follow-up of 9.6 years, with metabolic and associated liver disease conferring a similar risk (HR 1.40; 95%CI: 1.04-1.88). In younger populations, Kwon *et al*[48] studied 1135967 participants aged 20-39 years and demonstrated a dose-response relationship. Compared with those with fatty liver index (FLI) of 30, individuals with an FLI between 30-60 had a significantly higher risk of thyroid cancer (men: HR 1.71; women: HR 1.81). Participants with higher cumulative FLI points and progression of MASLD over time exhibited incrementally higher thyroid cancer risk[48]. A meta-analysis by Chen *et al*[49], pooling eight cohort studies with roughly 19 million participants in total, confirmed that MASLD is linked to an increased risk of thyroid cancer (HR 1.46; 95%CI: 1.14-1.86), with the association particularly strong in the Chinese population (HR 2.24; 95%CI: 1.32-3.81) and among overweight individuals. The Zhou *et al*[37] meta-analysis (2024) reported a similar significant association (HR 1.46; 95%CI: 1.02-2.09). Wang *et al*[50] found that in a large Chinese male cohort, MASLD was associated with thyroid cancer (HR 2.79; 95%CI: 1.25-6.21), and this association increased with higher alanine aminotransferase levels. The proposed mechanisms center on insulin resistance and thyroid-stimulating hormone

dysregulation, with thyroid nodularity potentially representing a shared endocrinopathy of the metabolic syndrome phenotype[25]. However, although the epidemiological signal is strengthening, further prospective studies are needed to clarify the degree and independence of this association across diverse populations.

Urinary system cancers (renal cell and bladder)

Urinary system cancers remain a relatively underexplored domain in the MASLD-malignancy literature. However, there is growing mechanistic plausibility and epidemiological support for associations with MASLD. The Zhou *et al*[37] meta-analysis (2024) reported a statistically significant association between MASLD and urinary malignancies (HR 1.45; 95%CI: 1.25-1.69), while a meta-analysis by Mantovani *et al*[12] found 1.2-1.5-fold increased risk. In a meta-analysis by Thomas *et al*[9], there was a significant effect of body mass index (BMI) on the association between MASLD and urinary system malignancies. This study suggested there may be an effect modifier of adiposity. Bu *et al*[18] from the UK Biobank confirmed a positive association between MASLD and kidney and bladder cancers over a median follow-up period of 13 years, and it was also noted that inflammation partially mediated the observed association by 11.2% to 31.0%. Liu *et al* [43] found that MASLD was significantly associated with kidney cancer (HR 1.77; 95%CI: 1.49-2.11) and bladder cancer (HR 1.26; 95%CI: 1.11-1.43), with kidney cancer remaining significant once adjusting for BMI, waist circumference, and metabolic syndrome. Adiposity-driven ROS generation, chronic renal inflammation, and altered adipokine signaling have all been proposed as candidate pathways linking MASLD to renal cell and bladder carcinogenesis[25,51]. Currently, the epidemiological evidence supports an association, but findings are heterogeneous across study populations, and dedicated mechanistic investigations are limited.

Lung cancer

Malignancy of the lung has been associated with MASLD in emerging biological and epidemiological data. The Mantovani *et al*[12] meta-analysis (2022) found that MASLD was associated with an increased risk of lung cancer, independent of confounders which included smoking. Thomas *et al*[9] reported that an umbrella meta-analysis also found a significantly increased risk of lung cancer in patients with MASLD. However, the more recent Zhou *et al*[37] meta-analysis of 18 cohort studies (2024) did not find a statistically significant association between lung cancer and MASLD (HR 1.01; 95%CI: 0.92-1.10), highlighting the heterogeneity in the current evidence base. Wang *et al*[50] found that MASLD was associated with lung cancer in a large Chinese male cohort (HR 1.23; 95%CI: 1.02-1.49), but this association was only seen in participants who smoked (HR 1.38; 95%CI: 1.03-1.84), suggesting that environmental exposures may confound the relationship. Bu *et al*[18], using United Kingdom Biobank data, demonstrated that inflammation partially mediates the MASLD-cancer association, providing a plausible biological link for lung cancer through systemic inflammation, adipokine-mediated alterations in bronchial epithelial biology, and environmental and metabolic exposures that affect pulmonary carcinogenesis. Further investigation to clarify confounding by smoking and other risk factors is needed.

Prostate cancer

The relationship between MASLD and prostate cancer has shown conflicting evidence. The Zhou *et al*[37] meta-analysis (2024) found no statistically significant association between MASLD and prostate cancer (HR 1.06; 95%CI: 0.94-1.19). Similarly, the Mantovani *et al*[12] meta-analysis did not demonstrate an independent association. Thomas *et al*[9] described that a meta-analysis with meta-regression showed a significant effect of BMI and pre-existing diabetes on the association between MASLD and prostate cancer, suggesting the relationship may be confounded or modified by these factors. Thomas *et al*[10] reported that prostate cancer was among the most frequently occurring extrahepatic cancers in MASLD by absolute incidence. However, the relative risk increase was modest and inconsistent across studies. Interestingly, a meta-analysis by Liu *et al*[11] reported a significant association between MASLD and prostate cancer, though the results have not been consistently replicated in larger, more recent meta-analyses. The mechanistic distinctions underlying these conflicting findings may correlate to androgen pathway complexities in the setting of MASLD. Testosterone deficiency, which is common in MASLD as well as obesity, may paradoxically attenuate androgen driven prostate carcinogenesis, thereby offsetting the pro-tumorigenic effects of insulin resistance and chronic inflammation[12]. This discordance underscores the need for a careful, critical appraisal and sex-specific mechanistic analysis when further evaluating cancer risk in the MASLD population.

TRENDS, DISEASE BURDEN, AND SPECIAL POPULATIONS

Overall trends in extrahepatic cancer risk in MASLD

The cumulative evidence linking MASLD to extrahepatic cancer risk has evolved rapidly over the past five years, with multiple large meta-analyses now demonstrating a consistent positive signal across cancer types and study populations. The Zhou *et al*[37] meta-analysis of 18 cohort studies (approximately 16.7 million participants) found significantly elevated MASLD-associated risk for gastric, colorectal, pancreatic, biliary tract, thyroid, urinary system, breast, and female genital organ cancers, with hazard ratios ranging from 1.17 to 1.47. The Chan *et al*[52] meta-analysis of 129 studies reported a pooled hazard ratio of 1.54 (95%CI: 1.35-1.76) for all cancers among MASLD patients compared with non-MASLD controls. Importantly, the Celsa *et al*[53] meta-analysis of 24 cohort studies (approximately 11.6 million individuals) found that extrahepatic cancer incidence had the lowest between-study heterogeneity (I^2 = 0%) of all outcomes examined, indicating that this association is remarkably consistent across geographic, methodological, and

populational variation. The Mantovani *et al*[12] meta-analysis in *Gut* reinforces this consistency, with a 1.5- to 2-fold increased GI cancer risk in NAFLD that is "independent of age, sex, smoking, obesity, diabetes, or other potential confounders".

Extrahepatic cancer in MASLD is also rising in absolute terms over time. The nationwide Korean cohort study by Park *et al*[54] followed 9298497 individuals (of whom 2500080 were diagnosed with MAFLD) over a median 10.3 years and identified 447880 incident extrahepatic malignancies (6.0% cumulative incidence), with both the diabetic MAFLD [adjusted subdistribution hazard ratio (aSHR) 1.13] and lean MAFLD (aSHR 1.12) subtypes carrying elevated risk. A prospective cohort study by Peng *et al*[55] similarly found rising cancer incidence in MASLD populations over follow-up, and an earlier systematic review by Liu *et al*[11] documented the same trend in pooled NAFLD analyses. Collectively, this evidence positions extrahepatic cancer as a first-tier complication of MASLD rather than a secondary or speculative association.

Young-onset and early-age MASLD and cancer risk

Earlier-onset MASLD carries disproportionately elevated cancer risk, a pattern consistently observed across recent large nationwide cohorts. In the matched cohort study by Liu *et al*[56], NAFLD onset before age 45 was associated with an average hazard ratio of 1.52 (95%CI: 1.09-2.12) for incident cancer, with risk declining as onset age increased. In patients under 45, 17.83% of cancer risk was attributable to NAFLD on population-attributable fraction analysis. The nationwide Korean cohort by Moon *et al*[57] (2877245 young adults aged 20-39) confirmed a significantly elevated all-cancer risk in young-adult MASLD (HR 1.19, 95%CI: 1.16-1.22), with cancer risk increasing in a graded, dose-dependent fashion as the number of accompanying cardiometabolic risk factors rose.

A finding of high clinical relevance comes from the Chung *et al*[58] cohort of 3536172 young adults followed over a median of 10.6 years. Persistent MASLD carried the highest cancer risk (HR 1.15, 95%CI: 1.13-1.18), while resolved MASLD showed no significant difference in cancer risk *vs* controls (HR 1.02, 95%CI: 0.98-1.05). This dynamic relationship provides direct evidence that MASLD is a modifiable cancer risk factor, since regression of MASLD appears to return cancer risk towards baseline. The finding supports an intervention paradigm centered on reversing hepatic steatosis early in the disease course. Given rising global MASLD prevalence among children and young adults, these findings have substantial public health implications for both screening and early therapeutic intervention.

Fibrosis severity and cancer risk gradient

The relationship between liver fibrosis stage and extrahepatic cancer risk in MASLD is asymmetric and clinically important. As developed in detail in the subsection on fibrosis severity as a cancer risk modifier, advanced non-cirrhotic fibrosis (F3) confers disproportionate extrahepatic cancer and vascular risk while cirrhosis (F4) shifts the risk profile toward liver-related mortality, fibrosis stage is the primary determinant of all-cause mortality, and overall cancer incidence rises across fibrosis stages but is driven chiefly by HCC with only a modest extrahepatic contribution[27-30].

These findings provide direct clinical relevance. Advanced non-cirrhotic fibrosis (F3) should be treated as a high-risk clinical state for extrahepatic cancer, warranting more intensive surveillance discussions even when hepatic outcomes appear stable. Equally important is the recognition that extrahepatic cancer risk in MASLD does not consistently amplify the transition from F3 to F4 and may be partially uncoupled from fibrosis at lower stages. Therefore, liver fibrosis stage alone cannot be used as the sole screening trigger for extrahepatic cancer surveillance in MASLD patients. A comprehensive risk stratification must incorporate metabolic burden, diabetes status, sex, age at onset, and disease duration.

Sex differences and dimorphic cancer risk

MASLD is a sexually dimorphic disease, and its extrahepatic cancer landscape mirrors this biology. Estrogens and androgens, along with the ratio between them, influence both MASLD pathogenesis and the spectrum of hormone-sensitive cancers that emerge as complications, particularly breast, endometrial, and ovarian cancer in women[26]. In the nationwide Korean cohort of 483279 women aged 40-60 by Lee *et al*[51], MASLD was not significantly associated with overall breast cancer risk; however, among postmenopausal women with BMI 25-30, MASLD was independently associated with elevated risk (adjusted HR 1.203, 95%CI: 1.029-1.407), identifying a clinically relevant subgroup at heightened risk. The nationwide cohort of 2376482 women aged 20-39 (Park *et al*[46], 2025) demonstrated an adjusted HR of 1.30 (95%CI: 1.16-1.45) for young-onset ovarian cancer in NAFLD, with a clear dose-response across NAFLD severity (severe NAFLD aHR 1.45; P for trend < 0.01).

Mechanistically, these sex-skewed risks reflect the convergence of peripheral aromatization-driven estrogen excess, leptin-mediated proliferative signaling, hyperinsulinemia, and adipokine dysregulation in the metabolic-hepatic milieu described in the subsection on adipose tissue and hormonal dysregulation[26]. In men, the picture is less uniform: MASLD-associated testosterone deficiency may reduce androgen-driven prostate carcinogenesis (consistent with the null or inverse prostate cancer signal observed in pooled analyses; see the discussion of prostate cancer), while shared hyperinsulinemia and gut-liver axis perturbations sustain elevated risk for colorectal and urologic cancers. Clinical risk stratification therefore should be sex-specific: Women with MASLD, particularly postmenopausal women with elevated BMI and young women with severe disease, warrant heightened attention to breast and gynecologic surveillance, while men with MASLD remain primarily at risk for the GI cancers detailed in the GI extrahepatic malignancies section.

MASLD comorbidities and compounding cancer risk

T2DM, obesity, or metabolic syndrome frequently co-occur with MASLD and contribute additively, and sometimes synergistically, to extrahepatic cancer risk. The central question is whether MASLD confers cancer risk independent of these comorbidities, or whether the observed associations are explained by confounding. The Allen *et al*[35] community-

based cohort followed over 21 years provides the clearest evidence. Patients with NAFLD had nearly a 2-fold increase in cancer incidence, predominantly consisting of liver, GI tract, and uterine. Critically, the cancer-risk association was stronger in NAFLD than in obesity alone, demonstrating that NAFLD confers independent oncologic risk beyond shared metabolic factors. The broader mechanistic framework, described by Renehan *et al*[15], shows that adiposity-driven oncogenic mechanisms, including altered sex hormone metabolism, insulin/IGF-1 bioavailability, and adipokine pathophysiology, are partially modifiable through intentional weight loss.

Among comorbidities, T2DM is the most consistent amplifier of MASLD-associated cancer risk. In the Park *et al*[41] nationwide BTC cohort, concomitant NAFLD and diabetes were associated with a 47% increase in BTC risk. The Golabi *et al*[59] analysis of NHANES III with follow-up through 2019 showed that NAFLD subjects with T2DM had the highest cumulative mortality among NAFLD phenotypes (41.3%). The 2026 ADA Standards of Care explicitly acknowledge this overlap, noting that CVD remains the leading cause of death in MASLD patients, "followed by certain extrahepatic cancers (primarily GI, breast, and gynecologic cancer)", framing the T2DM-MASLD connection as clinically relevant for cancer risk management[38]. The complementary 2025 ADA consensus report from Cusi *et al*[39] reinforces this framework and calls for fibrosis screening as a new standard of care in patients with prediabetes or T2DM. Integrated cardiometabolic-oncologic risk assessment, particularly in patients with both MASLD and T2DM, represents an emerging clinical priority[60].

DIAGNOSTIC APPROACHES AND SURVEILLANCE IMPLICATIONS

Identifying at-risk patients: Biomarkers and non-invasive tools

Effective cancer risk stratification in MASLD requires non-invasive tools that can identify high-risk individuals from within the large MASLD population. The FLI, a validated composite of triglycerides, BMI, waist circumference, and gamma-glutamyl transferase, has been shown in United Kingdom Biobank analyses to stratify not only the cross-sectional presence of NAFLD but also incident cardiometabolic disease, hepatic malignancy, "specific metabolism-related malignancies", and all-cause mortality[61]. When combined with either the NAFLD fibrosis score or FIB-4, FLI independently predicts mortality risk[61]. The clinical implication is that simple, widely available laboratory parameters can flag MASLD patients warranting enhanced cancer surveillance, even in primary care settings where transient elastography may not be accessible. Importantly, the Ratziu *et al*[62] validation study confirmed that the diagnostic accuracy of FIB-4 and vibration-controlled transient elastography is nearly identical when applied to NAFLD and MASLD defined populations, allowing historical NAFLD risk-stratification literature to be extrapolated without re-validation.

Beyond conventional biomarkers, omics-based technologies, including genomics, transcriptomics, metabolomics, and proteomics, are rapidly developing tools that "show great potential for discovering non-invasive markers" of MASLD-associated malignancies in both cirrhotic and non-cirrhotic patients[63]. Liquid biopsy approaches incorporating circulating tumor DNA, microRNAs, and EV-derived cargo (discussed in the subsection on hepatocyte-derived EVs and systemic crosstalk) may eventually allow individualized cancer surveillance in MASLD, although none of these approaches are yet clinically validated for routine use. In the interim, FIB-4 plus FLI represents the most pragmatic available framework for identifying MASLD patients at elevated cancer risk.

Current surveillance gaps and opportunities

Despite the developing evidence linking MASLD to extrahepatic malignancy, no consensus-endorsed cancer surveillance protocol specific to MASLD populations currently exists, representing an unmet clinical need. The Thomas *et al*[9] review explicitly highlights this gap, noting that "almost 30% of the world's adult population has NAFLD" and that deaths from extrahepatic cancers in this population already exceed those from HCC, yet "no established extrahepatic cancer surveillance guidance" exists for NAFLD/MASLD cohorts. The Kalligeros *et al*[64] synthesis describes understanding MASLD-cancer mechanisms as "crucial for developing targeted screening and prevention strategies", and the Kim and Seki[17] review underscores the timeliness of integrating hepatic microenvironmental and immune-response insights into clinical risk frameworks.

In the absence of MASLD-specific protocols, several practical measures are available within current clinical structures. Enhanced adherence to existing age-and-sex-appropriate cancer screening guidelines should be strongly emphasized in patients with MASLD. These include colonoscopy for CRC, mammography for breast cancer, and urologic surveillance for at-risk men. A further option is risk-stratified extension of standard screening intervals for MASLD patients with concentrated risk profiles, such as earlier colonoscopy in MASLD patients with diabetes, postmenopausal women with MASLD and BMI 25-30 for breast surveillance. Although, this requires guideline development and evidence support through more studies before widespread implementation. Finally, integrated cardiometabolic-oncologic risk assessment that links hepatology, endocrinology, and primary care offers a feasible structural approach to operationalize MASLD as a multisystem disease with oncologic implications[38,39]. Particular attention is warranted for lean MASLD, in which the colorectal cancer association may exceed that observed in obese individuals (see the discussion of colorectal cancer). With such patients unlikely to be flagged by body mass index-based triggers, colonoscopic surveillance decisions in this subgroup should rest on hepatic steatosis and metabolic criteria rather than adiposity alone.

Management of MASLD as cancer risk reduction

A pragmatic implication of the evidence reviewed throughout this manuscript is that effective MASLD management may

constitute a meaningful cancer-risk-reduction strategy, particularly given that MASLD is a modifiable cancer risk factor (as established in the subsection on young-onset and early-age MASLD and cancer risk)[58]. The strongest anchor for this principle is that resolution of MASLD returns cancer risk toward that of unaffected individuals, whereas persistent disease retains the highest risk[58]; each intervention below is therefore considered specifically for its capacity to reverse hepatic steatosis and the systemic metabolic milieu that drives extrahepatic carcinogenesis. The first-line intervention remains lifestyle modification: Weight loss of 3%-5% *via* dietary and exercise interventions improves hepatic steatosis with reversal of insulin resistance, while weight loss exceeding 10% achieves improvement in hepatic fibrosis[60]. Aerobic exercise of about 150 minutes per week of moderate-intensity [3-6 metabolic equivalent tasks (METs)] or 75-150 minutes per week of vigorous-intensity (> 6 METs) is recommended by the American Association for the Study of Liver Diseases guidance. In addition, 2-3 sessions of resistance training are also recommended. In patients with cirrhosis both aerobic and anaerobic exercise have shown to reduce portal pressure[65]. Patients with MASLD and cirrhosis should also focus on increased daily protein intake to improve frailty and reduce sarcopenia[66]. Some studies have shown that a Mediterranean dietary pattern is preferred in MASLD due to its low glycemic effects and cardiometabolic benefits[60]. Bariatric surgery improves steatosis, hepatic inflammation, and fibrosis through weight loss and microbiome modulation, providing rationale for surgical management in selected patients with severe obesity and MASLD[60].

The pharmacotherapeutic landscape has expanded substantially. Glucagon-like peptide-1 (GLP-1) receptor agonists (*e.g.*, semaglutide) achieve 2.0-11.4 kg weight loss with HbA1c reductions of 0.66%-2.3%, and tirzepatide (a dual GLP-1/glucose-dependent insulinotropic polypeptide receptor agonist) achieves 15%-20.9% weight loss from baseline[60]. Semaglutide and resmetirom (a thyroid hormone receptor-β agonist) are now conditionally United States Food and Drug Administration approved for adults with metabolic dysfunction-associated steatohepatitis and moderate-to-advanced fibrosis[44]. Farnesoid X receptor agonists (*e.g.*, obeticholic acid) reduce hepatic inflammation, fibrosis, and improve insulin sensitivity[60]. Reviews by Targher *et al*[67] and Stefan *et al*[16] highlight that the most effective non-pharmacological intervention remains weight loss, while pharmacotherapy is increasingly central to MASLD care as the disease's metabolic, cardiovascular, and oncologic burden becomes better understood.

While direct evidence that MASLD pharmacotherapy reduces extrahepatic cancer incidence is still emerging, the Chung *et al*[58] finding that resolved MASLD normalizes cancer risk provides indirect but powerful support for the principle that MASLD reversal constitutes cancer prevention. Notably, GLP-1 receptor agonists have shown signals of reduced incident cancer in obesity and T2DM populations more broadly, and the 2026 Wu *et al*[33] Delphi consensus explicitly identifies GLP-1 receptor agonists, weight loss, and bariatric surgery as risk-reduction strategies for MASLD-associated CRC.

Limitations

Several limitations temper these conclusions. The evidence base is heterogeneous in disease definition, spanning historical non-alcoholic fatty liver disease, metabolic dysfunction-associated fatty liver disease, and current MASLD criteria, and most studies rely on non-invasive surrogates such as the FLI or ultrasonography rather than histology, introducing exposure misclassification. Residual confounding by obesity, type 2 diabetes mellitus, smoking, and shared metabolic factors cannot be fully excluded in observational designs, which preclude causal inference. Much of the strongest signal, particularly for biliary tract, thyroid, ovarian, and young-onset cancers, derives from Asian and predominantly Korean nationwide cohorts, limiting generalizability to Western and other populations. Several associations rest on single cohorts or show between-study heterogeneity, and site- and histology-specific estimates remain sparse. These constraints argue for cautious interpretation of effect magnitudes and for prospective, multiethnic validation before MASLD-specific surveillance can be formally recommended.

CONCLUSION

MASLD is no longer a disease confined to the liver. The evidence reviewed here demonstrates a consistent and clinically meaningful association between MASLD and an expanded spectrum of extra-hepatic malignancies. These span GI cancers, most robustly colorectal cancer, with growing data for pancreatic, esophagogastric, and BTCs, as well as non-GI cancers, including breast, gynecologic, thyroid, urinary system, and lung cancers. The oncologic risk landscape of MASLD is driven by a convergence of systemic metabolic pathways, including insulin resistance, chronic inflammation, lipotoxicity, gut dysbiosis, and hormonal dysregulation, many of which are addressable through therapeutic intervention.

Rising trends in MASLD prevalence globally portend a parallel increase in associated cancer burden, with particular concern for younger populations and those with advanced hepatic fibrosis. Sex-specific differences in malignancy risk require tailored consideration. Despite the growing body of evidence, dedicated MASLD-specific cancer surveillance guidelines remain absent, representing an important unmet clinical need. Future prospective studies should prioritize establishing causal relationships, defining fibrosis thresholds for cancer screening initiation, and evaluating sex-stratified risk prediction tools. A multidisciplinary approach integrating hepatology, oncology, endocrinology, and primary care would be a key consideration to translating this evidence into actionable clinical practice.

REFERENCES

1 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg

JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [*RCA*] [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039] [FullText]

2 **Rinella ME**, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, Arrese M, Bataller R, Beuers U, Boursier J, Bugianesi E, Byrne CD, Castro Narro GE, Chowdhury A, Cortez-Pinto H, Cryer DR, Cusi K, El-Kassas M, Klein S, Eskridge W, Fan J, Gawrieh S, Guy CD, Harrison SA, Kim SU, Koot BG, Korenjak M, Kowdley KV, Lacaille F, Loomba R, Mitchell-Thain R, Morgan TR, Powell EE, Roden M, Romero-Gómez M, Silva M, Singh SP, Sookoian SC, Spearman CW, Tiniakos D, Valenti L, Vos MB, Wong VW, Xanthakos S, Yilmaz Y, Younossi Z, Hobbs A, Villota-Rivas M, Newsome PN; NAFLD Nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* 2023; **78**: 1966-1986 [*RCA*] [PMID: 37363821 DOI: 10.1097/HEP.0000000000000520] [FullText] [Full Text(PDF)]

3 **Younossi ZM**, Kalligeros M, Henry L. Epidemiology of metabolic dysfunction-associated steatotic liver disease. *Clin Mol Hepatol* 2025; **31**: S32-S50 [*RCA*] [PMID: 39159948 DOI: 10.3350/cmh.2024.0431] [FullText] [Full Text(PDF)]

4 **Wong VW**, Ekstedt M, Wong GL, Hagström H. Changing epidemiology, global trends and implications for outcomes of NAFLD. *J Hepatol* 2023; **79**: 842-852 [*RCA*] [PMID: 37169151 DOI: 10.1016/j.jhep.2023.04.036] [FullText]

5 **Byrne CD**, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; **62**: S47-S64 [*RCA*] [PMID: 25920090 DOI: 10.1016/j.jhep.2014.12.012] [FullText]

6 **Adams LA**, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017; **66**: 1138-1153 [*RCA*] [PMID: 28314735 DOI: 10.1136/gutjnl-2017-313884] [FullText]

7 **Targher G**, Byrne CD, Tilg H. MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. *Gut* 2024; **73**: 691-702 [*RCA*] [PMID: 38228377 DOI: 10.1136/gutjnl-2023-330595] [FullText]

8 **Wijarnpreecha K**, Aby ES, Ahmed A, Kim D. Evaluation and management of extrahepatic manifestations of nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2021; **27**: 221-235 [*RCA*] [PMID: 33317243 DOI: 10.3350/cmh.2020.0239] [FullText] [Full Text(PDF)]

9 **Thomas JA**, Kendall BJ, El-Serag HB, Thrift AP, Macdonald GA. Hepatocellular and extrahepatic cancer risk in people with non-alcoholic fatty liver disease. *Lancet Gastroenterol Hepatol* 2024; **9**: 159-169 [*RCA*] [PMID: 38215780 DOI: 10.1016/S2468-1253(23)00275-3] [Full Text]

10 **Thomas JA**, Kendall BJ, Dalais C, Macdonald GA, Thrift AP. Hepatocellular and extrahepatic cancers in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Eur J Cancer* 2022; **173**: 250-262 [*RCA*] [PMID: 35944373 DOI: 10.1016/j.ejca.2022.06.051] [FullText]

11 **Liu SS**, Ma XF, Zhao J, Du SX, Zhang J, Dong MZ, Xin YN. Association between nonalcoholic fatty liver disease and extrahepatic cancers: a systematic review and meta-analysis. *Lipids Health Dis* 2020; **19**: 118 [*RCA*] [PMID: 32475354 DOI: 10.1186/s12944-020-01288-6] [Full Text] [Full Text(PDF)]

12 **Mantovani A**, Petracca G, Beatrice G, Csermely A, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and increased risk of incident extrahepatic cancers: a meta-analysis of observational cohort studies. *Gut* 2022; **71**: 778-788 [*RCA*] [PMID: 33685968 DOI: 10.1136/gutjnl-2021-324191] [FullText]

13 **Mantovani A**, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism* 2020; **111S**: 154170 [*RCA*] [PMID: 32006558 DOI: 10.1016/j.metabol.2020.154170] [FullText]

14 **Kim D**, Adejumo AC, Yoo ER, Iqbal U, Li AA, Pham EA, Cholankeril G, Glenn JS, Ahmed A. Trends in Mortality From Extrahepatic Complications in Patients With Chronic Liver Disease, From 2007 Through 2017. *Gastroenterology* 2019; **157**: 1055-1066.e11 [*RCA*] [PMID: 31251928 DOI: 10.1053/j.gastro.2019.06.026] [FullText]

15 **Renehan AG**, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. *Nat Rev Cancer* 2015; **15**: 484-498 [*RCA*] [PMID: 26205341 DOI: 10.1038/nrc3967] [FullText]

16 **Stefan N**, Yki-Järvinen H, Neuschwander-Tetri BA. Metabolic dysfunction-associated steatotic liver disease: heterogeneous pathomechanisms and effectiveness of metabolism-based treatment. *Lancet Diabetes Endocrinol* 2025; **13**: 134-148 [*RCA*] [PMID: 39681121 DOI: 10.1016/S2213-8587(24)00318-8] [FullText]

17 **Kim J**, Seki E. Unveiling the cancer risk nexus of the steatotic liver. *Trends Endocrinol Metab* 2024; **35**: 708-719 [*RCA*] [PMID: 38531699 DOI: 10.1016/j.tem.2024.02.017] [FullText] [Full Text(PDF)]

18 **Bu J**, Mi N, Liang Z, Xie P, Long C, Yang M, Yuan J, Xia B, Liu A, Xiang S, He Q. The mediating role of inflammation in cancer risk among individuals with metabolic dysfunction-associated steatotic liver disease: a cohort study. *Int J Surg* 2025 [*RCA*] [PMID: 41342547 DOI: 10.1097/JS9.0000000000004181] [FullText]

19 **Zhao Y**, Zhou Y, Wang D, Huang Z, Xiao X, Zheng Q, Li S, Long D, Feng L. Mitochondrial Dysfunction in Metabolic Dysfunction Fatty Liver Disease (MAFLD). *Int J Mol Sci* 2023; **24**: 17514 [*RCA*] [PMID: 38139341 DOI: 10.3390/ijms242417514] [FullText] [Full Text(PDF)]

20 **Puchalska P**, Crawford PA. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. *Cell Metab* 2017; **25**: 262-284 [*RCA*] [PMID: 28178565 DOI: 10.1016/j.cmet.2016.12.022] [FullText] [Full Text(PDF)]

21 **Louis P**, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014; **12**: 661-672 [*RCA*] [PMID: 25198138 DOI: 10.1038/nrmicro3344] [FullText]

22 **Leung C**, Rivera L, Furness JB, Angus PW. The role of the gut microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 412-425 [*RCA*] [PMID: 27273168 DOI: 10.1038/nrgastro.2016.85] [FullText]

23 **Tsutsumi T**, Nakano D, Hashida R, Sano T, Kawaguchi M, Amano K, Kawaguchi T. The Inter-Organ Crosstalk Reveals an Inevitable Link between MAFLD and Extrahepatic Diseases. *Nutrients* 2023; **15**: 1123 [*RCA*] [PMID: 36904122 DOI: 10.3390/nu15051123] [FullText]

24 **Li S**, Cheng F, Zhang Z, Xu R, Shi H, Yan Y. The role of hepatocyte-derived extracellular vesicles in liver and extrahepatic diseases. *Biomed Pharmacother* 2024; **180**: 117502 [*RCA*] [PMID: 39357327 DOI: 10.1016/j.biopha.2024.117502] [FullText]

25 **Singeap AM**, Stanciu C, Huiban L, Muzica CM, Cuciureanu T, Girleanu I, Chiriac S, Zenovia S, Nastasa R, Sfarti C, Cojocariu C, Trifan A. Association between Nonalcoholic Fatty Liver Disease and Endocrinopathies: Clinical Implications. *Can J Gastroenterol Hepatol* 2021; **2021**: 6678142 [*RCA*] [PMID: 33505943 DOI: 10.1155/2021/6678142] [FullText] [Full Text(PDF)]

26 **Zhang X**, Nguyen MH. Metabolic dysfunction-associated steatotic liver disease: A sexually dimorphic disease and breast and gynecological cancer. *Metabolism* 2025; **167**: 156190 [*RCA*] [PMID: 40081614 DOI: 10.1016/j.metabol.2025.156190] [FullText]

27 **Vilar-Gomez E**, Calzadilla-Bertot L, Wai-Sun Wong V, Castellanos M, Aller-de la Fuente R, Metwally M, Eslam M, Gonzalez-Fabian L, Alvarez-Quiñones Sanz M, Conde-Martin AF, De Boer B, McLeod D, Hung Chan AW, Chalasani N, George J, Adams LA, Romero-Gomez

M. Fibrosis Severity as a Determinant of Cause-Specific Mortality in Patients With Advanced Nonalcoholic Fatty Liver Disease: A Multi-National Cohort Study. *Gastroenterology* 2018; **155**: 443-457.e17 [*RCA*] [PMID: 29733831 DOI: 10.1053/j.gastro.2018.04.034] [FullText]

28 **Sanyal AJ**, Van Natta ML, Clark J, Neuschwander-Tetri BA, Diehl A, Dasarathy S, Loomba R, Chalasani N, Kowdley K, Hameed B, Wilson LA, Yates KP, Belt P, Lazo M, Kleiner DE, Behling C, Tonascia J; NASH Clinical Research Network (CRN). Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease. *N Engl J Med* 2021; **385**: 1559-1569 [*RCA*] [PMID: 34670043 DOI: 10.1056/NEJMoa2029349] [FullText]

29 **Simon TG**, Roelstraete B, Sharma R, Khalili H, Hagström H, Ludvigsson JF. Cancer Risk in Patients With Biopsy-Confirmed Nonalcoholic Fatty Liver Disease: A Population-Based Cohort Study. *Hepatology* 2021; **74**: 2410-2423 [*RCA*] [PMID: 33811766 DOI: 10.1002/hep.31845] [FullText]

30 **Björkström K**, Widman L, Hagström H. Risk of hepatic and extrahepatic cancer in NAFLD: A population-based cohort study. *Liver Int* 2022; **42**: 820-828 [*RCA*] [PMID: 35152526 DOI: 10.1111/liv.15195] [FullText] [Full Text(PDF)]

31 **Zhao X**, Xue F, Wang S, Ding H, Li D, Rao H, Ji F, Jia J, Ma X, Hu P, Dou X, Xu K, Gao S, Yang M, Wei L. Hypertension Associated with the Risk of Extrahepatic Cancers in the Metabolic Dysfunction-associated Steatotic Liver Disease Population: A Multicenter Cross-sectional Study in China. *J Clin Transl Hepatol* 2026; **14**: 280-290 [*RCA*] [PMID: 41970195 DOI: 10.14218/JCTH.2025.00423] [FullText] [Full Text (PDF)]

32 **Celsa C**, Pennisi G, Tulone A, Ciancimino G, Vaccaro M, Pecorella F, Di Maria G, Enea M, Targher G, Valenti L, Romeo S, Cammà C, Petta S. PNPLA3 polymorphisms and risk of hepatic and extrahepatic outcomes in MASLD: a meta-analysis of observational studies. *JHEP Rep* 2026; 101918 [*RCA*] [PMID: 42250763 DOI: 10.1016/j.jhepr.2026.101918] [FullText]

33 **Wu CT**, Targher G, Byrne CD, Younossi ZM, Armstrong MJ, Luu HN, Lesmana CRA, Rustgi VK, Thomas JA, Okabayashi K, Lee JWJ, El-Serag HB, Kim W, Zhang HJ, Lonardo A, Souza M, George ES, Yang W, Yilmaz Y, Papatheodoridis G, Sookoian S, Romero-Gómez M, Sebastiani G, Schattenberg JM, El-Kassas M, Chan WK, Ocama P, Lupşor-Platon M, Perera N, Alswat K, Méndez-Sánchez N, Wong VW, Chai J, Tilg H, Sung JJ, Zheng MH. Colorectal cancer in metabolic dysfunction-associated steatotic liver disease: an international Delphi consensus statement. *Gut* 2026; gutjnl-2025 [*RCA*] [PMID: 41770836 DOI: 10.1136/gutjnl-2025-337374] [FullText]

34 **Azimi A**, Jolfayi AG, Rezayifar V, Ateen F, Fard HH, Mohammed SKM, Hosseinizadeh H, Faizabadi S, Keshavarzian A, Mansournia MA, Vosough M, Rahmanian M. The association between metabolic-associated fatty liver diseases and risk of colorectal polyps, neoplasia, and cancer: A systematic review and meta-analysis of over 56 million individuals. *Clin Res Hepatol Gastroenterol* 2025; **49**: 102652 [*RCA*] [PMID: 40706955 DOI: 10.1016/j.clinre.2025.102652] [FullText]

35 **Allen AM**, Hicks SB, Mara KC, Larson JJ, Therneau TM. The risk of incident extrahepatic cancers is higher in non-alcoholic fatty liver disease than obesity - A longitudinal cohort study. *J Hepatol* 2019; **71**: 1229-1236 [*RCA*] [PMID: 31470068 DOI: 10.1016/j.jhep.2019.08.018] [Full Text] [Full Text(PDF)]

36 **Mantovani A**, Lonardo A, Stefan N, Targher G. Metabolic dysfunction-associated steatotic liver disease and extrahepatic gastrointestinal cancers. *Metabolism* 2024; **160**: 156014 [*RCA*] [PMID: 39182602 DOI: 10.1016/j.metabol.2024.156014] [FullText]

37 **Zhou BG**, Jiang X, She Q, Ding YB. Association of MASLD with the risk of extrahepatic cancers: A systematic review and meta-analysis of 18 cohort studies. *Eur J Clin Invest* 2024; **54**: e14276 [*RCA*] [PMID: 38943276 DOI: 10.1111/eci.14276] [FullText]

38 **American Diabetes Association Professional Practice Committee for Diabetes***. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Care in Diabetes-2026. *Diabetes Care* 2026; **49**: S61-S88 [*RCA*] [PMID: 41358897 DOI: 10.2337/dc26-S004] [FullText] [Full Text(PDF)]

39 **Cusi K**, Abdelmalek MF, Apovian CM, Balapattabi K, Bannuru RR, Barb D, Bardsley JK, Beverly EA, Corbin KD, ElSayed NA, Isaacs S, Kanwal F, Pekas EJ, Richardson CR, Roden M, Sanyal AJ, Shubrook JH, Younossi ZM, Bajaj M. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) in People With Diabetes: The Need for Screening and Early Intervention. A Consensus Report of the American Diabetes Association. *Diabetes Care* 2025; **48**: 1057-1082 [*RCA*] [PMID: 40434108 DOI: 10.2337/dci24-0094] [FullText]

40 **Zou Q**, Tan HY, Li JC, Li YD, Yang K. Metabolic-associated fatty liver disease and risk of esophagogastric cancer: a systematic review and meta-analysis. *Jpn J Clin Oncol* 2023; **53**: 680-690 [*RCA*] [PMID: 37132637 DOI: 10.1093/jjco/hyad038] [FullText]

41 **Park JH**, Hong JY, Kwon M, Lee J, Han K, Han IW, Kang W, Park JK. Association between non-alcoholic fatty liver disease and the risk of biliary tract cancers: A South Korean nationwide cohort study. *Eur J Cancer* 2021; **150**: 73-82 [*RCA*] [PMID: 33892409 DOI: 10.1016/j.ejca.2021.03.024] [FullText]

42 **Kim GA**, Lee HC, Choe J, Kim MJ, Lee MJ, Chang HS, Bae IY, Kim HK, An J, Shim JH, Kim KM, Lim YS. Association between non-alcoholic fatty liver disease and cancer incidence rate. *J Hepatol* 2017; S0168-8278(17)32294 [*RCA*] [PMID: 29150142 DOI: 10.1016/j.jhep.2017.09.012] [FullText]

43 **Liu Z**, Lin C, Suo C, Zhao R, Jin L, Zhang T, Chen X. Metabolic dysfunction-associated fatty liver disease and the risk of 24 specific cancers. *Metabolism* 2022; **127**: 154955 [*RCA*] [PMID: 34915036 DOI: 10.1016/j.metabol.2021.154955] [FullText]

44 **Tilg H**, Petta S, Stefan N, Targher G. Metabolic Dysfunction-Associated Steatotic Liver Disease in Adults: A Review. *JAMA* 2026; **335**: 163-174 [*RCA*] [PMID: 41212550 DOI: 10.1001/jama.2025.19615] [FullText]

45 **Jeong MJ**, Lee YS, Choi YJ, Han KD. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Risk of Gynecologic Cancer: A Nationwide Cohort Study. *Cancers (Basel)* 2026; **18**: 894 [*RCA*] [PMID: 41899499 DOI: 10.3390/cancers18060894] [FullText] [Full Text (PDF)]

46 **Park JH**, Hong JY, Han K, Kang W, Shen JJ. Increased risk of young-onset ovarian cancer in women with non-alcoholic fatty liver disease: A nationwide cohort study of 2.3 million women aged 20-39 years. *Sci Rep* 2025; **15**: 14463 [*RCA*] [PMID: 40281103 DOI: 10.1038/s41598-025-99093-7] [FullText] [Full Text(PDF)]

47 **Moon SY**, Son M, Cho JH, Kim HI, Han JM, Bae JC, Suh S. Association Between Metabolic Dysfunction-Associated Steatotic Liver Disease and Thyroid Cancer. *Thyroid* 2025; **35**: 79-86 [*RCA*] [PMID: 39692617 DOI: 10.1089/thy.2024.0522] [FullText]

48 **Kwon H**, Han KD, Moon SJ, Park SE, Rhee EJ, Lee WY. Nonalcoholic Fatty Liver Disease and the Risk of Thyroid Cancer Among Young Adults in South Korea. *J Clin Endocrinol Metab* 2024; **109**: e1095-e1104 [*RCA*] [PMID: 37921093 DOI: 10.1210/clinem/dgad575] [FullText]

49 **Chen J**, Gao Y, Fu T, Gu Y, Du W. Association between metabolic dysfunction-associated steatotic liver disease and risk of thyroid cancer: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2025; **37**: 119-128 [*RCA*] [PMID: 39589817 DOI: 10.1097/MEG.0000000000002881] [FullText]

50 **Wang Z**, Zhao X, Chen S, Wang Y, Cao L, Liao W, Sun Y, Wang X, Zheng Y, Wu S, Wang L. Associations Between Nonalcoholic Fatty Liver Disease and Cancers in a Large Cohort in China. *Clin Gastroenterol Hepatol* 2021; **19**: 788-796.e4 [*RCA*] [PMID: 32407969 DOI:

10.1016/j.cgh.2020.05.009] [FullText]

51 **Lee JA**, Lee HS, Jeon S, Kim D, Lee YJ, Bae SY, Park WC, Lee JM, Yoon CI. Association between metabolic dysfunction-associated steatotic liver disease and breast cancer risk in Korean women: a nationwide population-based cohort study. *Breast Cancer Res* 2026; **28**: 37 [*RCA*] [PMID: 41501873 DOI: 10.1186/s13058-025-02211-6] [FullText] [Full Text(PDF)]

52 **Chan KE**, Ong EYH, Chung CH, Ong CEY, Koh B, Tan DJH, Lim WH, Yong JN, Xiao J, Wong ZY, Syn N, Kaewdech A, Teng M, Wang JW, Chew N, Young DY, Know A, Siddiqui MS, Huang DQ, Tamaki N, Wong VW, Mantzoros CS, Sanyal A, Noureddin M, Ng CH, Muthiah M. Longitudinal Outcomes Associated With Metabolic Dysfunction-Associated Steatotic Liver Disease: A Meta-analysis of 129 Studies. *Clin Gastroenterol Hepatol* 2024; **22**: 488-498.e14 [*RCA*] [PMID: 37775028 DOI: 10.1016/j.cgh.2023.09.018] [FullText]

53 **Celsa C**, Pennisi G, Tulone A, Ciancimino G, Vaccaro M, Pecorella F, Di Maria G, Enea M, Midiri F, Mantovani A, Targher G, Krag A, Rinella ME, Cammà C, Petta S. Risk of hepatic and extrahepatic outcomes associated with metabolic dysfunction-associated steatotic liver disease and metabolic dysfunction and alcohol-associated steatotic liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2025; **10**: 998-1012 [*RCA*] [PMID: 40953570 DOI: 10.1016/S2468-1253(25)00188-8] [FullText]

54 **Park MK**, Hur MH, Moon HS, Shin H, Chung SW, Won S, Lee YB, Cho EJ, Lee JH, Yu SJ, Yoon JH, Kim YJ. Extrahepatic malignancies in metabolic dysfunction-associated fatty liver disease: A nationwide cohort study. *Liver Int* 2024; **44**: 799-810 [*RCA*] [PMID: 38230848 DOI: 10.1111/liv.15832] [FullText]

55 **Peng Y**, Wang P, Liu F, Wang X, Si C, Gong J, Zhou H, Gu J, Qin A, Song W, Song F. Metabolic dysfunction-associated steatotic liver disease and cancer risk: A cohort study. *Diabetes Obes Metab* 2025; **27**: 1940-1949 [*RCA*] [PMID: 39781580 DOI: 10.1111/dom.16186] [Full Text]

56 **Liu C**, Liu T, Zhang Q, Jia P, Song M, Zhang Q, Ruan G, Ge Y, Lin S, Wang Z, Xie H, Shi J, Han R, Chen Y, Zheng X, Shen L, Deng L, Wu S, Shi H. New-Onset Age of Nonalcoholic Fatty Liver Disease and Cancer Risk. *JAMA Netw Open* 2023; **6**: e2335511 [*RCA*] [PMID: 37747732 DOI: 10.1001/jamanetworkopen.2023.35511] [FullText] [Full Text(PDF)]

57 **Moon JH**, Jeong S, Jang H, Lee DH, Joo SK, Koo BK, Xia Q, Sha M, Chang Y, Kim W. Young Adults With Metabolic Dysfunction-Associated Steatotic Liver Disease Have an Increased Risk of Early-Onset Cancer: A Nationwide Cohort Study Differentiating the Risk of 23 Site-Specific Cancers. *Clin Gastroenterol Hepatol* 2025; **23**: 2540-2549.e8 [*RCA*] [PMID: 40348107 DOI: 10.1016/j.cgh.2025.01.042] [Full Text]

58 **Chung GE**, Yu SJ, Park J, Kim YJ, Yoon JH, Han K, Cho EJ. Associations between changes in MASLD status and cancer development in young adults: A nationwide cohort study. *Eur J Cancer* 2026; **235**: 116228 [*RCA*] [PMID: 41534486 DOI: 10.1016/j.ejca.2026.116228] [Full Text]

59 **Golabi P**, Paik JM, Kumar A, Al Shabeeb R, Eberly KE, Cusi K, GunduRao N, Younossi ZM. Nonalcoholic fatty liver disease (NAFLD) and associated mortality in individuals with type 2 diabetes, pre-diabetes, metabolically unhealthy, and metabolically healthy individuals in the United States. *Metabolism* 2023; **146**: 155642 [*RCA*] [PMID: 37380016 DOI: 10.1016/j.metabol.2023.155642] [FullText]

60 **Venkatesan K**, Haroon NN. Management of Metabolic-Associated Fatty Liver Disease. *Endocrinol Metab Clin North Am* 2023; **52**: 547-557 [*RCA*] [PMID: 37495344 DOI: 10.1016/j.ecl.2023.02.002] [FullText]

61 **Ho B**, Thompson A, Jorgensen AL, Pirmohamed M. Role of fatty liver index in risk-stratifying comorbid disease outcomes in non-alcoholic fatty liver disease. *JHEP Rep* 2023; **5**: 100896 [*RCA*] [PMID: 37928746 DOI: 10.1016/j.jhepr.2023.100896] [FullText] [Full Text(PDF)]

62 **Ratziu V**, Boursier J; AFEF Group for the Study of Liver Fibrosis. Confirmatory biomarker diagnostic studies are not needed when transitioning from NAFLD to MASLD. *J Hepatol* 2024; **80**: e51-e52 [*RCA*] [PMID: 37543307 DOI: 10.1016/j.jhep.2023.07.017] [FullText]

63 **Lan T**, Tacke F. Diagnostics and omics technologies for the detection and prediction of metabolic dysfunction-associated steatotic liver disease-related malignancies. *Metabolism* 2024; **161**: 156015 [*RCA*] [PMID: 39216799 DOI: 10.1016/j.metabol.2024.156015] [FullText]

64 **Kalligeros M**, Henry L, Younossi ZM. Metabolic dysfunction-associated steatotic liver disease and its link to cancer. *Metabolism* 2024; **160**: 156004 [*RCA*] [PMID: 39182603 DOI: 10.1016/j.metabol.2024.156004] [FullText]

65 **Huang X**, Yu R, Guo M, Tan X, Xia Y, Zou H, Liu X, Qin C. Exercise-Based Interventions for Metabolic Dysfunction-Associated Fatty Liver Disease: An Umbrella Review of Systematic Reviews and Meta-Analyses. *Nutr Rev* 2026; **84**: 1418-1437 [*RCA*] [PMID: 41268875 DOI: 10.1093/nutrit/nuaf145] [FullText]

66 **Rinella ME**, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE, Loomba R. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology* 2023; **77**: 1797-1835 [*RCA*] [PMID: 36727674 DOI: 10.1097/HEP.0000000000000323] [FullText] [Full Text(PDF)]

67 **Targher G**, Valenti L, Byrne CD. Metabolic Dysfunction-Associated Steatotic Liver Disease. *N Engl J Med* 2025; **393**: 683-698 [*RCA*] [PMID: 40802944 DOI: 10.1056/NEJMra2412865] [FullText]

68 **Bril F**, Elbert A. Metabolic dysfunction-associated steatotic liver disease and urinary system cancers: Mere coincidence or reason for concern? *Metabolism* 2025; **162**: 156066 [*RCA*] [PMID: 39551388 DOI: 10.1016/j.metabol.2024.156066] [FullText]

69 **Pan Q**, Xu QY, Zhang LH, He YF. What is the role of nonalcoholic fatty liver disease in pulmonary carcinoma development? *World J Gastroenterol* 2025; **31**: 97500 [*RCA*] [PMID: 39926215 DOI: 10.3748/wjg.v31.i5.97500] [FullText] [Full Text(PDF)]

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Retrospective Study

Examination of bone mineral density in patients with varied liver diseases - a cross-sectional study

Oliver Rohland, Lysann Schwarzer, Laura Schwenk, Nicola Pollmann, Aladdin Ali Deeb, Utz Settmacher, Falk Rauchfuss, Felix Dondorf, Karin Amrein, Roland Kocijan, Michael Ardelt

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Abstract

BACKGROUND

Chronic liver disease is associated with profound alterations in bone metabolism, predisposing patients to secondary osteoporosis and fragility fractures - a condition commonly termed hepatic osteodystrophy. The pathogenesis of bone loss in chronic liver disease is multifactorial, involving increased osteoclastic activity, impaired osteoblast function, chronic inflammation, malnutrition, and deficiencies of vitamin D and K. Among these, cholestatic liver diseases such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are particularly prone to severe bone loss, whereas viral hepatitis and autoimmune hepatitis (AIH) may contribute through inflammatory and steroid-mediated mechanisms. Despite this, comparative data on bone mineral density (BMD) across different etiologies of end-stage liver disease remain limited, particularly in patients awaiting liver transplantation.

AIM
To compare BMD and bone turnover markers among patients with different etiologies of end-stage liver disease listed for liver transplantation.

METHODS
This retrospective, cross-sectional study included 82 patients with end-stage liver disease: AIH, hepatitis C virus infection, PBC, PSC, and polycystic liver disease. Areal BMD at the lumbar spine and femoral neck was measured using dual-energy X-ray absorptiometry. Standard hepatic parameters (bilirubin, international normalized ratio, creatinine, albumin), and bone turnover and micronutrient markers (25-hydroxyvitamin D, parathyroid hormone, osteocalcin, phosphate, calcium, magnesium, ferritin, zinc, and copper.) were analyzed from clinical laboratory data. The Model of End-stage Liver Disease-score and Child-Pugh scores were analyzed. Data on current or prior use of bisphosphonates and glucocorticoids were extracted from patient records.

RESULTS
All liver disease groups showed significantly reduced lumbar spine BMD compared to controls ($P < 0.01$), with the lowest lumbar spine T-scores in PBC (-2.5) and PSC (-2.1). Femoral BMD was less affected but still reduced in several groups. Elevated osteocalcin and parathormone levels were most pronounced in patients with hepatic cysts. AIH patients exhibited less severe bone loss, despite glucocorticoid exposure in some cases.

CONCLUSION
Reduced BMD is prevalent across hepatic disease entities in liver transplant candidates, particularly in cholestatic and cystic liver disease. Early screening and targeted bone-protective strategies are essential to mitigate fracture risk in this population.

Key Words: Bone mineral density; Chronic liver disease; Osteoporosis; Liver transplantation, Dual-energy X-ray absorptiometry

Core Tip: Patients with end-stage liver disease are at high risk of reduced bone mineral density, particularly in cholestatic liver diseases such as primary biliary cholangitis and primary sclerosing cholangitis. This study demonstrates that bone loss varies according to the underlying liver disease etiology. Given the high prevalence of osteoporosis in this population, early assessment using dual-energy X-ray absorptiometry should be considered in clinical routine to identify patients at risk and guide preventive strategies.

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INTRODUCTION

The liver plays a central role in numerous metabolic processes, including protein synthesis, vitamin and mineral homeostasis, and the regulation of inflammatory pathways. In the context of chronic liver disease (CLD), disruption of these physiological functions may result in systemic complications, including secondary osteoporosis[1].

Osteoporosis is a well-recognized complication of CLD and is associated with an increased risk of fragility fractures, leading to substantial morbidity and mortality[1,2]. Recent studies indicate that osteoporosis affects approximately 30%-50% of patients with advanced liver disease, while fracture prevalence may reach up to 40%-50%, particularly in patients awaiting liver transplantation. These findings underscore the clinical relevance of hepatic osteodystrophy as an increasingly recognized systemic complication of CLD[3].

The pathogenesis of hepatic osteodystrophy is multifactorial and involves both increased bone resorption and impaired bone formation. Chronic systemic inflammation promotes osteoclast activation through proinflammatory cytokines, while impaired hepatic synthesis of growth factors and hormones contributes to reduced osteoblast activity[4]. In addition, deficiencies in vitamin D and vitamin K, malnutrition, hypogonadism, and reduced physical activity further exacerbate bone loss in these patients[1,5].

Certain etiologies of CLD appear to have a particularly strong impact on bone metabolism. Cholestatic liver diseases, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), are consistently associated with reduced bone mineral density (BMD) and an increased risk of osteoporosis[1]. In contrast, viral liver diseases such as hepatitis C virus (HCV) infection are associated with systemic inflammation and cytokine-mediated bone loss, even in earlier disease stages[6].

Autoimmune hepatitis (AIH) has been less extensively studied in this context; however, long-term glucocorticoid therapy, which is commonly used in its management, represents an additional major risk factor for secondary osteoporosis[1,7].

Despite the recognized association between CLD and impaired bone health, comparative data on BMD across different etiologies of end-stage liver disease remain limited[8]. In particular, it is unclear to what extent the underlying liver disease contributes to bone loss independent of other risk factors such as age, sex, or glucocorticoid exposure.

Therefore, the aim of the present study was to evaluate BMD in patients with different etiologies of end-stage liver disease listed for liver transplantation and to compare these findings with a healthy control population. Secondary objectives included the assessment of disease-specific differences and the potential impact of clinical and biochemical parameters on bone health.

MATERIALS AND METHODS

Study design and patient selection

This retrospective, cross-sectional study was conducted at the University Hospital Jena. Clinical data were collected from 82 patients who underwent evaluation for liver transplantation between 2004 and 2016. Patients were grouped according to the etiology of their liver disease into five categories: AIH, HCV, PBC, PSC, and polycystic liver disease. A control cohort of 101 healthy individuals, matched for age and sex distribution where feasible, was recruited from the Platelet Collection Center at the Medical University of Graz.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of University Hospital Jena on 18th September 2025, approval No. 2025-3947-BO-D.

Inclusion and exclusion criteria

Inclusion criteria comprised adults of any sex and age with end-stage liver disease listed for transplantation. Exclusion criteria included alcohol-induced cirrhosis, hepatocellular carcinoma, overlap syndromes, and co-existing hepatic or systemic diseases known to affect bone metabolism. Specifically excluded were patients with diabetes mellitus, systemic inflammatory diseases (*e.g.*, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease), chronic kidney disease, human immunodeficiency virus infection, and hematologic malignancies such as lymphoma or multiple myeloma.

A total of 176 patients were initially identified and screened for eligibility. After application of the predefined inclusion and exclusion criteria, 94 patients were excluded from the analysis.

The most frequent reasons for exclusion were alcohol-related liver cirrhosis (n = 34) and hepatocellular carcinoma (n = 31), representing the largest proportion of excluded cases. End-stage kidney disease was present in 11 patients and led to exclusion due to its known impact on bone metabolism. In addition, 9 patients were excluded because of other active or prior malignant diseases. A further 9 patients were excluded due to overlapping liver disease etiologies or incomplete clinical data.

After applying all criteria, a total of 82 patients with end-stage liver disease of non-alcoholic etiology were included in the final analysis.

BMD assessment

Areal BMD (aBMD) was measured by dual-energy X-ray absorptiometry (DXA) using a Prodigy Advance system (GE Healthcare). Standardized scans were performed at the lumbar spine (L1-L4) and bilateral femoral necks. Results were reported as absolute BMD (g/cm^2) and T-scores based on reference values for young adults. According to World Health Organization criteria, osteopenia was defined as a T-score between -1.0 and -2.5, and osteoporosis as T-score < -2.5.

Biochemical analyses

Laboratory analyses included standard hepatic parameters (bilirubin, international normalized ratio, creatinine, albumin), which were used to calculate the Model of End-Stage Liver Disease (MELD) and Child-Pugh scores. Additional laboratory markers relevant to bone metabolism included serum levels of 25-hydroxyvitamin D, parathyroid hormone (PTH), osteocalcin, phosphate, calcium, magnesium, ferritin, zinc, and copper. Data on current or prior use of bisphosphonates and glucocorticoids were extracted from patient records.

Follow-up evaluations

Patients were followed longitudinally with clinical and laboratory reassessments every 6 months until transplantation or death. These follow-up visits included reevaluation of liver function, documentation of any prevalent fractures and medication adjustments. BMD is routinely assessed during the initial evaluation prior to liver transplantation and is subsequently re-evaluated every two years as part of the standardized follow-up protocol.

Control group

The control group consisted of 101 healthy individuals recruited from a platelet donation cohort at the Medical University of Graz. All control subjects fulfilled standard eligibility criteria, which include the absence of acute or chronic diseases, normal physical examination findings, and no history of relevant systemic disorders.

In addition, individuals with known conditions affecting bone metabolism were excluded from the control group. These included CLD, renal insufficiency, endocrine disorders, inflammatory diseases, malignancies, and the use of medications known to influence bone metabolism, such as glucocorticoids or antiresorptive therapies.

All control subjects underwent routine laboratory testing as part of the donation screening process and had no evidence of abnormal liver function or metabolic disturbances. Furthermore, only individuals without a history of osteoporosis or fragility fractures were included. This approach ensured that the control group represented a metabolically healthy population suitable for comparison with patients with end-stage liver disease.

As part of the donation protocol, bone-relevant parameters were measured, and DXA scans were performed under the same protocol as for the patient cohort.

Statistical analysis

Data analysis was conducted using SPSS software (version 24.0, IBM Corp., Armonk, NY, United States). Distribution of variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups were made using the Mann-Whitney *U* test or Fisher's exact test as appropriate. Correlations were evaluated using Pearson's coefficient. A P value < 0.05 was considered statistically significant. For clarity of presentation, group values are reported as medians with interquartile ranges (IQR), and graphs were plotted using mean values without standard deviation bars.

Match-pair-analysis

To investigate differences in bone-related parameters between liver transplant recipients with and without osteoporotic fractures, we performed a matched-pair analysis using a 1:1 nearest-neighbor matching algorithm. Patients with documented osteoporotic fractures either before or after liver transplantation (n = 9) were matched to patients without fractures (n = 9) based on the following variables: Age, sex, body mass index, MELD score, serum 25-hydroxyvitamin D, PTH, and osteocalcin. Matching was conducted using standardized Euclidean distance after z-transformation of continuous variables.

Following matching, differences between the two groups were assessed using paired statistical tests (Wilcoxon signed-rank test or paired *t*-test where appropriate). BMD values at the lumbar spine and femoral neck, T-scores, and biochemical markers of bone metabolism (including PTH, osteocalcin, and vitamin D) were compared between groups. Only matched pairs with available data for the respective variables were included in each test.

The analysis was performed in Python (pandas, sklearn, scipy), and missing values were excluded listwise.

Multivariable regression

To account for potential confounding due to the unequal sex distribution and to identify independent predictors of BMD, a multivariable linear regression analysis was performed. The dependent variables were aBMD of the lumbar spine (L1-L4, g/cm^2), the corresponding lumbar T-score, and aBMD at the femoral neck. Independent variables included sex (male/female), age (years), body mass index (kg/m^2), MELD score, Child-Pugh points, and liver disease etiology (AIH as reference category; dummy variables for PBC, PSC, HCV, and hepatic cystic disease).

A multiple ordinary least squares regression model was used to estimate regression coefficients (β) and 95% confidence intervals. Categorical variables were dummy-coded; continuous variables were entered in their original units. Observations with missing data in any of the included variables were excluded listwise. To reduce the influence of potential outliers, implausible lumbar spine BMD values (< 0.4 g/cm^2 or > 2.0 g/cm^2) were set to missing before analysis.

Model assumptions (linearity, homoscedasticity, and normality of residuals) were verified by visual inspection of residual plots. Multicollinearity was assessed using variance inflation factors. Statistical significance was defined as a two-sided P < 0.05.

RESULTS

Study population

A total of 82 patients with end-stage liver disease were included (53 female, 64.6%; 29 male, 35.4%), with a median age of 62 years (IQR: 54-70). The control group comprised 101 healthy individuals (16 females, 15.8%; 85 males, 84.2%), with a median age of 50 years (IQR: 48-53). The distribution of patients across disease subgroups was as follows: PBC (n = 15), PSC (n = 22), HCV (n = 11), AIH (n = 13), and hepatic cysts (n = 21). Baseline characteristics including sex distribution, body mass index (BMI), and bisphosphonate use are shown in Table 1. Liver function indices reflected varying degrees of hepatic impairment across disease groups. As expected, patients with cholestatic liver disease (PBC and PSC) exhibited the highest gamma-glutamyl transferase and bilirubin levels, accompanied by elevated MELD and Child-Pugh scores indicative of moderate hepatic dysfunction (median MELD ≈ 13-14; Child-Pugh class B). In contrast, patients with HCV- and AIH-related cirrhosis showed moderately increased transaminases but overall lower bilirubin values, corresponding to compensated liver disease (Child-Pugh A-B). The cystic liver disease cohort demonstrated only mild biochemical alterations and preserved hepatic function (median MELD 9; Child-Pugh A). Healthy controls displayed liver parameters within the normal range.

BMD and T-scores

At the time of evaluation, median T-scores at the lumbar spine were significantly lower in all patient groups compared to the control group (PBC: -2.5, PSC: -2.1, HCV: -1.8, hepatic cysts: -1.9; all P < 0.001; AIH: -1.1, P = 0.08 *vs* control -0.1). These

Table 1 Patient demographics, median (interquartile range)

Group	*n*	Median age	Female	Male	BMI (kg/m²)	Liver function panel	MELD-score	Child-Pugh score	Bisphosphonate use
PBC	15	64 (57-72)	11	4	28	ALT: 68 (45-92) U/L γ-GT: 460 (320-590) U/L Bilirubin: 3.2 (2.1-4.8) mg/dL	14 (11-17)	8 (7-9) (class B)	6
PSC	22	49 (42-63)	7	15	22	ALT: 74 (50-108) U/L γ-GT: 410 (280-570) U/L Bilirubin: 2.8 (1.9-4.3) mg/dL	-	7 (6-9) (class B)	3
HCV	11	66 (64-75)	7	4	27	ALT: 74 (52-106) U/L γ-GT: 220 (150-310) U/L Bilirubin: 2.0 (1.3-3.1) mg/dL	-	6 (5-7) (class A)	3
AIH	13	63 (53-70)	10	3	26	ALT: 102 (84-155) U/L γ-GT: 310 (220-420) U/L Bilirubin: 2.5 (1.4-3.9) mg/dL	-	7 (6-8) (class B)	2
Cysts	21	57 (49-63)	18	3	24	ALT: 45 (30-60) U/L γ-GT: 110 (85-150) U/L Bilirubin: 0.9 (0.5-1.4) mg/dL	-	5 (5-6) (class A)	1
Control	101	50 (48-53)	16	85	27	Within normal range	-	-	-

Baseline demographic characteristics of patients with different etiologies of end-stage liver disease and healthy controls. PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis; Cysts: Hepatic cystic disease; Control: Healthy control group; BMI: Body mass index; MELD: Model of End-Stage Liver Disease; ALT: Alanin aminotransferase; γ-GT: Gamma-glutamyl transferase.

findings indicate reduced bone mass in all liver disease groups, with the most pronounced changes in BMD seen in cholestatic liver diseases (PBC and PSC) (Figure 1).

Corresponding BMD values (g/cm²) in the lumbar spine were significantly reduced in all groups (PBC: 0.92, PSC: 0.95, AIH: 1.01, HCV: 0.94, cysts: 0.93; all $P < 0.05$ *vs* control: 1.21), confirming the presence of osteopenia or osteoporosis across the spectrum of liver pathologies (Figure 1, Table 2). The corresponding lumbar spine T-scores were -2.5 in PBC, -2.1 in PSC, -1.1 in AIH, -1.8 in HCV, and -1.9 in cystic liver disease compared to -0.1 in controls, highlighting the predominance of trabecular bone loss in cholestatic and cystic entities. At the femoral neck, BMD values were lower but less markedly affected (right femoral neck: PBC 0.91, PSC 0.96, AIH 0.93, HCV 0.79, cysts 0.93 g/cm² *vs* control 0.99 g/cm²; left femoral neck: PBC 0.88, PSC 0.94, AIH 0.92, HCV 0.98, cysts 0.94 g/cm² *vs* control 0.99 g/cm²), with corresponding T-scores of -0.8, -0.5, -0.4, -1.7, and -0.7, respectively (control -0.5). These findings indicate that cortical bone involvement at the proximal femoral neck is comparatively milder, consistent with the predominantly trabecular nature of hepatic osteodystrophy. BMD and T-scores at the femoral neck were consistently higher than those at the spine but followed a similar trend. Significant reductions in femoral BMD were noted particularly in the PBC and cyst groups (right femoral neck: $P = 0.01$ and 0.03, respectively; left femoral neck: $P = 0.00$ and 0.06, respectively).

Serum markers of bone metabolism

Osteocalcin levels were elevated across all liver disease groups, most markedly in patients with hepatic cysts (median 61.9 ng/mL *vs* 14-17 ng/mL in other groups), suggesting increased bone turnover. PTH concentrations were mildly elevated in most groups, but notably highest in the cyst cohort (85.8 ng/L), many of whom had concurrent polycystic

Table 2 Bone mineral density and T-scores

Parameter	PBC	*P* value PBC	PSC	*P* value PSC	AIH	*P* value AIH	HCV	*P* value HCV	Cysts	*P* value cysts	Control
BMD right femoral neck	0.91 (0.81; 0.96)	0.01	0.96 (0.91; 1.05)	0.3	0.93 (0.86; 0.99)	0.07	0.79 (0.72; 1.06)	0.1	0.93 (0.85; 1.04)	0.03	0.99 (0.92; 1.09)
T-score right femoral neck	-0.8 (-1.7; -0.3)	0.13	-0.5 (-1.4; -0.2)	0.49	-0.4 (-1.1; 0.3)	0.74	-1.7 (-2.4; -0.2)	0.07	-0.7 (-1.6; 0.1)	0.29	-0.5 (-1.1; 0.2)
BMD left femoral neck	0.88 (0.63; 0.95)	0.0	0.94 (0.87; 1.05)	0.06	0.92 (0.86; 1.04)	0.14	0.98 (0.78; 1.02)	0.25	0.94 (0.84; 1.04)	0.06	0.99 (0.92; 1.08)
T-score left femoral neck	-1.3 (-2.7; -0.4)	0.01	-0.7 (-1.4; -0.1)	0.09	-0.4(-1.2; 0.2)	0.99	-0.6 (-1.7; 1.0)	0.31	-0.7 (-1.5; 0.3)	0.33	-0.5 (-1.1; 0.2)
BMD lumbar spine	0.92 (0.74; 1.10)	0.0	0.95 (0.88; 1.01)	0.0	1.01 (0.93; 1.21)	0.01	0.94 (0.84; 1.00)	0.0	0.93 (0.80; 1.02)	0.0	1.21 (1.09; 1.30)
T-score lumbar spine	-2.5 (-3.1; -0.8)	0.0	-2.1 (-2.6; -1.2)	0.0	-1.1 (-2.1; 0.7)	0.08	-1.8 (-2.9; -0.6)	0.0	-1.9 (-3.0; -0.2)	0.0	-0.1 (-1.0; 0.6)

PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis; Cysts: Hepatic cystic disease; Control: Healthy control group; BMD: Bone mineral density.

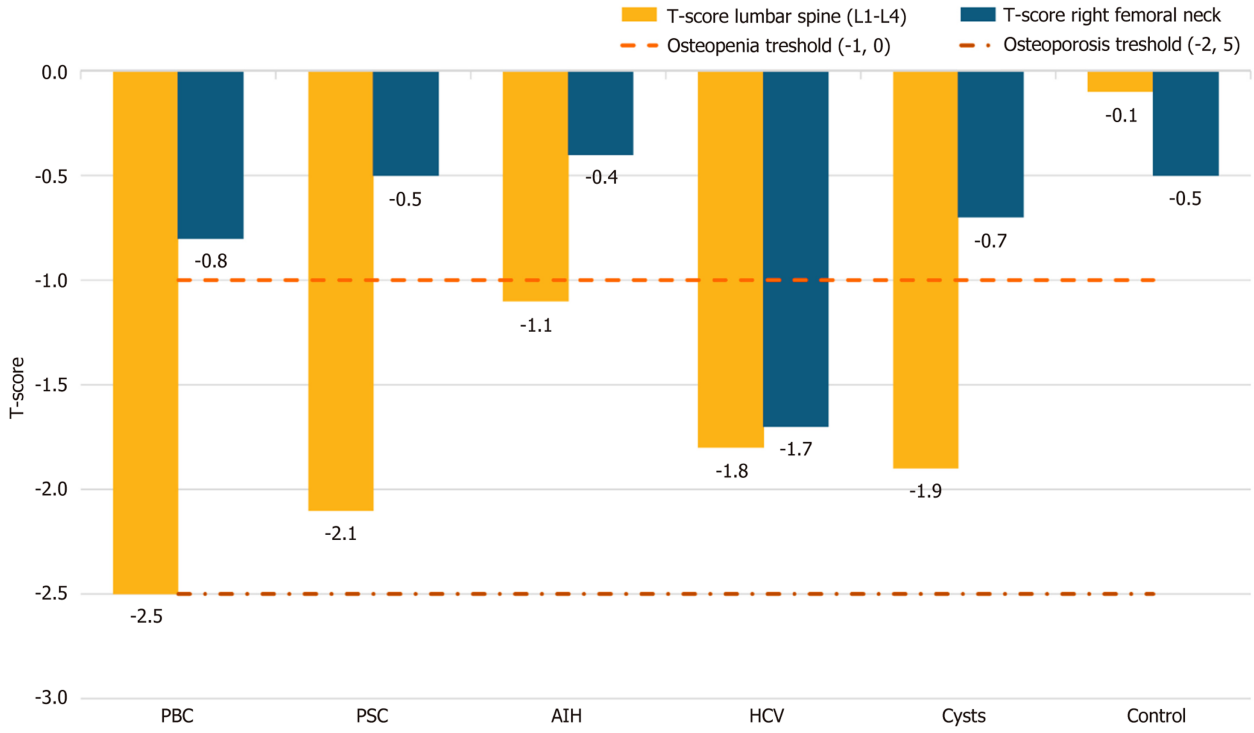


Figure 1 T-scores at lumbar spine (L1-L4) and right femoral neck by group. PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; AIH: Autoimmune hepatitis; HCV: Hepatitis C virus infection; Cysts: Hepatic cystic disease; Control: Healthy control group.

kidney disease and chronic kidney impairment.

Micronutrient analyses revealed increased serum copper in patients with cholestatic liver disease (PBC: 25.8 μmol/L, PSC: 27.6 μmol/L) and elevated zinc in PSC, HCV, and cyst patients. Magnesium and calcium levels were within normal range across all groups (Table 3).

Impact of glucocorticoids

Among AIH patients, 2/13 were receiving ongoing steroid therapy at the time of evaluation. No significant difference in lumbar BMD was observed compared to non-steroid-treated AIH patients, although small sample size limits interpretability. Nonetheless, AIH patients had the highest median BMD (1.01 g/cm^2, T-scores -1.1) at the lumbar spine and femoral neck (BMD, T-score), respectively.

Table 3 Bone metabolism markers

Marker	PBC	PSC	AIH	HCV	Cysts
Osteocalcin (ng/mL)	14.2	17.2	14.9	14.6	61.9
25-OH vitamin D (nmol/L)	142.4	60.3	49.9	45.9	79.0
PTH (ng/L)	34.7	39.0	44.8	49.7	85.8
Phosphate (mmol/L)	1.1	1.0	1.0	2.1	1.1
Copper (μmol/L)	25.8	27.6	19.9	18.8	27.5
Zinc (μmol/L)	7.1	8.8	8.0	7.6	9.8
Magnesium (mmol/L)	0.8	0.8	0.8	0.8	0.8
Calcium (mmol/L)	2.3	2.3	2.3	2.1	2.3
Creatinine (μmol/L)	88	95	83	91	310[1]
AST (U/L)	85	74	121	98	63
ALT (U/L)	72	69	115	104	52

[1]Several patients with hepatic cysts presented with concomitant polycystic kidney disease and dialysis-dependent renal failure, explaining the markedly elevated creatinine levels. PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis; Cysts: Hepatic cystic disease; PTH: Parathyroid hormone; AST: Aspartat aminotransferase; ALT: Alanin aminotransferase.

Group comparisons and statistical significance

Compared with controls, patients with PBC, PSC, HCV, and hepatic cysts showed significantly reduced BMD at the lumbar spine ($P < 0.01$ for all). In contrast, AIH patients did not significantly differ in T-score from controls ($P = 0.08$). At the femoral sites, the magnitude of BMD reduction was smaller, and statistical significance was limited, consistent with the known predominance of trabecular bone loss in hepatic osteodystrophy.

Match-pair-analysis

A total of 9 patients with documented osteoporotic fractures (pre- or post-transplantation) were successfully matched 1:1 to 9 fracture-free liver transplant recipients based on age, sex, BMI, MELD score, vitamin D, PTH, and osteocalcin levels. DXA analysis showed a consistent trend toward lower BMD and T-scores in the fracture group compared to matched controls. The mean T-score at the lumbar spine was -3.07 in the fracture group *vs* -1.29 in the control group ($P = 0.078$). Similarly, the mean overall T-score was -2.45 *vs* -0.81 ($P = 0.078$), and BMD at the left femoral neck was 0.77 g/cm^2 *vs* 0.94 g/cm^2 ($P = 0.094$), respectively (Tables 4, 5, and 6).

Although the differences did not reach statistical significance due to the small sample size, the results demonstrate a consistent pattern of reduced bone mass in the fracture cohort.

No significant differences were observed in serum vitamin D, PTH, or osteocalcin levels between the two groups within the matched pairs. In multivariable linear regression models adjusting for sex, age, BMI, MELD score, Child-Pugh points, and liver disease etiology, sex showed no significant association with any bone parameter (Tables 7, 8, 9, and 10). This indicates that the male predominance in the cohort did not confound the observed relationships between liver disease and bone status.

For lumbar spine BMD (Table 7), age (β = -0.0046 g/cm^2 per year, $P = 0.021$) and BMI (β = +0.0121 g/cm^2 per kg/m^2, $P = 0.020$) were independent predictors of bone density. MELD and Child-Pugh scores were not significantly associated with BMD. Among etiologic subgroups, none showed significant differences compared with AIH after adjustment.

For the lumbar spine T-score (Table 8), only PBC was independently associated with lower values compared to AIH (β = -1.59, $P = 0.036$), while PSC, HCV, and cystic liver disease were not significant. Age, BMI, and liver function parameters did not predict lumbar T-scores in the adjusted model.

At the femoral neck, both left and right sides yielded consistent findings (Tables 9 and 10). Higher BMI was positively associated with femoral BMD (left: B = +0.0121 g/cm^2 per kg/m^2, $P = 0.005$; right: B = +0.0124, $P = 0.004$). Age showed a trend toward lower femoral BMD (left: $P = 0.091$; right: $P = 0.08$). There was no significant effect of sex, MELD, or Child-Pugh scores on femoral BMD. Patients with PBC tended to have lower adjusted femoral BMD compared to AIH (left: $P = 0.06$; right: $P = 0.07$), although these associations did not reach statistical significance.

Across all models, adjusted coefficients of determination (R^2) ranged from 0.05 for lumbar spine BMD to 0.12 for femoral BMD, consistent with expected variability in clinical cross-sectional datasets. Sensitivity analyses excluding patients with hepatic cystic disease confirmed the robustness of these findings.

Table 4 Correlation between bone mineral density parameters and liver function scores

Variable pair	Spearman's ρ	Interpretation
Lumbar spine T-score *vs* MELD	-0.01	No correlation
Lumbar spine T-score *vs* Child-Pugh	+0.12	Weak positive, not significant
Lumbar spine BMD *vs* MELD	-0.06	No correlation
Lumbar spine BMD *vs* Child-Pugh	+0.09	Weak positive, not significant
Right femoral neck T-score *vs* MELD	+0.04	No correlation
Right femoral neck T-score *vs* Child-Pugh	+0.09	Weak positive
Right femoral neck BMD *vs* MELD	+0.01	No correlation
Right femoral neck BMD *vs* Child-Pugh	+0.01	No correlation
Left femoral neck T-score *vs* MELD	-0.02	No correlation
Left femoral neck T-score *vs* Child-Pugh	+0.06	Weak positive
Left femoral neck BMD *vs* MELD	-0.01	No correlation
Left femoral neck BMD *vs* Child-Pugh	+0.02	No correlation
Lumbar spine T-score *vs* MELD	-0.01	No correlation
Lumbar spine T-score *vs* Child-Pugh	+0.12	Weak positive, not significant
Lumbar spine BMD *vs* MELD	-0.06	No correlation
Lumbar spine BMD *vs* Child-Pugh	+0.09	Weak positive, not significant
Right femoral neck T-score *vs* MELD	+0.04	No correlation
Right femoral neck T-score *vs* Child-Pugh	+0.09	Weak positive
Right femoral neck BMD *vs* MELD	+0.01	No correlation
Right femoral neck BMD *vs* Child-Pugh	+0.01	No correlation
Left femoral neck T-score *vs* MELD	-0.02	No correlation
Left femoral neck T-score *vs* Child-Pugh	+0.06	Weak positive
Left femoral neck BMD *vs* MELD	-0.01	No correlation
Left femoral neck BMD *vs* Child-Pugh	+0.02	No correlation

Spearman's rank correlation coefficients (ρ) were calculated to assess the association between bone parameters (bone mineral density and T-scores at the lumbar spine and femoral neck) and liver function indices. No significant correlations were found between bone density measures and either Model of End-Stage Liver Disease or Child-Pugh scores. Weak, non-significant positive trends were observed between T-scores and Child-Pugh classification, indicating a potential but clinically minor association between better hepatic function and higher bone density. MELD: Model of End-Stage Liver Disease; BMD: Bone mineral density.

Table 5 Match-pair analysis of bone mineral density and T-scores in liver transplant recipients with and without osteoporotic fractures

Parameter	Test	*n*	Mean fracture	Mean control	*P* value
T-score lumbar spine	Wilcoxon	7	-3.07	-1.29	0.08
BMD lumbar spine	Wilcoxon	6	0.81	0.95	0.16
T-score left femoral neck	Wilcoxon	6	-1.9	-0.82	0.16
BMD left femoral neck	Wilcoxon	6	0.77	0.94	0.09

Comparison of lumbar spine and femoral neck bone parameters between patients with documented osteoporotic fractures and matched fracture-free controls. Matching was performed 1:1 based on age, sex, body mass index, Model of End-Stage Liver Disease score, serum 25-hydroxyvitamin D, parathyroid hormone, and osteocalcin levels. BMD: Bone mineral density.

Table 6 Match-pair analysis using Wilcoxon signed-rank test in 1:1 matched pairs

Parameter	Test	*n*	Mean fracture	Mean control	*P* value
T-score (lumbar spine)	Wilcoxon	7	-3.07	-1.29	0.08
BMD (lumbar spine)	Wilcoxon	6	0.81 g/cm²	0.95 g/cm²	0.16
T-score (left femoral neck)	Wilcoxon	6	-1.90	-0.82	0.16
BMD (left femoral neck)	Wilcoxon	6	0.77 g/cm²	0.94 g/cm²	0.09

Detailed comparison of T-scores and bone mineral density values at the lumbar spine and left femoral neck between fracture and control groups. Bone mineral density, expressed in g/cm²; T-score = standard deviation from the mean peak bone mass of a healthy young adult population. Matching criteria: Age, sex, body mass index, Model of End-Stage Liver Disease score, serum 25-hydroxyvitamin D, parathyroid hormone, and osteocalcin levels. All comparisons used the Wilcoxon signed-rank test for paired data. BMD: Bone mineral density.

Table 7 Regression coefficients for bone mineral density lumbar spine

Predictor	Regression coefficient (β)	*P* value	95%CI (lower bound)	95%CI (higher bound)	Standardized coefficient (β-standardized)
Sex (male)	-0.0159	0.7681	-0.1231	0.0914	-0.0414
Age (per year)	-0.0046	0.0215	-0.0084	-0.0007	-0.3115
BMI (kg/m²)	0.0121	0.0202	2	0.0223	0.3162
MELD score	-0.0046	0.4076	-0.0155	0.0064	-0.1312
Child-Pugh (points)	0.0082	0.6788	-31	0.0474	0.0726
PBC	0.1079	0.0917	-18	0.2338	-0.1033
PSC	0.1766	0.0002	0.0878	0.2655	0.0473
HCV	0.18	0.0129	0.0395	0.3205	0.0382
AIH	0.1775	0.0038	0.0596	0.2955	0.0387
Polycystic liver disease	0.1495	0.0023	0.0557	0.2433	-0.0209

Results from multiple linear regression identifying independent predictors of areal lumbar spine bone mineral density (g/cm²). CI: Confidence interval; BMI: Body mass index; MELD: Model of End-Stage Liver Disease; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis.

DISCUSSION

In this cross-sectional analysis of 82 patients with end stage liver disease (ESLD) awaiting transplantation, we observed a high prevalence of skeletal compromise across all hepatic disease etiologies, with lumbar spine T-scores ranging from -2.5 in PBC to -1.1 in AIH. Compared to healthy controls, all groups except AIH had significantly reduced spinal BMD, confirming the widespread presence of osteopenia or osteoporosis in this population. These findings align with prior reports citing osteoporosis prevalence rates up to 80% in advanced PBC and PSC[8,9].

The lumbar spine, with its high proportion of metabolically active trabecular bone, was consistently more affected than the femoral neck, mirroring known patterns in hepatic osteodystrophy[4]. This site-specific vulnerability is also reflected in high-resolution peripheral quantitative computed tomography (HR-pQCT) studies, where greater percentage losses in trabecular volumetric BMD (vBMD) were detected, compared to cortical-rich sites. A meta-analysis of HR-pQCT fracture studies demonstrated that fracture cases exhibited on average -12.6% lower trabecular vBMD, -8.8% lower trabecular number, and +9.7% higher trabecular separation compared to non-fracture controls - well above the least significant change for these parameters[1,10]. While HR-pQCT and DXA assess distinct aspects of bone quality and cannot be directly compared, the magnitude of lumbar spine T-score reduction observed in our PBC group (-2.4 SD *vs* controls) reflects a correspondingly severe skeletal deficit and thus underscores the clinical significance of bone loss in this population[11].

Patients with hepatic cystic disease demonstrated marked skeletal deficits (lumbar T-score -1.9; BMD 0.93 g/cm²), accompanied by substantially elevated osteocalcin (median 61.9 ng/mL) and PTH (85.8 ng/L) levels. More than half had coexisting polycystic kidney disease and end-stage renal failure - conditions associated with cortical thinning and trabecular deterioration on HR-pQCT. This suggests a multifactorial bone loss phenotype, combining features of hepatic and renal osteodystrophy. In HR-pQCT studies of dialysis patients, cortical area is reduced by ~8%-10% and cortical porosity increased by ~15% compared to matched controls, parameters that are independently predictive of fracture risk.

Table 8 Regression coefficients for T-score lumbar spine

Predictor	Regression coefficient (β)	*P* value	95%CI (lower bound)	95%CI (higher bound)	Standardized coefficient (β-standardized)
Sex (male)	-0.4431	0.3909	-1.4671	0.5809	-0.1138
Age (per year)	-0.0045	0.8165	-0.0432	0.0341	-0.0312
BMI (kg/m^2)	0.0753	0.1503	-28	0.1785	0.1906
MELD score	-38	0.4853	-0.1459	0.07	-0.1049
Child-Pugh (points)	0.1194	531	-259	0.4978	106
PBC	-1.3522	0.0379	-2.6265	-0.0778	-0.18
PSC	-0.4625	297	-1.3406	0.4156	0.0009
HCV	-0.7389	0.2776	-2.086	0.6083	-0.0497
AIH	0.2416	678	-0.9144	1.3977	0.1437
Polycystic liver disease	-0.1799	701	-1.1112	0.7513	0.0681

Results from multiple linear regression identifying independent predictors of areal lumbar spine for T-score. CI: Confidence interval; BMI: Body mass index; MELD: Model of End-Stage Liver Disease; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis.

Table 9 Regression coefficients for bone mineral density left femoral neck

Predictor	Regression coefficient (β)	*P* value	95%CI (lower bound)	95%CI (higher bound)	Standardized coefficient (β-standardized)
Sex (male)	-0.0047	0.9122	-0.0897	0.0803	-0.0147
Age (per year)	-0.0027	0.0914	-0.0058	0.0004	-0.2182
BMI (kg/m^2)	0.0121	0.0052	0.0038	0.0204	0.3727
MELD score	-0.0	0.9918	-0.0091	9	-0.0016
Child-Pugh (points)	-0.0056	729	-38	0.0267	-0.0593
PBC	0.0241	0.6421	-0.0789	0.1271	-0.2673
PSC	0.1671	0.0	0.0956	0.2386	0.1003
HCV	0.1548	0.0088	0.0404	0.2693	0.0432
AIH	0.1428	0.0044	0.0462	0.2394	0.0213
Cystic disease	0.1591	0.0001	0.0828	0.2355	73

Results from multiple linear regression identifying independent predictors of areal left femoral neck bone mineral density (g/cm^2). CI: Confidence interval; BMI: Body mass index; MELD: Model of End-Stage Liver Disease; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis.

HCV-related bone loss was also substantial (lumbar T-score -1.8; BMD 0.94 g/cm^2, $P < 0.01$), consistent with literature linking chronic viral hepatitis to systemic inflammation and elevated osteoclast activity[12]. AIH patients had the highest median BMD (1.01 g/cm^2) yet still subnormal T-scores (-1.1), likely reflecting cumulative glucocorticoid exposure. HR-pQCT has shown that steroid therapy can precipitate early trabecular perforation and cortical thinning before significant DXA changes appear, complicating attribution solely to hepatic pathology.

Our matched-pair analysis, though underpowered for statistical significance, revealed clinically meaningful differences: Fracture patients had mean lumbar spine T-scores 1.8 SD lower and femoral neck BMD 0.17 g/cm^2 lower than matched controls. This is directionally consistent with HR-pQCT-based prediction models - such as the Bone Microarchitecture International Consortium pooled analysis - which demonstrated that microarchitectural parameters (total vBMD, trabecular number, cortical area) improve fracture discrimination beyond hip aBMD, with net reclassification improvements of 17%-21%[13,14].

Biochemically, calcium and magnesium remained normal, while copper was elevated in cholestatic disease and zinc increased in several groups. The cyst group's high osteocalcin and PTH likely reflect high-turnover bone loss and secondary hyperparathyroidism, both of which are associated with greater microarchitectural deterioration in HR-pQCT

Table 10 Regression coefficients for bone mineral density right femoral neck

Predictor	Regression coefficient (β)	*P* value	95%CI (lower)	95%CI (upper)	Standardized coefficient (β-standardized)
Sex (male)	-9	0.86	-0.1	0.08	-15
Age (years)	-0.0029	0.08	-6	0.0003	-0.22
BMI (kg/m^2)	0.0124	4	4	21	0.37
MELD score	0.0002	0.94	-8	9	-2
Child-Pugh (points)	-6	0.71	-38	26	-0.06
PBC	-0.11	0.07	-0.23	0.01	-0.27
PSC	0.03	0.66	-0.1	0.15	0.1
HCV	0.01	0.89	-0.13	0.15	0.04
Cystic disease	0.02	0.8	-0.11	0.14	0.07

Results from multiple linear regression identifying independent predictors of areal right femoral neck bone mineral density (g/cm^2). CI: Confidence interval; BMI: Body mass index; MELD: Model of End-Stage Liver Disease; PBC: Primary biliary cholangitis; PSC: Primary sclerosing cholangitis; HCV: Hepatitis C virus infection; AIH: Autoimmune hepatitis.

studies[15-17].

In multivariable regression analyses (Tables 7, 8, 9, and 10), neither MELD nor Child-Pugh scores were independently associated with lumbar or femoral BMD, suggesting that the severity of liver dysfunction itself exerts a limited direct influence on skeletal integrity once other clinical factors are considered. Instead, age and BMI emerged as the principal determinants of BMD, consistent with previous studies identifying low body weight as a major risk factor for hepatic osteodystrophy[3,18]. The absence of a significant association with MELD or Child-Pugh points aligns with earlier findings that biochemical and hormonal factors, such as vitamin D deficiency, hypogonadism, and inflammation, better explain bone loss in liver disease than global hepatic function scores[3].

Spearman's correlation analyses confirmed these results, showing no significant correlations between BMD or T-score and MELD (ρ = -0.07, *P* = 0.58 for lumbar BMD; ρ = -0.01, *P* = 0.92 for lumbar T-score) or Child-Pugh points (ρ = +0.06, *P* = 0.60 and ρ = +0.12, *P* = 0.29, respectively). Femoral neck BMD also showed no relationship with either score (ρ = -0.01, *P* = 0.95 for MELD; ρ = +0.02, *P* = 0.88 for Child-Pugh). These findings indicate that bone loss occurs largely independent of the degree of hepatic decompensation, reinforcing that standard liver function indices are poor surrogate markers of skeletal risk in ESLD.

Importantly, after adjustment for age, BMI, and disease etiology, sex was not associated with BMD or T-score, ruling out gender imbalance as a confounder in the observed bone density differences. This emphasizes that the lower bone mass seen in cholestatic liver disease reflects disease-specific mechanisms rather than demographic bias.

From a pathophysiologic standpoint, the lack of correlation between BMD and liver severity scores supports the concept that hepatic osteodystrophy represents a parallel metabolic complication of liver disease rather than a simple sequela of liver failure. Bone loss may therefore begin early in the disease course and progress independently of hepatic function decline. Histomorphometric and HR-pQCT data from patients with early-stage cholestatic disorders have shown reduced trabecular bone volume and connectivity even before biochemical evidence of liver failure[18]. This highlights the need for early and systematic screening of bone health in patients with CLD, especially those with cholestatic or cystic etiologies where bone loss appears most severe.

Collectively, these results underscore that hepatic osteodystrophy is multifactorial in nature and only weakly related to overall liver function. Preventive and therapeutic strategies should therefore target modifiable risk factors - such as maintaining adequate body weight, optimizing vitamin D status, and minimizing steroid exposure - while integrating routine DXA assessment into the pre-transplant evaluation of all ESLD patients.

Clinical implications

Our findings, together with microarchitectural evidence from HR-pQCT studies, underscore the need for routine lumbar spine DXA screening in all patients with ESLD, particularly those with cholestatic or cystic etiologies such as PBC, PSC, or hepatic cystic disease. The consistent association of bone loss with age and BMI rather than MELD or Child-Pugh scores highlights that skeletal deterioration progresses largely independent of hepatic function. Therefore, bone assessment should be incorporated into transplant evaluation irrespective of disease stage or biochemical severity.

Given that conventional DXA captures only areal density and not microarchitectural quality, adjunctive tools such as the trabecular bone score or HR-pQCT may better identify patients at high fracture risk. This is particularly relevant in AIH patients receiving long-term corticosteroids, in whom DXA often underestimates true structural compromise. Integration of these advanced imaging modalities into pre-transplant screening could improve risk stratification and guide early intervention.

Limitations

This study has several limitations, including its retrospective single-center design, small etiologic subgroups, and the absence of vertebral fracture assessment or direct microarchitectural imaging. The cross-sectional nature of the analysis precludes conclusions regarding longitudinal bone loss. Moreover, serum markers of bone turnover and vitamin D metabolism were only partially available. Despite these limitations, the consistency of our findings with established HR-pQCT-based microarchitectural thresholds suggests that the observed DXA deficits represent clinically meaningful skeletal deterioration.

Future directions

Future work should aim to integrate advanced imaging modalities - such as HR-pQCT and trabecular bone score - into pre- and post-transplant protocols to quantify both trabecular and cortical deficits. Prospective longitudinal studies are warranted to clarify the temporal relationship between liver disease progression, transplantation, and bone microarchitecture. In addition, early initiation of anti-osteoporotic therapy, ideally with anabolic-first sequences followed by antiresorptives, should be evaluated for its potential to preserve bone mass and prevent fractures in this vulnerable population.

Summary

Across all disease groups, at least osteopenic changes were evident, with PBC and PSC showing the most pronounced reductions in lumbar spine BMD. The hepatic cyst group demonstrated similarly low bone density despite preserved hepatic function, likely reflecting combined hepatic and renal osteodystrophy. Elevated bone turnover markers and micronutrient imbalances were common but heterogeneous across etiologies. Neither MELD nor Child-Pugh scores correlated significantly with bone parameters, emphasizing that liver function indices are poor predictors of skeletal health in ESLD.

CONCLUSION

In this retrospective cross-sectional study, reduced BMD was observed across all investigated etiologies of end-stage liver disease, with the most pronounced impairment in patients with cholestatic liver disorders. While these findings support the concept of hepatic osteodystrophy as a relevant complication of advanced liver disease, the extent of bone loss appears to vary depending on the underlying etiology. Given the retrospective design and the limited sample size of certain subgroups, these results should be interpreted with caution. Nevertheless, the data highlight the importance of routine bone health assessment in patients with ESLD, particularly in those with known risk factors. Future prospective studies are warranted to further clarify disease-specific mechanisms and to evaluate targeted preventive and therapeutic strategies.

REFERENCES

1 **Handzlik-Orlik G**, Holecki M, Wilczyński K, Duława J. Osteoporosis in liver disease: pathogenesis and management. *Ther Adv Endocrinol Metab* 2016; **7**: 128-135 [*RCA*] [PMID: 27293541 DOI: 10.1177/2042018816641351] [FullText]

2 **Guañabens N**, Parés A. Osteoporosis in chronic liver disease. *Liver Int* 2018; **38**: 776-785 [*RCA*] [PMID: 29479832 DOI: 10.1111/liv.13730] [FullText]

3 **Shirinezhad A**, Eshlaghi FM, Salabat D, Azarboo A, Ardakani ZF, Esmaeili S, Hoveidaei AH, Ghaseminejad-Raeini A. Prevalent osteoporosis and fracture risk in patients with hepatic cirrhosis: a systematic review and meta-analysis. *BMC Gastroenterol* 2025; **25**: 115 [*RCA*] [PMID: 40000980 DOI: 10.1186/s12876-025-03720-6] [FullText] [Full Text(PDF)]

4 **Angulo P**, Grandison GA, Fong DG, Keach JC, Lindor KD, Bjornsson E, Koch A. Bone disease in patients with primary sclerosing cholangitis. *Gastroenterology* 2011; **140**: 180-188 [*RCA*] [PMID: 20955707 DOI: 10.1053/j.gastro.2010.10.014] [FullText] [Full Text(PDF)]

5 **Jeong HM**, Kim DJ. Bone Diseases in Patients with Chronic Liver Disease. *Int J Mol Sci* 2019; **20**: 4270 [*RCA*] [PMID: 31480433 DOI: 10.3390/ijms20174270] [FullText] [Full Text(PDF)]

6 **Yang YJ**, Kim DJ. An Overview of the Molecular Mechanisms Contributing to Musculoskeletal Disorders in Chronic Liver Disease: Osteoporosis, Sarcopenia, and Osteoporotic Sarcopenia. *Int J Mol Sci* 2021; **22**: 2604 [*RCA*] [PMID: 33807573 DOI: 10.3390/ijms22052604] [FullText] [Full Text(PDF)]

7 **Zheng JP**, Miao HX, Zheng SW, Liu WL, Chen CQ, Zhong HB, Li SF, Fang YP, Sun CH. Risk factors for osteoporosis in liver cirrhosis patients measured by transient elastography. *Medicine (Baltimore)* 2018; **97**: e10645 [*RCA*] [PMID: 29768330 DOI: 10.1097/MD.0000000000010645] [FullText] [Full Text(PDF)]

8 **Danford CJ**, Trivedi HD, Bonder A. Bone Health in Patients With Liver Diseases. *J Clin Densitom* 2020; **23**: 212-222 [*RCA*] [PMID: 30744928 DOI: 10.1016/j.jocd.2019.01.004] [FullText]

9 **Guañabens N**, Parés A, Mariñoso L, Brancós MA, Piera C, Serrano S, Rivera F, Rodés J. Factors influencing the development of metabolic bone disease in primary biliary cirrhosis. *Am J Gastroenterol* 1990; **85**: 1356-1362 [*RCA*] [PMID: 2220729] [FullText]

10 **Collier JD**, Ninkovic M, Compston JE. Guidelines on the management of osteoporosis associated with chronic liver disease. *Gut* 2002; **50** Suppl 1: i1-i9 [*RCA*] [PMID: 11788576 DOI: 10.1136/gut.50.suppl_1.i1] [FullText]

11 **Kocijan R**, Muschitz C, Haschka J, Hans D, Nia A, Geroldinger A, Ardelt M, Wakolbinger R, Resch H. Bone structure assessed by HR-pQCT, TBS and DXL in adult patients with different types of osteogenesis imperfecta. *Osteoporos Int* 2015; **26**: 2431-2440 [*RCA*] [DOI: 10.1007/s00198-015-3156-4] [FullText]

12 **Raszeja-Wyszomirska J**, Miazgowski T. Osteoporosis in primary biliary cirrhosis of the liver. *Prz Gastroenterol* 2014; **9**: 82-87 [*RCA*]

[PMID: 25061487 DOI: 10.5114/pg.2014.42502] [FullText] [Full Text(PDF)]

13 **De Vries F**, Bracke M, Leufkens HG, Lammers JW, Cooper C, Van Staa TP. Fracture risk with intermittent high-dose oral glucocorticoid therapy. *Arthritis Rheum* 2007; **56**: 208-214 [*RCA*] [PMID: 17195223 DOI: 10.1002/art.22294] [FullText]

14 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Autoimmune hepatitis. *J Hepatol* 2015; **63**: 971-1004 [*RCA*] [PMID: 26341719 DOI: 10.1016/j.jhep.2015.06.030] [FullText]

15 **Collier J**. Bone disorders in chronic liver disease. *Hepatology* 2007; **46**: 1271-1278 [*RCA*] [PMID: 17886334 DOI: 10.1002/hep.21852] [Full Text]

16 **Kaemmerer D**, Schmidt B, Lehmann G, Wolf G, Settmacher U, Hommann M. Treatment of bone loss in patients with chronic liver disease awaiting liver transplantation. *Transplant Res* 2012; **1**: 7 [*RCA*] [PMID: 23369371 DOI: 10.1186/2047-1440-1-7] [FullText] [Full Text(PDF)]

17 **Hommann M**, Kämmerer D, Lehmann G, Kornberg A, Küpper B, Daffner W, Wolf G, Settmacher U. Prevention of early loss of bone mineral density after liver transplantation by prostaglandin E1. *Transplant Proc* 2007; **39**: 540-543 [*RCA*] [PMID: 17362777 DOI: 10.1016/j.transproceed.2006.12.016] [FullText]

18 **Carey E**, Balan V. Metabolic bone disease in patients with liver disease. *Curr Gastroenterol Rep* 2003; **5**: 71-77 [*RCA*] [PMID: 12530951 DOI: 10.1007/s11894-003-0012-z] [FullText]

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Acute hepatitis B in the vaccination era: Predictors of functional cure and chronicity

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Abstract

BACKGROUND

Despite the existence of an effective vaccine and treatment for the hepatitis B virus (HBV) more than 880000 people die each year from complications of cirrhosis and hepatocellular carcinoma associated with chronic HBV (CHB) infection. Limited information exists regarding the effect of antiviral treatment or the effect of patient background and viral factors on acute HBV outcome in a population with wide vaccine availability. Previous studies have demonstrated that widespread availability of the vaccine in developed countries has shifted the epidemiology of acute HBV infection to vulnerable subpopulations.

AIM

To evaluate the hypothesis that this epidemiologic shift in susceptibility of popu-

lations to acute HBV infection may alter the immune responses during acute infection and affect the rates of functional cure (FC) and viral persistence.

METHODS

We performed a retrospective evaluation of all patients above the age of 18 with acute HBV infection in Southern Israel between the years 2004-2022. Evaluated outcomes were FC, defined as hepatitis B surface antigen (HBsAg) clearance, or CHB infection defined as positive HBsAg at 6 months past the acute event. Clinical, biochemical, metabolic, and socioeconomic variables were analyzed.

RESULTS

Among 78 patients with acute HBV infection, 51 (65.3%) achieved FC, while 27 (34.6%) progressed to CHB infection. Patients who achieved FC had significantly higher peak alanine aminotransferase levels at presentation than those who developed CHB infection (2117 ± 1509 IU/mL *vs* 1103 ± 1540 IU/mL; $P = 0.01$). No differences in early initiation of nucleos(t)ide analogue therapy were found between patients who achieved FC and patients who developed CHB. Progression to CHB infection was significantly more common among patients with a background of low socioeconomic status (SES; 70% *vs* 41%; $P = 0.04$) and diabetes mellitus (DM; 26% *vs* 3.9%; $P = 0.007$). In univariable analysis, DM presence and a high SES were associated with a significant effect on HBsAg persistence (odds ratio = 8.57 and 0.46, respectively).

CONCLUSION

In the vaccination era, we postulate that acute HBV carries a higher-than-expected risk of chronicity, particularly among patients with metabolic disease and those from socioeconomic disadvantage backgrounds. Early risk stratification, targeted follow-up for high-risk cases, and further study of metabolic and social factors in FC are warranted.

Key Words: Hepatitis B; Diabetes mellitus; Socioeconomic status; Vaccination; Nucleos(t)ide analogues

Core Tip: The widespread availability of the universal hepatitis B virus (HBV) vaccination reduced acute HBV incidence in developed countries. Acute HBV usually resolves with functional cure in > 95% of adults. In our study we evaluated all acute HBV cases in southern Israel between 2004-2022 and found a striking 34% chronicity rate. Further analysis revealed that a background of diabetes and low socioeconomic status were found to strongly predict chronic infection. These findings highlight the need for risk stratification in acute HBV patients from vulnerable populations.

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INTRODUCTION

Hepatitis B virus (HBV) is a small hepatotropic DNA virus that persistently remains a cause of chronic liver disease around the globe[1]. It is estimated that more than 2 billion persons have been exposed worldwide to HBV of whom approximately 240 million have chronic infection[2]. Although an effective vaccine and treatment exist, more than 880000 people die each year from complications of cirrhosis and hepatocellular carcinoma (HCC)[3]. Widespread availability of the vaccine in developed countries[4] has shifted the epidemiology of acute HBV in developed countries to vulnerable subpopulations[5].

Chronic HBV (CHB) is defined by the persistence of hepatitis B surface antigen (HBsAg) > 6 months following acute HBV infection[6]. The evolution of acute HBV infection to CHB likely involves both host and viral factors[7,8] although precise mechanisms are not well defined[9-13]. An intriguing novel host factor is the early introduction of nucleos(t)ide analogues (NAs) during the acute infection. American and European Liver society guidelines recommend supportive treatment during acute HBV and advocate for treatment with NAs only in patients with severe hepatitis potentially progressing towards acute liver failure[14,15]. However, there is a paucity of data regarding the effect of antiviral therapy [16] administered during acute HBV infection on the risk of progression to CHB. Furthermore, little is known regarding the impact of a low socioeconomic status (SES) and metabolic comorbidities such as obesity and diabetes mellitus (DM), which are often overrepresented among vulnerable unvaccinated populations[17-20], on the transition from acute to CHB.

In this study, we aimed to evaluate the hypothesis that the likelihood of achieving a functional cure (FC) *vs* progression from acute HBV infection to CHB in adult patients is influenced by patient background characteristics, pre-existing medical conditions and NA treatment.

MATERIALS AND METHODS

Study population

We performed a retrospective evaluation of all male and female patients, members of Clalit Health Services (CHS) in the Southern Israel district, above the age of 18 years who were diagnosed with an acute HBV infection between January 2004 to December 2022.

CHS provides medical services for a population of nearly 1 million people living in Southern Israel and has a comprehensive computerized database with records of medical history, laboratory and imaging tests. In Israel, where HBV genotype D[21,22] is the predominant genotype, a universal 3 dose vaccination program beginning within 12 hours from birth has been implemented since 1992[23] and has achieved widespread coverage.

Clinical definitions and data sources

A diagnosis of an acute HBV infection was established when a patient presented with newly positive tests for HBsAg and hepatitis B core (HBc) IgM antibody, in the absence of any prior evidence of HBV infection or immunity, including previous positivity for anti-hepatitis B surface, HBsAg, IgG or IgM anti-HBc, hepatitis B envelope antigen (HBeAg) or anti-hepatitis B envelope. Included patients were stratified into two groups: Those who demonstrated HBsAg loss with/without seroconversion within 6 months following acute HBV diagnosis (FC) and those who retained HBsAg 6 months or longer after acute HBV diagnosis (CHB). Patients lacking sufficient data to determine progression to CHB or achievement of FC following acute HBV infection, were excluded. Patients presenting with acute HBV infection while receiving immunosuppressive treatment in whom HBV reactivation could not be ruled out, were also excluded from the analysis. Patients with antibodies against hepatitis delta were excluded. Validation of the results was performed through manual review of HBsAg status in the CHS patient records. Patients with a PCR positive diagnosis of hepatitis C virus (HCV) or antibodies to HCV without a known negative PCR result were excluded. Patients with a diagnosis of human immunodeficiency virus were also excluded.

Clinical parameters including demographic characteristics, diagnostic information and laboratory data were collected if obtained within 1 month of the acute HBV infection. For laboratory markers with multiple measurements surrounding the acute event, peak values were selected to appropriately represent severity of disease. SES was assigned according to the Central Bureau of Statistics index, based on each patient's residential zip code. DM was defined based on an HgbA1C score of at least 6.5% or use of anti-glycemic medications. Dyslipidemia and hypertension were identified based on documented diagnoses in the medical record or the use of corresponding pharmacologic therapies. Detection of anti-HBc and HBsAg was performed with the Alinity system from Abbot, Illinois, United States. HBV DNA was quantified *via* Real-Time Qualitative PCR assay, on the GeneXpert system (Cepheid, California, United States). HBV DNA testing was performed only in patients who provided a second blood sample following a new diagnosis of acute HBV. Anti-HDV antibodies were detected using the LIAISON® XL MUREX Anti-HDV (DiaSorin, Saluggia, Italy). Data were extracted using the Clalit Research Data Sharing Platform powered by MDClone.

Study outcomes

The primary clinical outcome evaluated was HBsAg positivity following 6 months of the acute HBV infection. Secondary outcomes included comparisons of clinical and demographic characteristics between patients who achieved FC and those who progressed to CHB infection.

Ethical statement

The study was approved by the institutional review board of Soroka University Medical Center and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. For this study of existing patient data, the Institutional Review Board of Soroka University Medical Center granted a full waiver of informed consent (Approval No. 0138-23-SOR) on September 26, 2023, under study protocol SCRC23018.

Statistical analysis

Patient characteristics were summarized using frequencies and percentages for categorical variables and means with standard deviations for continuous variables. Univariate comparisons, were performed using the χ^2 test or Fisher's exact for categorical variables appropriate, and the Student's *t* test, Welch two-sample *t*-test, or the Wilcoxon rank-sum test for continuous variables, depending on the data distribution and. A directed acyclic graph (DAG) was constructed based on clinical knowledge and prior literature, to formalize assumed causal relationships and to identify potential confounder. Odds ratios (ORs) with 95% confidence intervals (95%CIs) were calculated to quantify the strength and direction of associations. Based on DAG framework, age, sex, and SES were identified as confounders and included in a DAG-informed multivariable logistic regression model. After testing several iterations of the model, we ultimately retained SES as a continuous variable (1 = low, 3 = high) and excluded age from the final model. Variables not identified as confounders or considered downstream of the exposure were not adjusted for to avoid overadjustment bias. A two-sided P value ≤ 0.05 was considered statistically significant. All analyses were conducted using R (version 2023.09.1+494). Key packages, including survival, survminer, and gtsummary, were used for survival analysis, data visualization, and the generation of regression tables.

RESULTS

Study population and acute HBV outcome

During the study period from January 2004 to December 2022, the Soroka University Medical Center virology laboratory identified 181 patients who tested positive for HBsAg and IgM anti-HBc (Figure 1). Eighty patients were excluded due to prior positive HBV serology or missing data regarding HBV infection outcomes. A total of 78 patients were included in the final analysis. Of these, 51 patients (65.3%) achieved FC while 27 patients (34.6%) progressed to CHB. In both the CHB and FC groups the majority of patients were male (85% and 63% respectively, $P = 0.03$, Table 1). The mean age at the time of acute HBV infection was 37.7 years (range: 19.9-71.8 years) in the FC group and 42.06 years (range: 18.8-78.5 years) in the CHB group ($P = 0.3$). The majority of patients in both groups were Israeli born (63% in the FC group and 59% in the CHB group; $P = 0.4$). A significant difference in SES was observed between the groups. Patients in the CHB were significantly more likely to come from a low SES compared to those in the FC group (70% *vs* 41%, $P = 0.04$; Figure 2). DM was significantly more prevalent in the CHB group than the FC group (26% *vs* 3.9%, $P = 0.007$). No significant differences were observed between the groups with respect to ethnicity, smoking status, body mass index (BMI), diagnosis of hypertension or dyslipidemia, history of alcohol abuse or intravenous drug use.

Clinical differences between patients who achieved FC and those who progressed to CHB infection

Patients in the FC group presented with a more severe hepatitis compared with those in the CHB group (Table 2). Peak alanine aminotransferase (ALT) levels were significantly higher in the FC group than in the CHB group (2117 ± 1509 IU/mL *vs* 1103 ± 1540 IU/mL, $P = 0.01$, Figure 3). Peak aspartate aminotransferase (AST) levels were also higher in the FC group than in the CHB group, although this difference did not reach statistical significance (1378 ± 1046 IU/mL *vs* 838 ± 1166 IU/mL, $P = 0.058$). Peak total bilirubin levels and peak alkaline phosphatase levels were also significantly higher in the FC group than in the CHB group (12.0 ± 8.5 mg/dL *vs* 6.5 ± 8.6 mg/dL, $P = 0.01$ and 207 ± 105 IU/mL *vs* 159 ± 87 IU/mL, $P = 0.04$ respectively). No statistically significant differences were observed in gamma glutamyl transferase levels or markers of hepatic synthetic function (Table 2). A comparison of the mean liver enzyme levels during the acute presentation rather than the peak values, yielded similar results (Supplementary Table 1). In both the FC and CHB groups, the majority of patients were positive for HBeAg (63% *vs* 67%, $P = 0.8$). HBV DNA results were available for 25.9% of the CHB group and 58.8% of patients in the CHB group. Among patients with available PCR data, no statistically significant difference was observed in mean HBV DNA levels between the groups ($P = 0.8$). No significant differences were observed between the groups with respect to initiation of NA therapy. During the first month following the diagnosis of acute HBV infection, 7.8% of patients in the FC group and 11% of patients in the CHB group initiated NA treatment ($P = 0.4$). In both groups, entecavir was the most frequently prescribed NA. No statistically significant difference was observed in the MELD-3 scores between the CHB and the FC groups (8.19 ± 3.07 *vs* 9.34 ± 3.17, $P = 0.2$).

Factors associated with the progression to CHB infection

A pre-existing diagnosis of DM at the time of acute HBV infection was associated with a significantly increased risk of progression to chronic infection (OR = 8.57, 95%CI: 1.88-61.01). High SES was significantly associated with a reduced risk of chronic infection (OR = 0.46; 95%CI: 0.22-0.89). Male gender was associated with an increased risk of chronic infection (OR = 3.41; 95%CI: 1.11-12.97). In contrast, age and BMI were not significantly associated with progression to chronic infection (Table 3). Initiation of NA therapy near the onset of acute HBV infection was not significantly associated with progression to chronic infection (OR = 1.47, 95%CI: 0.27-7.19, $P = 0.63$). However, the analysis was substantially underpowered because only a very small number of patients in either group received antiviral therapy limiting any meaningful inference regarding treatment effect. To formalize assumed causal relationships and to identify potential confounders, a DAG-informed multivariable logistic regression analysis was performed. However, due to the relatively small sample size, the multivariable model did not achieve sufficient precision or reliability (Supplementary Figure 1). In an additional sensitivity analysis, excluding patients with either DM or low SES did not materially alter the association between these factors and the risk of CHB infection (Supplementary Table 2). However, male sex was no longer an independently associated with the outcome after the exclusion of patients with low SES. A subgroup analysis of only the CHB and FC patients with HBeAg positivity (62.8% of the cohort) revealed results consistent with the full cohort analysis (Supplementary Figure 2 and Supplementary Table 3).

DISCUSSION

In this study, we retrospectively evaluated a cohort of patients diagnosed with acute HBV in a setting with an established vaccination program, with the aim of identifying factors associated with the progression to CHB infection. In comparison with historical cohorts[6], our study identified a notably high proportion of adult patients (34%) who remained HBsAg positive and progressed to CHB infection according to current disease definitions. DM was identified as a significant risk factor for progression to CHB infection with an OR of 8.57. Conversely, higher SES was associated with a significantly reduced risk of progression to CHB with an OR of 0.46. Despite the small sample size and wide confidence interval, these findings suggest that in a setting with widespread vaccine availability, unvaccinated individuals from socioeconomically disadvantaged and medically vulnerable populations may be at increased risk of persistent HBsAg positivity. Additionally, patients who achieved FC exhibited more severe hepatitis, as reflected by substantially higher ALT and AST levels, compared with patients who remained HBsAg positive.

Table 1 Patient characteristics, *n* (%) or mean ± SD

	Total (*n* = 78)	Chronic HBV (*n* = 27)	Functional cure (*n* = 51)	*P* value[1]
Male	55 (71)	23 (85)	32 (63)	0.03
Ethnicity				0.3
Arab	20 (26)	9(33)	11 (22)	
Jewish	58 (74)	18 (67)	40 (78)	
Age (years)	39.2 ± 15.5	42.0 ± 17.2	37.7 ± 14.6	0.3
Region of birth				0.4
Israel	48 (62)	16 (59)	32 (63)	
Africa	8 (10)	5 (19)	3(5.9)	
Middle East	7 (9)	3 (11)	4 (7.8)	
Russia/Former Union of Soviet Socialist Republics	8 (10)	1 (3.7)	7 (14)	
Europe	2 (2.6)	1 (3.7)	1 (2)	
Other	5 (6.4)	1 (3.7)	4 (7.8)	
History of alcohol use	7 (9)	2 (7.4)	5 (9.8)	> 0.9
Active smoking	8 (16)	2 (15)	6 (17)	0.3
IV drug use	5 (6.4)	2 (7.4)	3 (5.9)	> 0.9
BMI (kg/m^2)	25.4 ± 7.1	26.7 ± 6.1	24.7 ± 7.5	0.2
Diabetes mellitus	9 (12)	7 (26)	2 (3.9)	0.007
Hypertension	17 (22)	7 (26)	10 (20)	0.5
Dyslipidemia	5 (6.4)	3 (11)	2 (3.9)	0.3
Anti-HBs positive	30 (48)	8 (33)	22 (58)	0.059
HBeAg positive	49 (64)	18 (67)	31 (62)	0.7
HBV DNA (IU/mL)[2]	$1.48 \times 10^6 \pm 3.5 \times 10^6$	$1.25 \times 10^6 \pm 9.2 \times 10^5$	$1.54 \times 10^6 \pm 3.9 \times 10^6$	0.8

[1]Pearson's χ^2 test; Wilcoxon rank sum test.
[2]Data available for only 25.9% and 58.8% of patients in the chronic hepatitis B virus and functional cure groups respectively.
HBV: Hepatitis B virus; BMI: Body mass index; HBeAg: Hepatitis B envelope antigen; Anti-HBs: Anti-hepatitis B surface.

Although complete eradication of HBV is not achievable due to the persistence of covalently closed circular DNA and integrated viral DNA within hepatocytes[24], FC is typically achieved in more than 95% of adults following acute infection[14,15]. In contrast, we observed an approximately sixfold higher rate of HBsAg persistence at 6 months compared with historically reported rates. This unexpectedly high rate of HBsAg persistence, was confirmed by extensive manual review to exclude misclassifications or data inaccuracies. In addition, a subgroup analysis of only the HBeAg positive patients of our cohort produced comparable findings. Notably, A similarly high rate of HBsAg persistence was recently reported in a North American cohort of adults with acute HBV infection, prompting the authors to question the adequacy of current definitions of acute HBV resolution[17]. Together, these observations suggest that contemporary host- and population-level factors may meaningfully influence the immune response to acute HBV infection and warrant focused investigation.

DM represents a biologically plausible host-level factor contributing to impaired viral clearance following acute HBV infection. DM has been associated with dysfunction of both adaptive and innate immune responses, including impaired T-cell, B-cell, Macrophage and Natural killer cell function[25-28], as well as with worse clinical outcomes in CHB, including cirrhosis and HCC[29,30]. Consistent with these observations a recent large retrospective study from Israel demonstrated a higher prevalence of DM among patients with CHB infection[18]. However, data specifically addressing the impact of DM on HBsAg clearance following acute HBV infection remain limited. In this context, our findings suggest that diabetes-associated immune dysfunction may impair effective viral control during acute infection, thereby increasing the likelihood of HBsAg persistence. This hypothesis is further supported by the observation that patients achieving FC demonstrated higher ALT levels than those progressing to chronic infection, consistent with a more vigorous immune response.

Our findings also suggest that low SES may be an independent significant risk factor for HBsAg persistence. While CHB is known to disproportionately affect socioeconomically disadvantaged populations[19,20], the role of SES in viral clearance following acute infection has not been previously described. Potential explanations include the higher preva-

Table 2 Clinical presentation of patients with chronic hepatitis B virus and patients who achieved functional cure, *n* (%) or mean ± SD

	Total (*n* = 78)	Chronic HBV (*n* = 27)	Functional cure (*n* = 51)	*P* value[1]
ALT (IU/mL)	1770 ± 1585	1103 ± 1540	2117 ± 1509	0.01
AST (IU/mL)	1193 ± 1111	838 ± 1166	1378 ± 1046	0.058
Total bilirubin (mg/dL)	10.2 ± 8.87	6.52 ± 8.62	12.0 ± 8.5	0.01
Direct bilirubin (mg/dL)	4.31 ± 3.98	4.26 ± 5.89	6.87 ± 4.99	0.07
Alkaline phosphatase (IU/mL)	190 ± 101	159 ± 87	207 ± 105	0.04
GGT (IU/mL)	219 ± 144	175 ± 128	236 ± 148	0.12
Albumin (g/dL)	3.78 ± 0.57	3.86 ± 0.54	3.74 ± 0.59	0.4
INR	1.39 ± 0.85	1.24 ± 0.42	1.46 ± 1.0	0.2
Creatinine (mg/dL)	0.81 ± 0.31	0.89 ± 0.43	0.77 ± 0.23	0.2
Hemoglobin (g/dL)	13.78 ± 1.88	14.26 ± 1.82	13.53 ± 1.89	0.1
Platelets (× 10^3/micL)	230 ± 99	216 ± 121	238 ± 85	0.4
Na (mEq/L)	138.5 ± 3.0	138.7 ± 3.1	138.4 ± 2.9	0.7
MELD-3	8.97 ± 3.16	8.19 ± 3.07	9.34 ± 3.17	0.2
NA treatment				0.4
Entecavir	6 (7.6)	2 (7.4)	4 (7.8)	
Lamivudine	1 (1)	1 (3.4)	0 (0)	
No treatment	71 (91)	24 (89)	47 (92)	

[1]Welch two sample *t*-test.

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma glutamyl transferase; INR: International normalized ratio; NA: Nucleos(t)ide analogue; HBV: Hepatitis B virus.

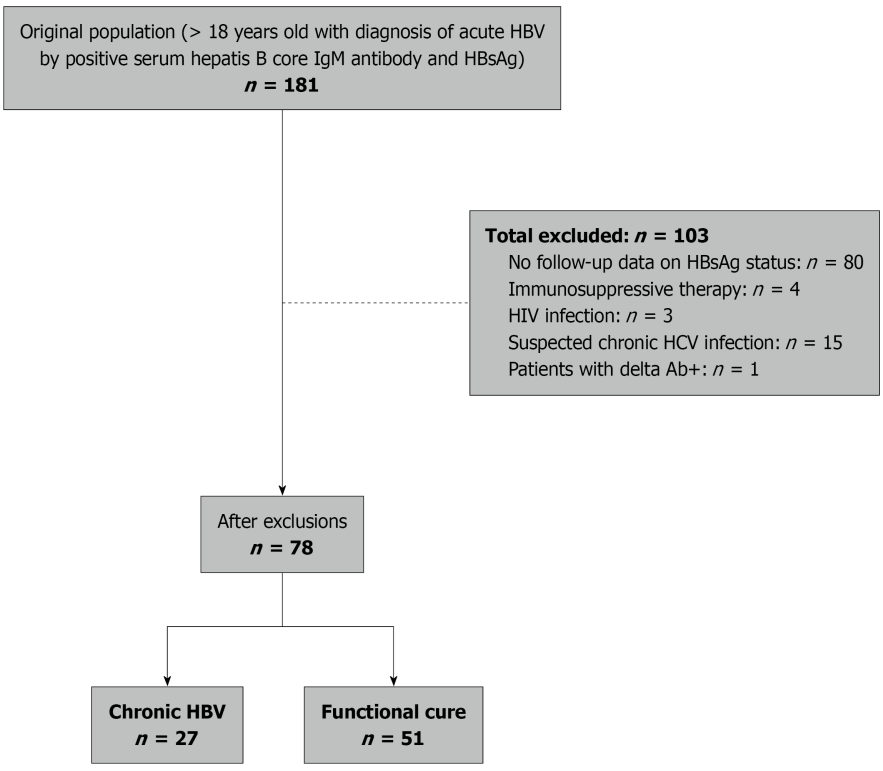


Figure 1 Study population flow chart. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus.

Table 3 Univariate analysis odds ratio analysis of factors leading to hepatitis B surface antigen persistence

Predictor	Odds ratio (confidence interval)	*P* value
Age	1.02 (0.99-1.05)	0.243
Gender	3.41 (1.11-12.97)	0.0458
BMI	1.04 (0.97-1.12)	0.264
Diabetes	8.57 (1.88-61.01)	0.0109
SES (high)	0.46 (0.22-0.89)	0.0289

BMI: Body mass index; SES: Socioeconomic status.

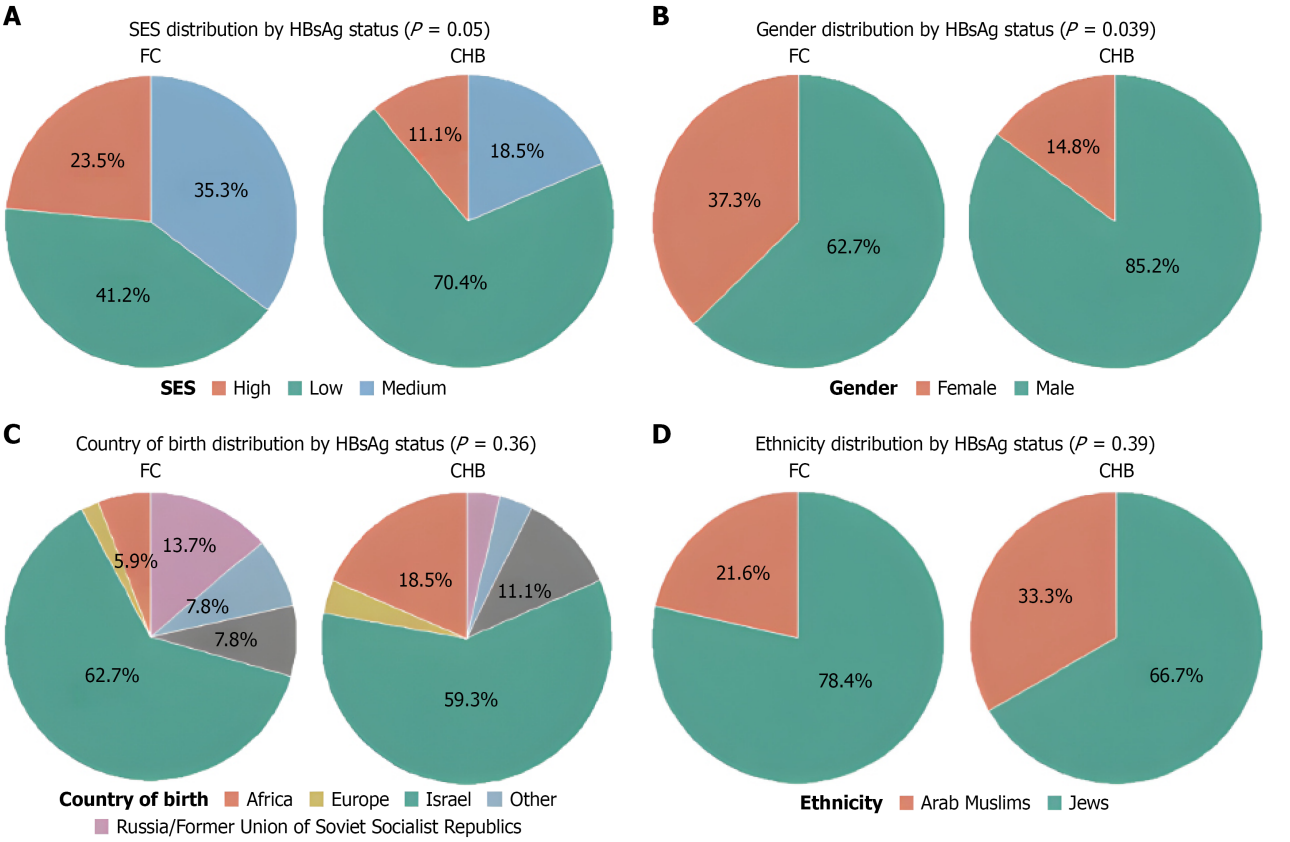


Figure 2 Distribution of patients within the functional cure and chronic hepatitis B virus groups according to socioeconomic status, country of birth, gender and ethnicity. A: Socioeconomic status distribution by hepatitis B surface antigen (HBsAg) status; B: Gender distribution by HBsAg status; C: Country of birth distribution by HBsAg status; D: Ethnicity distribution by HBsAg status. HBsAg: Hepatitis B surface antigen; SES: Socioeconomic status; FC: Functional cure; CHB: Chronic hepatitis B virus.

lence of comorbidities such as obesity[31], DM[32] and malnutrition[33] in low-SES populations, which may collectively contribute to impaired immune function, as well as emerging evidence linking gut microbiota composition to immune-mediated HBV clearance. Differences in gut microbiome composition across socioeconomic strata may theoretically partially contribute at least in part to the observed disparities and warrant further investigation[34-36]. Finally, we cannot rule out the possibility of residual confounding arising from differences in health care access health literacy, or other unmeasured socioeconomic factors that may have contributed to this association.

The principal strengths of this study include a rigorously validated cohort defined by serologic criteria, comprehensive longitudinal follow-up and access to unified electronic medical records capturing SES, comorbidities, laboratory data, and antiviral therapy. As the sole tertiary referral center in Southern Israel, our institution provides centralized care for acute HBV minimizing lost to follow up and ensuring uniform ascertainment of clinical outcomes.

Acute HBV infection remains uncommon in adults and the cohort size is comparable to contemporary studies[17]. However, key limitations of this study include its retrospective design and modest sample size, which limited statistical power particularly for evaluating the impact of NA therapy during the acute phase, and contributed to wide confidence intervals observed for key associations, including DM and SES. Additionally, although multivariable analysis was per-

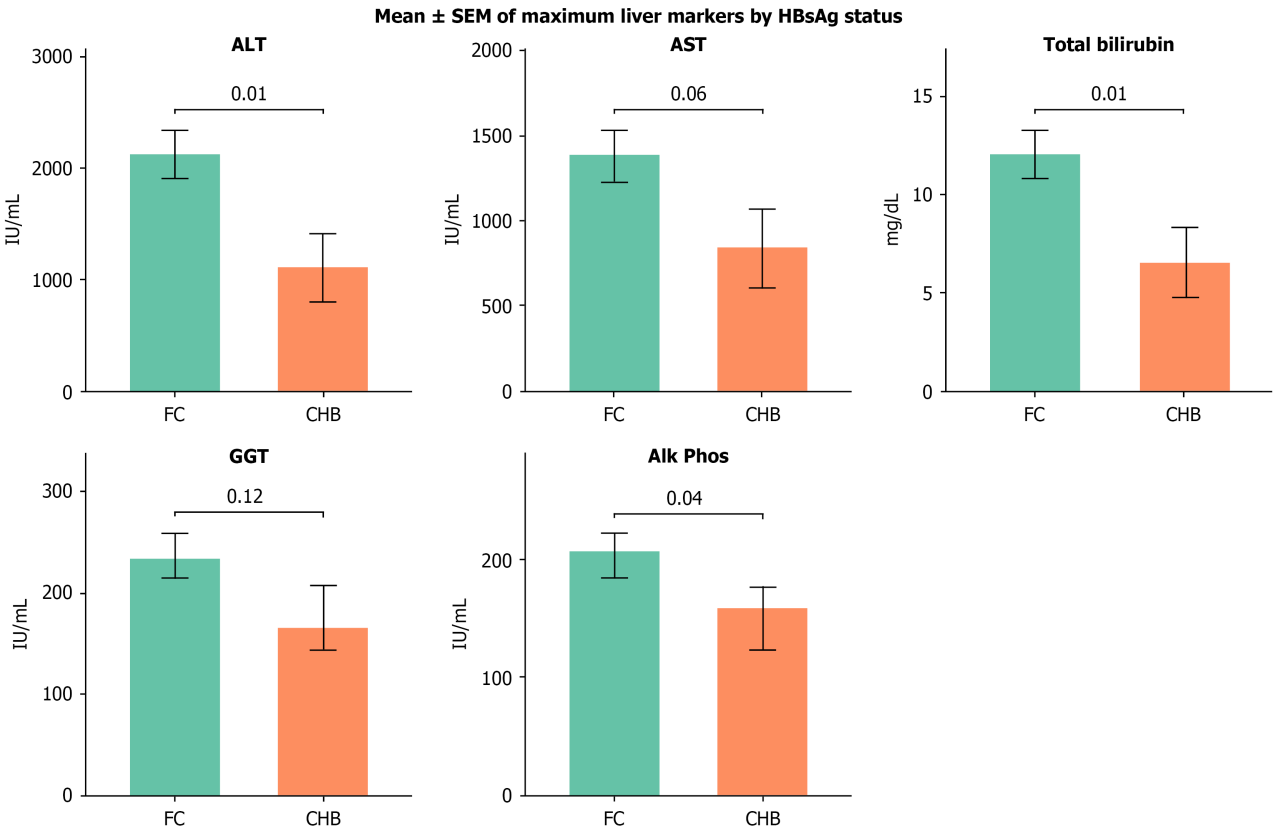


Figure 3 Comparison of liver enzymes and total bilirubin during acute hepatitis B virus presentation in patients who achieved functional cure and patients with chronic hepatitis B virus. HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma glutamyl transferase; Alk Phos: Alkaline phosphatase; FC: Functional cure; CHB: Chronic hepatitis B virus.

formed and demonstrated similar directional trends, the stability of these estimates was limited, further underscoring the need for larger, adequately powered cohorts to more precisely define risk relationship and mitigate residual confounding. Residual confounding cannot be excluded and the absence of data on HBV genotype (presumed to be predominantly genotype D), escape mutants, viral kinetics, transmission routes and interval between symptom onset and clinical presentation further limits mechanistic interpretation of our findings. In addition, despite the manual validation of all acute HBV cases included in our study, the possibility that some cases represented unrecognized HBV reactivation rather than true acute HBV infection cannot be completely excluded. Finally, the single center design and the region-specific characteristics of the study population may limit the generalizability of our findings.

CONCLUSION

This study demonstrates an unexpectedly high rate of progression from acute to CHB infection in adults, with DM and low SES emerging as significant risk factors for HBsAg persistence. These findings highlight the need for prospective studies examining metabolic, socioeconomic, and host-microbiome determinants of immune response during acute HBV infection and underscore the ongoing importance of comprehensive HBV vaccination strategies, to prevent chronic disease particularly in vulnerable populations.

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REFERENCES

1 **Yardeni D**, Ghany MG. Review article: hepatitis B-current and emerging therapies. *Aliment Pharmacol Ther* 2022; **55**: 805-819 [*RCA*] [PMID: 35224760 DOI: 10.1111/apt.16828] [FullText]

2 **The CDA Foundation**. Polaris Database Query. [cited 8 February 2026]. Available from: https://cdafound.org/polaris/database-query/

3 **Lozano R**, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [*RCA*] [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0] [FullText]

4 **Dekker SE**, Green EW, Ahn J. Treatment and Prevention of Acute Hepatitis B Virus. *Clin Liver Dis* 2021; **25**: 711-724 [*RCA*] [PMID: 34593149 DOI: 10.1016/j.cld.2021.06.002] [FullText]

5 **Hsu YC**, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. *Nat Rev Gastroenterol Hepatol* 2023; **20**: 524-537 [*RCA*] [PMID: 37024566 DOI: 10.1038/s41575-023-00760-9] [FullText]

6 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [*RCA*] [PMID: 15014185 DOI: 10.1056/NEJMra031087] [FullText]

7 **Suzuki Y**, Kobayashi M, Ikeda K, Suzuki F, Arfase Y, Akuta N, Hosaka T, Saitoh S, Kobayashi M, Someya T, Matsuda M, Sato J, Watabiki S, Miyakawa Y, Kumada H. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. *J Med Virol* 2005; **76**: 33-39 [*RCA*] [PMID: 15779048 DOI: 10.1002/jmv.20320] [FullText]

8 **Jose-Abrego A**, Roman S, Laguna-Meraz S, Panduro A. Host and HBV Interactions and Their Potential Impact on Clinical Outcomes. *Pathogens* 2023; **12**: 1146 [*RCA*] [PMID: 37764954 DOI: 10.3390/pathogens12091146] [FullText] [Full Text(PDF)]

9 **Ferrari C**, Boni C, Rossi M, Vecchi A, Barili V, Laccabue D, Fisicaro P, Missale G. T cell regulation in HBV-related chronic liver disease. *J Hepatol* 2017; **66**: 1096-1098 [*RCA*] [PMID: 28189261 DOI: 10.1016/j.jhep.2016.10.002] [FullText]

10 **Burton AR**, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, Swadling L, Alberts E, Davidson BR, Kennedy PT, Gill US, Mauri C, Blair PA, Pelletier N, Maini MK. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. *J Clin Invest* 2018; **128**: 4588-4603 [*RCA*] [PMID: 30091725 DOI: 10.1172/JCI121960] [FullText]

11 **Ben-Ari Z**, Mor E, Papo O, Kfir B, Sulkes J, Tambur AR, Tur-Kaspa R, Klein T. Cytokine gene polymorphisms in patients infected with hepatitis B virus. *Am J Gastroenterol* 2003; **98**: 144-150 [*RCA*] [PMID: 12526950 DOI: 10.1111/j.1572-0241.2003.07179.x] [FullText]

12 **Thio CL**, Mosbruger TL, Kaslow RA, Karp CL, Strathdee SA, Vlahov D, O'Brien SJ, Astemborski J, Thomas DL. Cytotoxic T-lymphocyte antigen 4 gene and recovery from hepatitis B virus infection. *J Virol* 2004; **78**: 11258-11262 [*RCA*] [PMID: 15452244 DOI: 10.1128/JVI.78.20.11258-11262.2004] [FullText]

13 **Godkin A**, Davenport M, Hill AV. Molecular analysis of HLA class II associations with hepatitis B virus clearance and vaccine nonresponsiveness. *Hepatology* 2005; **41**: 1383-1390 [*RCA*] [PMID: 15915462 DOI: 10.1002/hep.20716] [FullText]

14 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [*RCA*] [PMID: 29405329 DOI: 10.1002/hep.29800] [FullText] [Full Text(PDF)]

15 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2025; **83**: 502-583 [*RCA*] [PMID: 40348683 DOI: 10.1016/j.jhep.2025.03.018] [FullText]

16 **Henriquez-Camacho C**, Hijas-Gomez AI, Risco Risco C, Ruiz Lapuente MA, Escudero-Sanchez R, Cuerda VM. Lamivudine and Entecavir for Acute Hepatitis B: A Systematic Review and Meta-Analysis. *Viruses* 2023; **15**: 2241 [*RCA*] [PMID: 38005918 DOI: 10.3390/v15112241] [FullText] [Full Text(PDF)]

17 **Sterling RK**, Wahed AS, Cloherty G, Hoofnagle JH, Lee WM; Hepatitis B Research Network Investigators. Acute Hepatitis B Virus Infection in North American Adults. *Clin Gastroenterol Hepatol* 2023; **21**: 1881-1892.e4 [*RCA*] [PMID: 36116752 DOI: 10.1016/j.cgh.2022.09.004] [FullText]

18 **Abu Baker F**, Zeina AR, Natour RT, Mouch SA, Kopelman Y, Shibolet O, Israel A. Prevalence and Risk Factors of Type 2 Diabetes Mellitus Among Hepatitis B Virus Patients: A Large Retrospective Cohort Study. *Int J Med Sci* 2025; **22**: 716-722 [*RCA*] [PMID: 39898253 DOI: 10.7150/ijms.104839] [FullText] [Full Text(PDF)]

19 **Lee C**, Emeto TI, Walsh N. Prevalence of hepatitis B virus amongst refugees, asylum seekers and internally displaced persons in low- and middle-income countries: A systematic review. *J Viral Hepat* 2023; **30**: 4-18 [*RCA*] [PMID: 36357172 DOI: 10.1111/jvh.13770] [FullText] [Full Text(PDF)]

20 **Zeng DY**, Li JM, Lin S, Dong X, You J, Xing QQ, Ren YD, Chen WM, Cai YY, Fang K, Hong MZ, Zhu Y, Pan JS. Global burden of acute viral hepatitis and its association with socioeconomic development status, 1990-2019. *J Hepatol* 2021; **75**: 547-556 [*RCA*] [PMID: 33961940 DOI: 10.1016/j.jhep.2021.04.035] [FullText]

21 **Sant'Anna TB**, Araujo NM. Hepatitis B Virus Genotype D: An Overview of Molecular Epidemiology, Evolutionary History, and Clinical Characteristics. *Microorganisms* 2023; **11**: 1101 [*RCA*] [PMID: 37317074 DOI: 10.3390/microorganisms11051101] [FullText] [Full Text (PDF)]

22 **Shirazi R**, Ram D, Rakovsky A, Bucris E, Gozlan Y, Lustig Y, Shaked-Mishan P, Picard O, Shemer-Avni Y, Ben-Zvi H, Halutz O, Lurie Y, Veizman E, Carlebach M, Braun M, Naftaly MC, Shlomai A, Safadi R, Mendelson E, Sklan EH, Ben-Ari Z, Mor O. Characterization of hepatitis B and delta coinfection in Israel. *BMC Infect Dis* 2018; **18**: 97 [*RCA*] [PMID: 29486716 DOI: 10.1186/s12879-018-3008-x] [FullText]

[Full Text(PDF)]

23 **Israel Ministry of Health**. Hepatitis B vaccine schedule. [cited 24 June 2026]. Available from: https://me.health.gov.il/en/parenting/raising-children/immunization-schedule/vaccines-up-to-age-six/hepatitis-b-vaccination-

24 **Bertoletti A**. The immune response in chronic HBV infection. *J Viral Hepat* 2024; **31** Suppl 2: 43-55 [*RCA*] [PMID: 38845402 DOI: 10.1111/jvh.13962] [FullText]

25 **Berbudi A**, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 Diabetes and its Impact on the Immune System. *Curr Diabetes Rev* 2020; **16**: 442-449 [*RCA*] [PMID: 31657690 DOI: 10.2174/1573399815666191024085838] [FullText] [Full Text(PDF)]

26 **Saadh MJ**, Allela OQB, Kareem RA, Kyada A, Malathi H, Nathiya D, Bhanot D, Sameer HN, Hamad AK, Athab ZH, Adil M. Immune cell dysfunction: A critical player in development of diabetes complications. *Curr Res Transl Med* 2025; **73**: 103510 [*RCA*] [PMID: 40339429 DOI: 10.1016/j.retram.2025.103510] [FullText]

27 **Sakowicz-Burkiewicz M**, Kocbuch K, Grden M, Maciejewska I, Szutowicz A, Pawelczyk T. High glucose concentration impairs ATP outflow and immunoglobulin production by human peripheral B lymphocytes: involvement of P2X7 receptor. *Immunobiology* 2013; **218**: 591-601 [*RCA*] [PMID: 22883563 DOI: 10.1016/j.imbio.2012.07.010] [FullText]

28 **Berrou J**, Fougeray S, Venot M, Chardiny V, Gautier JF, Dulphy N, Toubert A, Peraldi MN. Natural killer cell function, an important target for infection and tumor protection, is impaired in type 2 diabetes. *PLoS One* 2013; **8**: e62418 [*RCA*] [PMID: 23638076 DOI: 10.1371/journal.pone.0062418] [FullText] [Full Text(PDF)]

29 **Xu C**, Chen J, Zhang PA. Relationship Between Diabetes Mellitus and Cirrhosis Risk in Chronic Hepatitis B Patients in Wuhan, China. *Med Sci Monit* 2019; **25**: 8112-8119 [*RCA*] [PMID: 31661471 DOI: 10.12659/MSM.917000] [FullText]

30 **Tan Y**, Wei S, Zhang W, Yang J, Yang J, Yan L. Type 2 diabetes mellitus increases the risk of hepatocellular carcinoma in subjects with chronic hepatitis B virus infection: a meta-analysis and systematic review. *Cancer Manag Res* 2019; **11**: 705-713 [*RCA*] [PMID: 30679924 DOI: 10.2147/CMAR.S188238] [FullText] [Full Text(PDF)]

31 **Claassen MA**, Klein O, Bratanova B, Claes N, Corneille O. A systematic review of psychosocial explanations for the relationship between socioeconomic status and body mass index. *Appetite* 2019; **132**: 208-221 [*RCA*] [PMID: 30217475 DOI: 10.1016/j.appet.2018.07.017] [Full Text]

32 **Volaco A**, Cavalcanti AM, Filho RP, Précoma DB. Socioeconomic Status: The Missing Link Between Obesity and Diabetes Mellitus? *Curr Diabetes Rev* 2018; **14**: 321-326 [*RCA*] [PMID: 28637406 DOI: 10.2174/1573399813666170621123227] [FullText]

33 **Popkin BM**, Corvalan C, Grummer-Strawn LM. Dynamics of the double burden of malnutrition and the changing nutrition reality. *Lancet* 2020; **395**: 65-74 [*RCA*] [PMID: 31852602 DOI: 10.1016/S0140-6736(19)32497-3] [FullText]

34 **Kwak S**, Usyk M, Beggs D, Choi H, Ahdoot D, Wu F, Maceda L, Li H, Im EO, Han HR, Lee E, Wu AH, Hayes RB, Ahn J. Sociobiome - Individual and neighborhood socioeconomic status influence the gut microbiome in a multi-ethnic population in the US. *NPJ Biofilms Microbiomes* 2024; **10**: 19 [*RCA*] [PMID: 38467678 DOI: 10.1038/s41522-024-00491-y] [FullText] [Full Text(PDF)]

35 **Chou HH**, Chien WH, Wu LL, Cheng CH, Chung CH, Horng JH, Ni YH, Tseng HT, Wu D, Lu X, Wang HY, Chen PJ, Chen DS. Age-related immune clearance of hepatitis B virus infection requires the establishment of gut microbiota. *Proc Natl Acad Sci U S A* 2015; **112**: 2175-2180 [*RCA*] [PMID: 25646429 DOI: 10.1073/pnas.1424775112] [FullText] [Full Text(PDF)]

36 **Wang J**, Zhou X, Li X, Guo W, Zhu Q, Zhu B, Lu Y, Zheng X, Yang D, Wang B. Fecal Microbiota Transplantation Alters the Outcome of Hepatitis B Virus Infection in Mice. *Front Cell Infect Microbiol* 2022; **12**: 844132 [*RCA*] [PMID: 35601099 DOI: 10.3389/fcimb.2022.844132] [FullText] [Full Text(PDF)]

FOOTNOTES

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Country of origin: Israel

Author contributions: Yardeni D and Etzion O contributed to the conception and design of the study as well as writing of manuscript and approval of final draft; Gershbaum Tobin J and Itkowitz B contributed to the data analysis as well as editing, writing and review of the manuscript and performed the formal statistical analysis; Lipnizkiy I, Abu Juma'a A, Abu-Freha N, Keren-Naus A, Turgeman M and Nevo-Shor A provided resources and performed a review and editing of the manuscript; Gish RG performed a revision of the manuscript and approved the final draft.

AI contribution statement: AI tools were not used during the preparation of this study.

Institutional review board statement: The study was approved by the Institutional Review Board of Soroka University Medical Center and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements.

Informed consent statement: For this study of existing patient data, the Institutional Review Board of Soroka University Medical Center granted a full waiver of informed consent (Approval No. 0138-23-SOR) on September 26, 2023, under study protocol SCRC23018.

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Data sharing statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Retrospective Study

Age-male-albumin-bilirubin-platelet score stratifies hepatocellular carcinoma risk after sustained virological response in hepatitis C-related compensated advanced chronic liver disease

Sirajuk Khongviwatsathien, Tawesak Tanwandee

Peer review: Externally peer reviewed.
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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) remains an important long-term complication after sustained virological response (SVR) in patients with hepatitis C virus (HCV)-related compensated advanced chronic liver disease (cACLD). Current guidelines generally recommend lifelong semiannual surveillance for patients with advanced fibrosis or cirrhosis; however, the residual risk of HCC after viral eradication is heterogeneous. We hypothesized that simple noninvasive scores could identify a very-low-risk subgroup suitable for individualized surveillance intensity.

AIM

To compare the performance of noninvasive scores for predicting HCC after SVR and to identify a very-low-risk subgroup.

METHODS

This single-center retrospective cohort study included 571 adults with HCV-related cACLD who achieved SVR at 12 weeks after treatment completion following interferon-free sofosbuvir-based direct-acting antiviral therapy between 2013 and 2023. The fibrosis-4 index, aspartate aminotransferase-to-platelet ratio index, albumin-bilirubin score, and age-male-albumin-bilirubin-platelet (aMAP) score were calculated. HCC risk was evaluated using Kaplan-Meier analysis, Cox proportional hazards regression, and time-dependent receiver operating characteristic curves at 1 year, 3 years, 5 years, and 8 years.

RESULTS

During a median follow-up of 4.59 years, 78 patients (13.7%) developed *de novo* HCC [annual incidence rate: 3.12%; 95% confidence interval (CI): 2.50-3.80]. Compared with those who did not develop HCC, patients who developed HCC had higher baseline liver stiffness, higher prevalence of diabetes mellitus and esophageal varices, lower platelet counts and albumin levels, and higher bilirubin levels. Among the evaluated scores, the aMAP score showed the highest discriminatory performance, with an 8-year area under the receiver operating characteristic curve of 0.74 (95%CI: 0.67-0.80). An exploratory aMAP threshold of < 55 identified 18.6% of patients with no observed HCC events, whereas patients with an aMAP score ≥ 60 had the highest annual HCC incidence of 6.42% per year.

CONCLUSION

The aMAP score effectively stratifies the risk of HCC after SVR and may support individualized surveillance intensity in patients with HCV-related cACLD. However, the exploratory aMAP threshold requires external validation.

Key Words: Hepatitis C virus; Compensated advanced chronic liver disease; Direct-acting antiviral therapy; Sustained virological response; Hepatocellular carcinoma; Age-male-albumin-bilirubin-platelet score; Risk stratification; Surveillance de-escalation

Core Tip: This retrospective cohort study evaluated the age-male-albumin-bilirubin-platelet (aMAP) score for predicting hepatocellular carcinoma (HCC) risk after sustained virological response in patients with hepatitis C virus-related compensated advanced chronic liver disease. Among 571 patients followed for a median of 4.59 years, 78 developed HCC. The aMAP score outperformed other noninvasive scoring systems, and an exploratory threshold of < 55 identified 18.6% of patients with no observed HCC events. These findings suggest that the aMAP score may support individualized surveillance intensity; however, the threshold requires external validation before routine clinical implementation.

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection remains a major global health concern, affecting an estimated 58 million individuals worldwide and causing approximately 290000 deaths annually, predominantly from cirrhosis and hepatocellular carcinoma (HCC)[1]. HCV-related cirrhosis contributes substantially to the global HCC burden, particularly in Western Europe, the Mediterranean/North Africa, Japan, and the Asia-Pacific region[2-5]. Cirrhosis and clinically significant portal hypertension together provide the biological basis for hepatocarcinogenesis[4].

The therapeutic landscape of HCV infection has been transformed by interferon-free, all-oral direct-acting antiviral (DAA) regimens. Historically, pegylated interferon plus ribavirin achieved only modest sustained virological response (SVR) rates and was limited by significant adverse effects[6]. In contrast, sofosbuvir-based DAA regimens routinely achieve SVR rates exceeding 95% across HCV genotypes, including in patients with advanced fibrosis or compensated cirrhosis, and are endorsed as first-line therapy by major liver societies[7,8]. Real-world studies from diverse settings have further confirmed their excellent efficacy and tolerability[9].

SVR is associated with improvements in liver histology and function. Prospective studies have demonstrated significant regression of fibrosis and lower incidence of HCC among patients with advanced fibrosis (stage F3) after DAA therapy[10]. Similarly, real-world cohorts have demonstrated significant reductions in liver stiffness after viral eradication, even in patients with cirrhosis[11]. Observational longitudinal studies—including the large European cohort by van der Meer *et al*[12]—have shown that viral eradication in patients with advanced fibrosis markedly reduces the risk of liver-related complications and mortality compared with individuals who fail to achieve SVR. Furthermore, large real-world studies of patients with advanced liver disease have demonstrated that achieving SVR with DAA therapy significantly lowers both all-cause and liver-related mortality[13].

Nevertheless, patients with advanced fibrosis or cirrhosis remain at risk of developing *de novo* HCC after achieving SVR. Multiple cohorts in the DAA era have consistently demonstrated that SVR reduces—but does not eliminate—HCC risk[14-20]. In a cohort of 636 patients with cirrhosis treated with DAAs, D'Ambrosio *et al*[14] reported a 5-year cumulative HCC incidence of 7.7%. Similarly, Caviglia *et al*[21] reported an HCC incidence of 9.9% over a median follow-up of approximately 45 months in 575 patients with HCV-related cirrhosis. Additional large observational studies have confirmed persistent HCC risk even after successful viral eradication[16-20].

Recognizing this persistent risk, major international guidelines—including those from the European Association for the Study of the Liver (EASL), American Association for the Study of Liver Diseases (AASLD), and Asian Pacific Association for the Study of the Liver (APASL)—recommend lifelong semiannual ultrasound-based HCC surveillance in patients with cirrhosis or compensated advanced chronic liver disease (cACLD), irrespective of SVR status[22-24]. However, this universal strategy is resource-intensive. Recent modeling studies suggest that surveillance may be cost-effective only when the annual HCC incidence exceeds specific thresholds, prompting intense interest in individualized, risk-stratified surveillance strategies[25].

Several widely available noninvasive indices have been proposed to refine HCC risk stratification, including the fibrosis-4 (FIB-4) index[26], aspartate aminotransferase-to-platelet ratio index (APRI)[27], albumin-bilirubin (ALBI) score [28,29], and the age-male-albumin-bilirubin-platelet (aMAP) score[30]. The aMAP model, which incorporates age, sex, ALBI score, and platelet count, was validated in 17374 patients across 11 international cohorts, including patients with HCV-related cirrhosis who achieved SVR, and stratifies patients into low-, intermediate-, and high-risk groups[30]. The ALBI score has also been identified as an independent predictor of *de novo* HCC after SVR in several studies[21,31].

Given the substantial burden of lifelong HCC surveillance, identifying a truly very-low-risk subgroup among patients who achieve SVR has become a priority. However, evidence supporting surveillance de-escalation in real-world populations with cACLD remains limited. Therefore, this study aimed to: (1) Determine the long-term incidence of *de novo* HCC in patients with HCV-related cACLD after SVR; (2) Compare the predictive performance of the FIB-4 index, APRI, ALBI score, and aMAP score over up to 8 years of follow-up; and (3) Evaluate whether these noninvasive scores can identify a very-low-risk subgroup that may support future surveillance de-escalation after external validation.

MATERIALS AND METHODS

Study design and population

This retrospective cohort study was conducted at Siriraj Hospital, a tertiary referral center in Thailand. Consecutive adult patients with HCV-related cACLD who initiated sofosbuvir-based DAA therapy between 2013 and 2023 were identified. Eligible patients had confirmed cACLD, defined by the Baveno VII consensus as histological stage F3/F4 fibrosis, vibration-controlled transient elastography (VCTE) ≥ 10 kPa, or clinical evidence of portal hypertension, and achieved SVR at 12 weeks after treatment completion (SVR12)[32]. This observational, longitudinal follow-up of real-world patients with HCV-related advanced fibrosis or cirrhosis after DAA therapy is consistent with previous studies evaluating HCC risk after SVR[14,15,18,19,21].

Ethical considerations

This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (Certificate of Approval No. Si 034/2024). The study was conducted in accordance with the principles of the Declaration of Helsinki. Owing to the retrospective nature of the study, the requirement for obtaining written informed consent was waived by the Institutional Review Board.

Study population, and inclusion and exclusion criteria

Eligible patients were required to meet all of the following criteria: Age ≥ 18 years; chronic HCV infection treated with DAA therapy with documented SVR12[7,8]; and evidence of advanced fibrosis or cirrhosis consistent with cACLD before DAA therapy, as defined by the Baveno VII consensus[32]. Briefly, patients were considered to have cACLD if they fulfilled at least one of the following criteria: Histological stage F3 or F4 fibrosis on liver biopsy; liver stiffness measurement by VCTE ≥ 10 kPa; radiological features consistent with cirrhosis or portal hypertension on ultrasonography, contrast-enhanced computed tomography, or contrast-enhanced magnetic resonance imaging; or clinical or endoscopic evidence of portal hypertension.

Radiological features were not used to diagnose histological stage F3 fibrosis but were used only to support the presence of cirrhosis or portal hypertension. Radiological features consistent with cirrhosis or portal hypertension included a nodular or irregular liver surface, morphological features of cirrhosis, splenomegaly, ascites, or portosystemic collaterals. Clinical or endoscopic evidence of portal hypertension included esophageal or gastric varices, portal hypertensive gastropathy, ascites, or other documented findings of portal hypertension. All patients were required to have a minimum follow-up of 12 months after achieving SVR.

Patients were excluded if they met any of the following criteria: (1) Incomplete or missing medical records precluding outcome assessment; (2) Known or suspected HCC before initiating DAA therapy; or (3) Failure to achieve SVR12.

Baseline data collection

Baseline demographic, clinical, and laboratory data were abstracted from electronic medical records. The collected variables included age, sex, metabolic comorbidities such as type 2 diabetes mellitus and dyslipidemia, prior HCV treatment history, routine laboratory parameters (complete blood count, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transferase, albumin, total bilirubin, and prothrombin time/international normalized ratio), and indicators of portal hypertension and hepatic functional reserve, including platelet count and the presence of esophageal varices. These variables—including markers of portal hypertension, thrombocytopenia, and impaired synthetic function—have been associated with subsequent liver-related events and *de novo* HCC in patients with HCV-related cirrhosis who achieved SVR[14,21,31,33].

Follow-up and HCC surveillance

After completion of DAA therapy, patients were followed in dedicated hepatology clinics with standardized laboratory monitoring and imaging-based HCC surveillance. Abdominal ultrasonography, with or without measurement of serum alpha-fetoprotein, was performed every 6 months in accordance with recommendations from the EASL, the AASLD, and the APASL for ongoing HCC surveillance in patients with cirrhosis or cACLD, even after achieving SVR[22-24].

Noninvasive scoring systems

Four noninvasive scoring systems were evaluated using laboratory data obtained at the SVR12 visit: APRI, FIB-4 index, ALBI, and aMAP score. The FIB-4 index[26] and APRI[27] have been widely validated for noninvasive fibrosis assessment in patients with chronic viral hepatitis, including chronic hepatitis B[34], whereas the ALBI score[28,29] and aMAP score [30] assess hepatic functional reserve and composite HCC risk, respectively. All scores were calculated using laboratory values obtained at the SVR12 visit.

Outcome and analysis

The primary outcome was the development of *de novo* HCC. The FIB-4 index, APRI, ALBI score, and aMAP score were calculated at SVR12. Kaplan-Meier survival analysis and time-dependent receiver operating characteristic curve analysis were performed to assess the predictive performance of these scoring systems for HCC at 1 year, 3 years, 5 years, and 8 years. The discriminatory performance of the aMAP score was evaluated using both the originally published cutoffs proposed by Fan *et al*[30] (< 50, 50 to < 60, and ≥ 60) and exploratory cohort-derived cutoff values identified in the present study (< 55, 55 to < 60, and ≥ 60). The exploratory cutoff of < 55 was selected because it represented the highest cutoff value that identified a subgroup with no observed *de novo* HCC during long-term follow-up while expanding the very-low-risk subgroup compared with the originally validated cutoff of < 50.

Statistical analysis

Baseline characteristics were summarized using descriptive statistics. Continuous variables were expressed as the mean ± SD for normally distributed data or as the median [interquartile range (IQR)] for nonnormally distributed data. Categorical variables were summarized as frequencies and percentages. Comparisons between the HCC and non-HCC groups were performed using the χ^2 or Fisher's exact test for categorical variables and the Student's *t*-test or Mann-Whitney *U* test for continuous variables, as appropriate.

Time-to-event analyses for the first occurrence of *de novo* HCC were performed using the Kaplan-Meier method, and differences in HCC-free survival were compared using the log-rank test. Cox proportional hazards regression analysis was performed to estimate hazard ratios and 95% confidence intervals (CIs) for the associations between baseline variables, including comorbidities, features of portal hypertension, hepatic synthetic function, and noninvasive scores, and the risk of HCC.

To evaluate the discriminatory performance of each noninvasive scoring system (APRI, FIB-4 index, ALBI, aMAP score), time-dependent receiver operating characteristic curves for HCC occurrence at 1 year, 3 years, 5 years, and 8 years of follow-up were generated, and the area under the curve (AUC) with 95%CIs was calculated. Patients were also stratified into aMAP-based risk categories using exploratory cohort-derived cutoff values (< 55, 55 to < 60, ≥ 60) and compared with the prespecified risk groups proposed by Fan *et al*[30] (< 50, 50 to < 60, ≥ 60). A two-sided *P* value < 0.05 was considered statistically significant.

All statistical analyses were performed using SPSS version 19 (IBM Corp., Armonk, NY, United States).

RESULTS

Patient selection and HCC incidence

Of 2971 patients with chronic HCV infection and evidence of advanced fibrosis or cirrhosis consistent with cACLD who were screened, 2179 were excluded because they did not receive interferon-free oral DAA therapy. The remaining 792 patients received oral DAA therapy. Before inclusion in the post-SVR outcome cohort, 204 patients were excluded: 203 who had inadequate long-term follow-up after DAA therapy owing to loss to follow-up or referral back to primary care or local hospitals, and 1 who did not achieve SVR12. Therefore, 588 patients achieved SVR12 and had adequate follow-up data for assessment of post-SVR HCC outcomes. Among these, 17 patients were excluded because of known or suspected HCC before initiation of DAA therapy. The final study population therefore comprised 571 patients (Figure 1).

As Siriraj Hospital is a tertiary referral center, some patients were referred from other hospitals for specialist assessment and antiviral treatment and were subsequently referred back to primary care or local hospitals for long-term follow-up after completion of DAA therapy. Therefore, the large number of excluded patients was mainly attributable to non-receipt of oral DAA therapy and inadequate long-term post-treatment follow-up rather than missing baseline data.

The diagnostic criteria supporting cACLD in the final analytic cohort are summarized in Table 1. Among the 571 patients included in the final analysis, liver biopsy data were available for 62 patients (10.9%), including 19 (3.3%) with histological stage F3 fibrosis and 43 (7.5%) with histological stage F4 cirrhosis. VCTE ≥ 10 kPa was documented in 293 patients (51.3%). Radiological features consistent with cirrhosis or portal hypertension were present in 518 patients (90.7%), and clinical or endoscopic evidence of portal hypertension was present in 77 patients (13.5%). These criteria were not mutually exclusive.

Table 1 Diagnostic criteria supporting compensated advanced chronic liver disease in the final study cohort

Diagnostic criterion supporting compensated advanced chronic liver disease	*n* (%)
Liver biopsy available	62 (10.9)
Histologic stage F3 fibrosis	19 (3.3)
Histologic stage F4 cirrhosis	43 (7.5)
Vibration-controlled transient elastography ≥ 10 kPa	293 (51.3)
Radiological features consistent with cirrhosis or portal hypertension	518 (90.7)
Clinical or endoscopic evidence of portal hypertension	77 (13.5)

Percentages were calculated using the final study cohort as the denominator (n = 571). Categories were not mutually exclusive and should not be summed. Histological stage F3 fibrosis and histological stage F4 cirrhosis were assigned only when liver biopsy data were available. Radiological features were used to support the presence of cirrhosis or portal hypertension and were not used to diagnose histological stage F3 fibrosis.

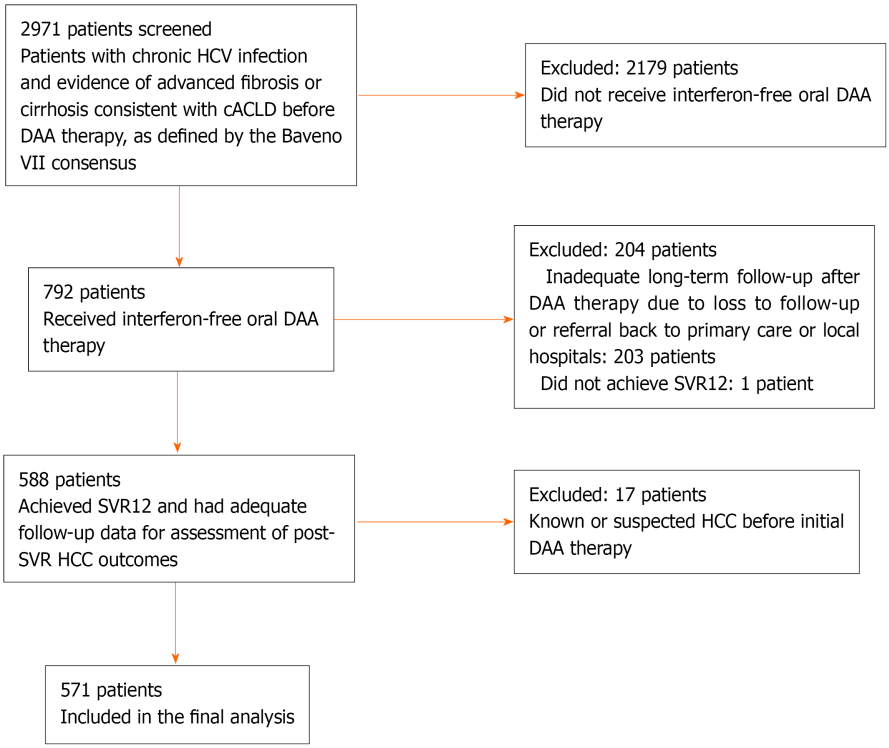


Figure 1 Flowchart of patient selection. cACLD: Compensated advanced chronic liver disease; DAA: Direct-acting antiviral; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; SVR12: Sustained virological response at 12 weeks after treatment completion; SVR: Sustained virological response.

During a median follow-up of 4.59 years, 78 of the 571 patients (13.7%) developed *de novo* HCC. This corresponded to an annual incidence rate of 3.12% (95%CI: 2.50-3.80), indicating that a clinically significant risk of HCC persisted despite achieving SVR12 in this population with HCV-related cACLD.

Baseline characteristics

The sex distribution did not differ significantly between patients who developed HCC and those who did not (P = 0.177). Patients who developed HCC tended to be older on average than those who did not, although this difference did not reach statistical significance.

Metabolic and portal hypertension-related features differed between the two groups. Patients who subsequently developed HCC had a significantly higher prevalence of type 2 diabetes mellitus and esophageal varices (both P < 0.05). These clinical features are consistent with prior studies showing that portal hypertension, impaired hepatic reserve, and metabolic comorbidities are associated with an increased risk of HCC after SVR in patients with HCV-related cirrhosis[14,21,31,33]. Baseline VCTE (E, kPa) before DAA therapy was significantly higher in the HCC group than in the non-HCC group [median (IQR), 21.3 (15.7-35.3) kPa *vs* 15.1 (10.5-23.3) kPa; P < 0.001].

All patients in the cohort received one of three standard-of-care sofosbuvir-based DAA regimens commonly used in routine practice: Sofosbuvir 400 mg plus ledipasvir 90 mg [sofosbuvir (SOF)/ledipasvir (LDV)], sofosbuvir 400 mg plus

velpatasvir 100 mg (SOF/velpatasvir), or sofosbuvir 400 mg plus daclatasvir 60 mg (SOF/daclatasvir). These interferon-free, all-oral regimens are consistent with current guideline recommendations for first-line treatment of chronic HCV infection[7,8].

At SVR12, all patients had undetectable HCV RNA levels. Laboratory parameters at SVR12 differed significantly between the two groups. Compared with patients who did not develop HCC, those who developed HCC had higher total bilirubin levels [median (IQR), 0.90 (0.54-1.47) mg/dL *vs* 0.71 (0.47-1.01) mg/dL; $P = 0.028$] and higher aspartate aminotransferase levels [34 (26-47) U/L *vs* 30 (23-40) U/L; $P = 0.006$]. Platelet counts were significantly lower in the HCC group [88 (68-150) × 10^9/L *vs* 149 (96-195) × 10^9/L; $P < 0.001$], and serum albumin levels were also significantly lower (3.77 ± 0.56 g/dL *vs* 4.08 ± 0.54 g/dL; $P < 0.001$). These findings, summarized in Table 2, are consistent with previous studies showing that thrombocytopenia, hyperbilirubinemia, hypoalbuminemia, and other surrogate markers of portal hypertension and impaired hepatic synthetic function are associated with an increased risk of subsequent HCC in patients with HCV-related cirrhosis who achieve SVR[14,21,31,33].

Each of the four noninvasive scoring systems—the FIB-4 index, APRI, ALBI, and aMAP score—calculated at SVR12 was significantly higher in the group that later developed HCC compared with the group that remained HCC-free. This finding is consistent with previous studies of patients with HCV-related cirrhosis who achieved SVR, in which these scoring systems were associated with the risk of *de novo* HCC during long-term follow-up[21,30,31].

When the discriminatory performance of the four noninvasive scoring systems was evaluated over time, the aMAP score demonstrated the highest predictive accuracy for overall HCC development, with an 8-year AUC of 0.74 (95%CI: 0.67-0.80). At earlier time points (1 year, 3 years, and 5 years), the aMAP score consistently achieved higher AUC values compared with FIB-4 index, APRI, and ALBI (Table 3, Figure 2). This pattern is consistent with the original multicohort validation of the aMAP score, which demonstrated strong discrimination for incident HCC across multiple etiologies of chronic liver disease and stable predictive performance across follow-up periods of up to 5 years[30].

Patients were stratified according to exploratory cohort-derived aMAP cutoffs (< 55, 55 to < 60, and ≥ 60). Patients with an aMAP score < 55 experienced no HCC events during follow-up, whereas those with aMAP scores between 55 and < 60 and ≥ 60 had annual HCC incidence rates of 2.42 and 6.42 per 100 person-years, respectively (95%CI: 1.21-4.84 and 4.81-8.57, respectively) (Table 4). These patterns were corroborated by the Kaplan-Meier analysis, which demonstrated clear separation of cumulative HCC risk across the three strata, with the aMAP < 55 group maintaining a flat risk trajectory throughout follow-up (Figure 3A).

To contextualize these findings, we additionally applied the originally validated aMAP thresholds proposed by Fan *et al*[30] (< 50, 50 to < 60, and ≥ 60). Consistent with the exploratory analysis, patients with an aMAP score < 50 also exhibited no observed HCC events, while those with aMAP scores of 50 to < 60 and ≥ 60 showed progressively higher HCC incidence rates (Table 5). The corresponding Kaplan-Meier curves demonstrated comparable stepwise risk stratification, supporting the robustness of the discriminatory performance of the aMAP score in this cohort of patients with cACLD (Figure 3B). Collectively, both the exploratory (< 55) and previously validated (< 50) cutoff values identified very-low-risk subgroups with no observed HCC events in this cohort.

DISCUSSION

In this longitudinal cohort of patients with HCV-related cACLD, a clinically meaningful risk of HCC persisted despite achievement of SVR, with an annual incidence of 3.12%. Among the evaluated noninvasive scoring systems, the aMAP score demonstrated the highest discriminatory performance. Importantly, an exploratory cutoff of < 55 identified a very-low-risk subgroup comprising 18.6% of the cohort, in whom no HCC events were observed during long-term follow-up. This finding supports the potential role of the aMAP score as a risk-stratification and rule-out tool to support individualized surveillance strategies rather than as a definitive basis for discontinuing HCC surveillance.

While the FIB-4 index and APRI are widely used for fibrosis staging, their utility in predicting HCC after SVR appears limited compared with the aMAP score. In our analysis, the aMAP score consistently achieved the highest AUC values across all follow-up time points. This superior performance likely reflects the variables of the score. The aMAP score incorporates age and sex, which are nonmodifiable risk factors, together with the ALBI score, which reflects hepatic function, and platelet count, a surrogate marker of portal hypertension. After SVR, transaminase-based indices such as APRI and the FIB-4 index often decline rapidly as hepatic necroinflammation resolves, potentially underestimating the residual risk of HCC. In contrast, the aMAP score captures persistent physiological derangements—particularly hepatic synthetic dysfunction and portal hypertension—that drive hepatocarcinogenesis in patients with cirrhosis.

The originally validated aMAP score cutoff of < 50 proposed by Fan *et al*[30] also identified a subgroup with no observed HCC events in our cohort, although this group represented only 6.6% of the study population. In the exploratory cohort-based analysis, a cutoff value of < 55 was the highest cutoff that still identified a subgroup with no observed HCC events while expanding the very-low-risk subgroup to 18.6% of the cohort. This threshold should therefore be interpreted as hypothesis-generating and cohort-derived, not as a validated criterion for discontinuing HCC surveillance. Prospective multicenter external validation is required before it can be used to guide surveillance de-escalation in routine clinical practice.

The relatively high incidence of HCC observed in this cohort may partly reflect the advanced liver disease profile of patients treated and followed at a tertiary referral center. Compared with patients who did not develop HCC, those who developed HCC had higher baseline liver stiffness, lower platelet counts, lower serum albumin levels, higher bilirubin levels, and a higher prevalence of esophageal varices, indicating more advanced portal hypertension and impaired hepatic functional reserve. In addition, type 2 diabetes mellitus was common and was more prevalent among patients

Table 2 Baseline characteristics, comorbidities, liver stiffness before direct-acting antiviral therapy, laboratory parameters at sustained virological response at 12 weeks after treatment completion, and noninvasive scoring systems, *n* (%)/median (interquartile range)

Characteristics	Overall	No HCC	*De novo* HCC	*P* value
Patients	571	493 (86.3)	78 (13.7)	
Sex, male	267	225 (45.6)	42 (53.8)	0.177
Age (years), mean ± SD	59.75 ± 10.00	59.53 ± 10.24	61.18 ± 8.22	0.175
Comorbidities				
T2DM	209	172 (34.9)	37 (47.4)	0.033
DLP	228	205 (41.6)	23 (29.5)	0.043
HT	315	270 (54.8)	45 (57.7)	0.629
Thalassemia	23	22 (4.5)	1 (1.3)	0.346
Esophageal varices without bleeding	87	67 (13.6)	20 (25.6)	0.006
Transient elastography				
Baseline liver stiffness by VCTE (kPa)	15.8 (11.1-25.7)	15.1 (10.5-23.3)	21.3 (15.7-35.3)	< 0.001
SOF-based treatment	571 (100)	493 (86.3)	78 (13.7)	0.045
SOF/LDV	186 (32.6)	153 (31.0)	33 (42.3)	
SOF/VEL	163 (28.5)	149 (30.2)	14 (17.9)	
SOF/DAC	222 (38.9)	191 (38.7)	31 (39.7)	
Laboratory parameters at SVR12				
Total bilirubin (mg/dL)	0.71 (0.49-1.10)	0.71 (0.47-1.01)	0.90 (0.54-1.47)	0.028
Direct bilirubin (mg/dL)	0.31 (0.20-0.49)	0.28 (0.19-0.46)	0.37 (0.26-0.62)	0.046
AST (U/L)	30 (24-40)	30 (23-40)	34 (26-47)	0.006
ALT (U/L)	22 (16-31)	21 (16-31)	23.5 (17-31)	0.275
ALP (U/L)	91 (67.5-120.5)	91 (68-124)	85 (64-107)	0.193
GGT (U/L)	44.5 (27-92)	43 (27-92)	59 (59-59)	0.641
Platelet count (× 10^9/L)	140 (92-191)	149 (96-195)	88 (68-150)	< 0.001
Total protein (g/dL), mean ± SD	7.69 ± 0.74	7.71 ± 0.74	7.61 ± 0.71	0.393
Albumin (g/dL), mean ± SD	4.04 ± 0.56	4.08 ± 0.54	3.77 ± 0.56	< 0.001
Globulin (g/dL), mean ± SD	3.70 ± 0.71	3.68 ± 0.68	3.84 ± 0.85	0.170
Noninvasive scoring systems at SVR12				
FIB-4	2.97 (1.81-4.97)	2.66 (1.76-4.51)	5.24 (2.79-7.46)	< 0.001
APRI	0.58 (0.35-1.08)	0.54 (0.34-0.96)	1.01 (0.51-1.65)	< 0.001
ALBI	-2.72 (-3.04 to -2.26)	-2.78 (-3.06 to -2.31)	-2.43 (-2.87 to -1.92)	0.001
aMAP	61.79 (56.59-66.22)	60.78 (55.45-65.31)	65.05 (62.29-68.57)	< 0.001

ALBI: Albumin-bilirubin score; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; aMAP: Age-male-albumin-bilirubin-platelet score; APRI: Aspartate aminotransferase-to-platelet ratio index; AST: Aspartate aminotransferase; DLP: Dyslipidemia; FIB-4: Fibrosis-4 index; GGT: Γ-Glutamyl transferase; HCC: Hepatocellular carcinoma; HT: Hypertension; IQR: Interquartile range; SD: Standard deviation; SOF: Sofosbuvir; DAC: Daclatasvir; LDV: Ledipasvir; VEL: Velpatasvir; SVR12: Sustained virological response at 12 weeks after treatment completion; T2DM: Type 2 diabetes mellitus; VCTE: Vibration-controlled transient elastography.

who subsequently developed HCC. These findings suggest that metabolic dysfunction may contribute to the residual risk of HCC after viral eradication.

Metabolic dysfunction-associated steatotic liver disease and metabolic dysfunction-associated steatohepatitis are increasingly recognized as contributors to hepatocarcinogenesis, including in patients who achieve SVR for HCV infection. In patients with pre-existing advanced fibrosis or cirrhosis, metabolic risk factors such as diabetes mellitus, obesity, insulin resistance, and steatotic liver disease may sustain hepatic inflammation, fibrogenesis, and carcinogenic risk despite SVR. However, as systematic data on hepatic steatosis, body mass index, controlled attenuation parameter,

Table 3 Time-dependent diagnostic performance of four noninvasive scoring systems for predicting *de novo* hepatocellular carcinoma at 1-year, 3-year, 5-year, and 8-year follow-up

Noninvasive scoring system at SVR12	1-year FU AUC (95%CI)	3-year FU AUC (95%CI)	5-year FU AUC (95%CI)	8-year FU AUC (95%CI)
FIB-4	0.64 (0.49-0.78)	0.69 (0.59-0.79)	0.69 (0.60-0.78)	0.69 (0.61-0.78)
APRI	0.61 (0.46-0.75)	0.67 (0.57-0.77)	0.65 (0.56-0.75)	0.66 (0.57-0.75)
ALBI	0.59 (0.44-0.73)	0.67 (0.58-0.76)	0.66 (0.57-0.74)	0.65 (0.56-0.74)
aMAP	0.74 (0.62-0.85)	0.76 (0.68-0.83)	0.74 (0.67-0.82)	0.74 (0.67-0.80)

ALBI: Albumin-bilirubin score; aMAP: Age-male-albumin-bilirubin-platelet score; APRI: Aspartate aminotransferase-to-platelet ratio index; AUC: Area under the curve; CI: Confidence interval; FIB-4: Fibrosis-4 index; FU: Follow-up; SVR12, sustained virological response at 12 weeks after treatment completion.

Table 4 Exploratory age-male-albumin-bilirubin-platelet score strata for hepatocellular carcinoma risk

Risk of developing *de novo* HCC	aMAP	Annual HCC incidence % (95%CI)	Proportion of the cohort, %
Low	< 55	0.0	18.55
Intermediate	55 to < 60	2.42 (1.21-4.84)	22.96
High	≥ 60	6.42 (4.81-8.57)	58.49

aMAP: Age-male-albumin-bilirubin-platelet score; HCC: Hepatocellular carcinoma.

Table 5 Risk stratification based on previously validated age-male-albumin-bilirubin-platelet score thresholds

Risk of developing *de novo* HCC	aMAP score	Annual HCC incidence % (95%CI)	Proportion of the cohort, %
Low	< 50	0.0	6.60
Intermediate	50 to <60	1.58 (0.79-3.16)	34.91
High	≥ 60	6.42 (4.81-8.57)	58.49

aMAP: Age-male-albumin-bilirubin-platelet score; HCC: Hepatocellular carcinoma.

and histologic steatohepatitis were not uniformly available, we could not directly quantify the independent contributions of metabolic dysfunction-associated steatotic liver disease and metabolic dysfunction-associated steatohepatitis to the residual risk of HCC.

Current cost-effectiveness analyses suggest that HCC surveillance is cost-effective when the annual HCC incidence exceeds approximately 1.32%-1.5%[25]. The overall HCC incidence in our cohort (3.12% per year) strongly supports the current standard of surveillance in unselected patients with cACLD who achieve SVR. However, the marked risk stratification provided by the aMAP score suggests that a universal, one-size-fits-all surveillance strategy may be inefficient for some very-low-risk subgroups.

The aMAP score should also be interpreted as a dynamic risk stratification tool. As it incorporates serum albumin, bilirubin, and platelet count, the score may change over time as hepatic functional reserve, portal hypertension, fibrosis burden, or metabolic comorbidities evolve after SVR. Therefore, patients classified as being at very low risk should continue to undergo routine clinical assessment and laboratory monitoring, with periodic reassessment of the score rather than relying on a one-time permanent classification. As abdominal ultrasonography is inexpensive, widely available, noninvasive, and integrated into routine clinical practice, the findings of this study should not be interpreted as supporting the immediate universal discontinuation of ultrasound-based HCC surveillance. Instead, a more practical implication is that patients at very low risk may be considered for individualized surveillance intensity and may avoid unnecessary expensive cross-sectional imaging unless clinically, biochemically, or ultrasonographically indicated.

In our cohort, patients with an aMAP score ≥ 60 had an annual HCC incidence of 6.42%, supporting continued standard semiannual HCC surveillance and careful clinical follow-up in this high-risk subgroup. These findings may help optimize surveillance resource allocation by identifying patients who remain at high risk while generating a testable very-low-risk subgroup for future studies evaluating surveillance de-escalation.

Genomic-clinical risk models and biomarker-based approaches may provide additional risk discrimination, particularly in selected high-risk populations or in settings where genomic testing is readily available and affordable. However,

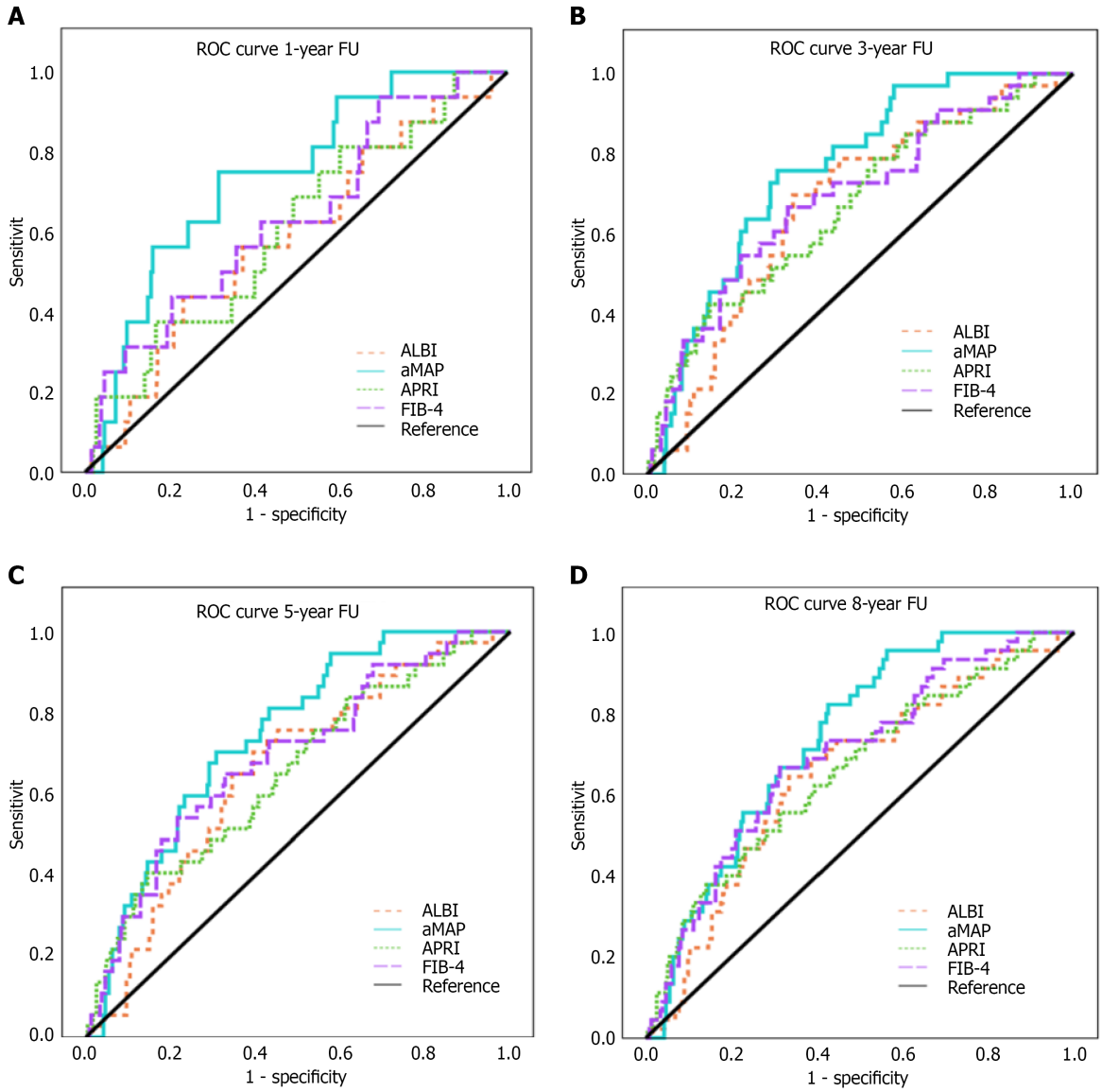


Figure 2 Time-dependent receiver operating characteristic curves of four noninvasive scoring systems for predicting *de novo* hepatocellular carcinoma at 1-year, 3-year, 5-year, and 8-year follow-up. A: 1-year follow-up; B: 3-year follow-up; C: 5-year follow-up; D: 8-year follow-up. ALBI: Albumin-bilirubin score; aMAP: Age-male-albumin-bilirubin-platelet score; APRI: Aspartate aminotransferase-to-platelet ratio index; FIB-4: Fibrosis-4 index; ROC: Receiver operating characteristic; FU: Follow-up.

such approaches may increase costs and may not be feasible in all healthcare systems. The aMAP score should therefore be viewed as a simple, low-cost, and complementary risk-stratification tool based on routinely available clinical and laboratory parameters rather than as a replacement for genomic-clinical models, advanced biomarkers, or guideline-recommended imaging-based HCC surveillance. Future studies should compare the aMAP score with genomic-clinical and biomarker-based models and evaluate their comparative cost-effectiveness across different healthcare settings.

Taken together, these findings support a practical risk-stratified framework rather than an immediate change in current guideline-recommended surveillance. Patients with higher aMAP scores, particularly those with scores ≥ 60, remain appropriate candidates for standard semiannual HCC surveillance and careful clinical follow-up. In contrast, patients with scores < 55 may represent a very-low-risk subgroup in whom future studies could evaluate less intensive surveillance strategies, periodic risk reassessment, and avoidance of unnecessary cross-sectional imaging. Thus, the main clinical contribution of this study is not to establish a criterion for discontinuing surveillance but to identify a reproducible low-risk subgroup that may inform individualized HCC surveillance intensity after external validation.

This study has several limitations. First, it was a single-center retrospective study conducted at a tertiary referral center, which may limit the generalizability of the findings and may have resulted in a cohort enriched with patients with more advanced liver disease. Second, although the absence of HCC events in the subgroup with an aMAP score < 55 is clinically encouraging, rare events cannot be excluded without validation in larger prospective multicenter cohorts. Third, the < 55 cutoff was exploratory and cohort-derived and should not be interpreted as a universally validated cutoff. Fourth, all patients received sofosbuvir-based regimens; therefore, the findings may not fully extrapolate to other DAA regimens, non-Asian populations, or populations with different etiological and metabolic risk profiles. Fifth, systematic

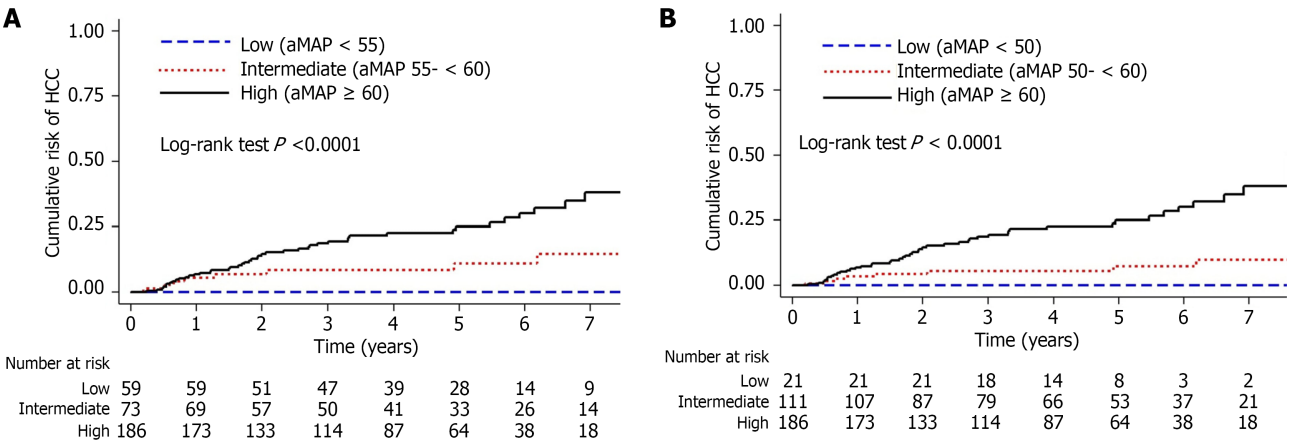


Figure 3 Kaplan-Meier curves. A: Kaplan-Meier curves of hepatocellular carcinoma risk according to empirically derived age-male-albumin-bilirubin-platelet (aMAP) score strata. Patients were stratified into low-, intermediate-, and high-risk groups using thresholds of < 55, 55 to < 60, and ≥ 60, respectively; B: Kaplan-Meier curves of hepatocellular carcinoma risk according to the originally validated aMAP score strata. Patients were stratified into low-, intermediate-, and high-risk groups using thresholds of < 50, 50 to < 60, and ≥ 60, respectively. aMAP: Age-male-albumin-bilirubin-platelet score.

data on hepatic steatosis, body mass index, controlled attenuation parameter, alcohol consumption, and histological evidence of steatohepatitis were not uniformly available. Therefore, the independent contributions of metabolic dysfunction-associated steatotic liver disease and metabolic dysfunction-associated steatohepatitis could not be quantified. Finally, serial changes in the aMAP score were not evaluated, and future studies should investigate the value of dynamic risk reassessment over time.

CONCLUSION

The aMAP score assessed at SVR12 is a simple and readily available tool for stratifying HCC risk in patients with HCV-related cACLD. An exploratory threshold of < 55 identified a very-low-risk subgroup with no observed HCC events in this cohort and may support individualized HCC surveillance intensity or potential surveillance de-escalation strategies. However, prospective multicenter external validation is required before this threshold can be implemented in routine clinical practice.

REFERENCES

1 **Fleurence RL**, Alter HJ, Collins FS, Ward JW. Global Elimination of Hepatitis C Virus. *Annu Rev Med* 2025; **76**: 29-41 [*RCA*] [PMID: 39485830 DOI: 10.1146/annurev-med-050223-111239] [FullText]

2 **Abu-Freha N**, Mathew Jacob B, Elhoashla A, Afawi Z, Abu-Hammad T, Elsana F, Paz S, Etzion O. Chronic hepatitis C: Diagnosis and treatment made easy. *Eur J Gen Pract* 2022; **28**: 102-108 [*RCA*] [PMID: 35579223 DOI: 10.1080/13814788.2022.2056161] [FullText] [Full Text(PDF)]

3 **Topi S**, Gaxhja E, Charitos IA, Colella M, Santacroce L. Hepatitis C Virus: History and Current Knowledge. *Gastroenterol Insights* 2024; **15**: 676-707 [DOI: 10.3390/gastroent15030049] [FullText]

4 **Llovet JM**, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2021; **7**: 6 [*RCA*] [PMID: 33479224 DOI: 10.1038/s41572-020-00240-3] [FullText]

5 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [*RCA*] [PMID: 30207593 DOI: 10.3322/caac.21492] [FullText]

6 **Falade-Nwulia O**, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB, Sulkowski MS. Oral Direct-Acting Agent Therapy for Hepatitis C Virus Infection: A Systematic Review. *Ann Intern Med* 2017; **166**: 637-648 [*RCA*] [PMID: 28319996 DOI: 10.7326/M16-2575] [FullText]

7 **European Association for the Study of the Liver**; Clinical Practice Guidelines Panel: Chair; EASL Governing Board representative; Panel members. EASL recommendations on treatment of hepatitis C: Final update of the series(☆). *J Hepatol* 2020; **73**: 1170-1218 [*RCA*] [PMID: 32956768 DOI: 10.1016/j.jhep.2020.08.018] [FullText]

8 **Ghany MG**, Morgan TR; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* 2020; **71**: 686-721 [*RCA*] [PMID: 31816111 DOI: 10.1002/hep.31060] [FullText] [Full Text(PDF)]

9 **Rout G**, Nayak B, Patel AH, Gunjan D, Singh V, Kedia S, Shalimar. Therapy with Oral Directly Acting Agents in Hepatitis C Infection Is Associated with Reduction in Fibrosis and Increase in Hepatic Steatosis on Transient Elastography. *J Clin Exp Hepatol* 2019; **9**: 207-214 [*RCA*] [PMID: 31024203 DOI: 10.1016/j.jceh.2018.06.009] [FullText]

10 **Shiha G**, Soliman R, Hassan AA, Mikhail NN. Changes in hepatic fibrosis and incidence of HCC following direct-acting antiviral treatment of F3 chronic hepatitis c patients: a prospective observational study. *Hepatoma Res* 2022; **8**: 29 [*RCA*] [DOI: 10.20517/2394-5079.2022.13] [Full

Text]
11 **Zakareya T**, Elhelbawy M, Elzohry H, Eltabbakh M, Deif M, Abbasy M. Long-Term Impact of Hepatitis C Virus Eradication on Liver Stiffness in Egyptian Patients. *Can J Gastroenterol Hepatol* 2021; **2021**: 4961919 [*RCA*] [PMID: 34589447 DOI: 10.1155/2021/4961919] [Full Text] [Full Text(PDF)]
12 **van der Meer AJ**, Feld JJ, Hofer H, Almasio PL, Calvaruso V, Fernández-Rodríguez CM, Aleman S, Ganne-Carrié N, D'Ambrosio R, Pol S, Trapero-Marugan M, Maan R, Moreno-Otero R, Mallet V, Hultcrantz R, Weiland O, Rutter K, Di Marco V, Alonso S, Bruno S, Colombo M, de Knegt RJ, Veldt BJ, Hansen BE, Janssen HLA. Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. *J Hepatol* 2017; **66**: 485-493 [*RCA*] [PMID: 27780714 DOI: 10.1016/j.jhep.2016.10.017] [FullText]
13 **Backus LI**, Belperio PS, Shahoumian TA, Mole LA. Impact of Sustained Virologic Response with Direct-Acting Antiviral Treatment on Mortality in Patients with Advanced Liver Disease. *Hepatology* 2019; **69**: 487-497 [*RCA*] [PMID: 28749564 DOI: 10.1002/hep.29408] [Full Text]
14 **D'Ambrosio R**, Degasperi E, Anolli MP, Fanetti I, Borghi M, Soffredini R, Iavarone M, Tosetti G, Perbellini R, Sangiovanni A, Sypsa V, Lampertico P. Incidence of liver- and non-liver-related outcomes in patients with HCV-cirrhosis after SVR. *J Hepatol* 2022; **76**: 302-310 [*RCA*] [PMID: 34592366 DOI: 10.1016/j.jhep.2021.09.013] [FullText]
15 **Shiha G**, Mousa N, Soliman R, Nnh Mikhail N, Adel Elbasiony M, Khattab M. Incidence of HCC in chronic hepatitis C patients with advanced hepatic fibrosis who achieved SVR following DAAs: A prospective study. *J Viral Hepat* 2020; **27**: 671-679 [*RCA*] [PMID: 32050037 DOI: 10.1111/jvh.13276] [FullText]
16 **Butt AA**, Yan P, Shaikh OS, Lo Re V 3rd, Abou-Samra AB, Sherman KE. Treatment of HCV reduces viral hepatitis-associated liver-related mortality in patients: An ERCHIVES study. *J Hepatol* 2020; **73**: 277-284 [*RCA*] [PMID: 32145260 DOI: 10.1016/j.jhep.2020.02.022] [Full Text]
17 **Ioannou GN**, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017; S0168-8278(17)32273 [*RCA*] [PMID: 28887168 DOI: 10.1016/j.jhep.2017.08.030] [FullText] [Full Text(PDF)]
18 **Kanwal F**, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents. *Gastroenterology* 2017; **153**: 996-1005.e1 [*RCA*] [PMID: 28642197 DOI: 10.1053/j.gastro.2017.06.012] [FullText]
19 **Waziry R**, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017; **67**: 1204-1212 [*RCA*] [PMID: 28802876 DOI: 10.1016/j.jhep.2017.07.025] [FullText]
20 **Singh S**, Nautiyal A, Loke YK. Oral direct-acting antivirals and the incidence or recurrence of hepatocellular carcinoma: a systematic review and meta-analysis. *Frontline Gastroenterol* 2018; **9**: 262-270 [*RCA*] [PMID: 30245788 DOI: 10.1136/flgastro-2018-101017] [FullText]
21 **Caviglia GP**, Troshina G, Santaniello U, Rosati G, Bombaci F, Birolo G, Nicolosi A, Saracco GM, Ciancio A. Long-Term Hepatocellular Carcinoma Development and Predictive Ability of Non-Invasive Scoring Systems in Patients with HCV-Related Cirrhosis Treated with Direct-Acting Antivirals. *Cancers (Basel)* 2022; **14**: 828 [*RCA*] [PMID: 35159094 DOI: 10.3390/cancers14030828] [FullText] [Full Text(PDF)]
22 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; 69: 182-236 [*RCA*] [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019] [FullText]
23 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [*RCA*] [PMID: 29624699 DOI: 10.1002/hep.29913] [FullText]
24 **Omata M**, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017; **11**: 317-370 [*RCA*] [PMID: 28620797 DOI: 10.1007/s12072-017-9799-9] [FullText] [Full Text(PDF)]
25 **Farhang Zangneh H**, Wong WWL, Sander B, Bell CM, Mumtaz K, Kowgier M, van der Meer AJ, Cleary SP, Janssen HLA, Chan KKW, Feld JJ. Cost Effectiveness of Hepatocellular Carcinoma Surveillance After a Sustained Virologic Response to Therapy in Patients With Hepatitis C Virus Infection and Advanced Fibrosis. *Clin Gastroenterol Hepatol* 2019; **17**: 1840-1849.e16 [*RCA*] [PMID: 30580095 DOI: 10.1016/j.cgh.2018.12.018] [FullText]
26 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [*RCA*] [PMID: 16729309 DOI: 10.1002/hep.21178] [FullText]
27 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [*RCA*] [PMID: 12883497 DOI: 10.1053/jhep.2003.50346] [FullText]
28 **Johnson PJ**, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, O'Beirne J, Fox R, Skowronska A, Palmer D, Yeo W, Mo F, Lai P, Iñarrairaegui M, Chan SL, Sangro B, Miksad R, Tada T, Kumada T, Toyoda H. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015; **33**: 550-558 [*RCA*] [PMID: 25512453 DOI: 10.1200/JCO.2014.57.9151] [FullText]
29 **Toyoda H**, Johnson PJ. The ALBI score: From liver function in patients with HCC to a general measure of liver function. *JHEP Rep* 2022; **4**: 100557 [*RCA*] [PMID: 36124124 DOI: 10.1016/j.jhepr.2022.100557] [FullText] [Full Text(PDF)]
30 **Fan R**, Papatheodoridis G, Sun J, Innes H, Toyoda H, Xie Q, Mo S, Sypsa V, Guha IN, Kumada T, Niu J, Dalekos G, Yasuda S, Barnes E, Lian J, Suri V, Idilman R, Barclay ST, Dou X, Berg T, Hayes PC, Flaherty JF, Zhou Y, Zhang Z, Buti M, Hutchinson SJ, Guo Y, Calleja JL, Lin L, Zhao L, Chen Y, Janssen HLA, Zhu C, Shi L, Tang X, Gaggar A, Wei L, Jia J, Irving WL, Johnson PJ, Lampertico P, Hou J. aMAP risk score predicts hepatocellular carcinoma development in patients with chronic hepatitis. *J Hepatol* 2020; **73**: 1368-1378 [*RCA*] [PMID: 32707225 DOI: 10.1016/j.jhep.2020.07.025] [FullText]
31 **Casadei Gardini A**, Foschi FG, Conti F, Petracci E, Vukotic R, Marisi G, Buonfiglioli F, Vitale G, Ravaioli F, Gitto S, Verucchi G, Lenzi M, Bolondi L, Mazzella G, Brillanti S, Andreone P; member of the Bologna DAA group. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. *Dig Liver Dis* 2019; **51**: 681-688 [*RCA*] [PMID: 30327251 DOI: 10.1016/j.dld.2018.09.016] [FullText]
32 **de Franchis R**, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. Baveno VII - Renewing consensus in portal hypertension. *J Hepatol* 2022; **76**: 959-974 [*RCA*] [PMID: 35120736 DOI: 10.1016/j.jhep.2021.12.022] [FullText] [Full Text(PDF)]
33 **Lleo A**, Aglitti A, Aghemo A, Maisonneuve P, Bruno S, Persico M; collaborators. Predictors of hepatocellular carcinoma in HCV cirrhotic

patients treated with direct acting antivirals. *Dig Liver Dis* 2019; **51**: 310-317 [*RCA*] [PMID: 30473220 DOI: 10.1016/j.dld.2018.10.014] [Full Text]

34 **Kim WR**, Berg T, Asselah T, Flisiak R, Fung S, Gordon SC, Janssen HL, Lampertico P, Lau D, Bornstein JD, Schall RE, Dinh P, Yee LJ, Martins EB, Lim SG, Loomba R, Petersen J, Buti M, Marcellin P. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. *J Hepatol* 2016; **64**: 773-780 [*RCA*] [PMID: 26626497 DOI: 10.1016/j.jhep.2015.11.012] [FullText]

FOOTNOTES

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ORIGINAL ARTICLE

Clinical Trials Study

Effect of hepatic venous pressure gradient in different hepatic venous branches on assessing portal hypertension in cirrhotic patients

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Abstract

BACKGROUND

Data regarding the hepatic venous pressure gradient (HVPG) in different hepatic venous branches remain insufficient.

AIM

To assess portal hypertension using HVPG measurements from the right HVPG (R-HVPG), middle HVPG (M-HVPG), and left HVPG (L-HVPG) hepatic veins, we conducted two sub-studies: Sub-study 1 evaluated the diagnostic value of R-HVPG, M-HVPG and L-HVPG, while sub-study 2 explored the correlation between M-HVPG and portal pressure gradient (PPG) to validate the results of sub-

study 1.

METHODS
We prospectively enrolled 88 patients who underwent HVPG measurements in different hepatic venous branches in sub-study 1. Sub-group 2 covered 16 cirrhotic patients for M-HVPG and PPG comparisons (NCT06358092). Agreement was analyzed using intraclass correlation coefficients (ICC) and Bland-Altman plots.

RESULTS
In sub-study 1, M-HVPG demonstrated a significantly higher technical success rate (96.6%) than standard R-HVPG (83.0%) and L-HVPG (93.2%) ($P < 0.05$). After excluding 15 patients with collateral shunts in the R-HVPG, 73 were included in the final analysis. The mean R-HVPG, M-HVPG and L-HVPG were 16.43 (5.34), 16.98 (5.69) and 15.29 (5.22) mmHg, respectively, with no statistically significant difference ($P > 0.05$). Agreement between the R-HVPG and M-HVPG was substantial [ICC: 0.70 (0.56-0.80)], superior to the moderate R-HVPG/L-HVPG agreement [ICC: 0.56 (0.37-0.70)]. In sub-study 2, the M-HVPG and PPG showed substantial agreement [ICC: 0.80 (0.52-0.92)].

CONCLUSION
Our study demonstrates that M-HVPG achieves the highest technical success rate and superior agreement with R-HVPG, which supports the priority of technical feasibility of HVPG proposed in Baveno VII consensus. Furthermore, the strong correlation with PPG suggests that the M-HVPG potentially reflects accurate portal pressure. Consequently, M-HVPG may be considered a preferred alternative when R-HVPG is unfeasible or unreliable.

Key Words: Cirrhosis; Portal hypertension; Agreement; Hepatic venous pressure gradient; Portal pressure gradient

Core Tip: Middle hepatic venous pressure gradient (M-HVPG) achieves the highest technical success rate and superior agreement with right hepatic venous pressure gradient (HVPG) than that of left HVPG. M-HVPG potentially reflects accurate portal pressure. M-HVPG may be considered a preferred alternative when right HVPG is unfeasible or unreliable.

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INTRODUCTION

Portal hypertension is the main driver of decompensation and liver-related mortality in cirrhosis, making its assessment crucial for pathophysiological and therapeutic research[1]. While direct measurement of portal vein pressure is the physiological ideal, it is technically challenging because of the vessel's anatomical position between the hepatic sinusoids and splanchnic capillaries. Consequently, the hepatic venous pressure gradient (HVPG), calculated as the difference between wedged hepatic venous pressure (WHVP) and free hepatic venous pressure (FHVP), has emerged as the standard minimally invasive surrogate[2]. It correlates strongly with direct portal pressure, particularly in patients with viral hepatitis- and alcohol-related cirrhosis[3].

The Baveno VII consensus emphasizes the necessity of stratifying patients based on liver disease severity and portal pressure dynamics to guide personalized management[1]. As the gold standard for this assessment, HVPG provides critical prognostic utility through well-established risk thresholds. Specifically, an HVPG ≥ 10 mmHg defines clinically significant portal hypertension, serving as an independent predictor of hepatic decompensation, with each one mmHg increase escalating the relative risk of decompensation by approximately 11%[1,4]. Furthermore, exceeding the 12 mmHg threshold significantly elevates the risk of variceal bleeding, while reducing pressure below this level offers protective benefits[5-7]. Higher thresholds of 16 mmHg and 20 mmHg are indicative of increased mortality risk and treatment failure (such as early rebleeding) during acute variceal bleeding episodes, respectively[8-10]. Given these precise clinical implications, ensuring the technical feasibility and accuracy of HVPG measurement is paramount for both clinical prognostication and drug development.

Standard HVPG measurement typically targets the right HVPG (R-HVPG)[11,12]. However, R-HVPG cannulation can be technically unsuccessful in certain patients owing to anatomical variations, such as ostial stenosis or right posterior lobe atrophy associated with venous malformation. Furthermore, the presence of intrahepatic veno-venous shunts can introduce systematic errors, resulting in falsely low HVPG readings that do not reflect the true portal pressure gradient (PPG)[13]. In such case, the middle HVPG (M-HVPG) and left HVPG (L-HVPG) serve as potential alternative access sites. Nevertheless, the degree of hepatic fibrosis may be heterogeneously distributed, and pressure readings could theoretically fluctuate between different liver segments[14]. Currently, there is a lack of evidence demonstrating HVPG in different hepatic venous branches on assessing portal hypertension.

Therefore, we performed two prospective sub-studies. Sub-study 1 aimed to determine the effect of HVPG in different hepatic venous branches on assessing portal hypertension in patients with hepatitis B virus (HBV)- and alcohol-related cirrhosis. Sub-study 2 explored the correlation between M-HVPG and PPG to validate sub-study 1.

MATERIALS AND METHODS

Study population and design

This prospective study recruited cirrhotic patients consecutively at the Third Affiliated Hospital of Sun Yat-Sen University from January 2024 to December 2025 (approval No. ZSSYXHNK2401; ClinicalTrials.gov identifier: NCT06358092).

In sub-study 1, we enrolled patients who underwent HVPG measurements across three hepatic veins as standalone assessment on the risk of portal hypertension. The inclusion criteria were as follows: (1) Adult patients (age, 18-75 years); (2) Confirmed cirrhosis based on previous compatible clinical, biochemical, and radiological findings; (3) HBV- and alcohol-related cirrhosis; (4) Scheduled to undergo clinically indicated transjugular HVPG measurement by means of catheterization of a hepatic vein; and (5) Written informed consent. The exclusion criteria were as follows: (1) Severe coagulation dysfunction; (2) Unfeasible HVPG measurement *via* the R-HVPG due to stenosis or shunt; (3) Prior transjugular intrahepatic portosystemic stent shunt surgery; (4) Hepatic carcinoma; (5) Prior liver resection or splenectomy; (6) Prior devascularization surgery; (7) Prior liver transplant; (8) Ongoing treatment with vasoactive drugs; and (9) Any active, serious, or life-threatening disease.

Sub-study 2 to observe the correlation between M-HVPG and PPG enrolled cirrhotic patients scheduled to undergo transjugular intrahepatic portosystemic stent shunt at the Third Affiliated Hospital of Sun Yat-sen University at the same time. The inclusion criteria were as follows: (1) Adult patients (age 18-75 years); (2) Confirmed cirrhosis based on previous compatible clinical, biochemical, and radiological findings; (3) HBV- and alcohol-related cirrhosis; (4) Scheduled to undergo clinically indicated transjugular intrahepatic portosystemic stent; and (5) Written informed consent. The exclusion criteria were as follows: (1) Severe coagulation dysfunction; (2) Hepatocellular carcinoma; (3) Prior liver resection or splenectomy; (4) Prior devascularization surgery; (5) Prior liver transplant; (6) Ongoing treatment with vasoactive drugs; and (7) Any active, serious, or life-threatening disease.

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University (approval No. RG2024-195-01). Written informed consent was obtained from all enrolled participants.

Measurement of HVPG and PPG

All HVPG and PPG measurements were performed by trained interventional radiologists with over 10 years of experience following the standard operating procedure (Wu C, Chang BY and Zhou CR)[1,15]. Strict standards were used to obtain an accurate HVPG, including the thickness of the catheter, the position of the catheter tip, the method and times of measurement, and so on, factors that could affect venous pressure were avoided before the operation, including discontinuation of drugs that affect portal pressure, such as nonselective beta-blockers and propofol, which can affect the HVPG and PPG during deep sedation[16].

The puncture site was disinfected and local routine sterile drapes were then placed. Under local anesthesia, HVPG measurements were conducted using a RUPS-100 set (COOK Medical, IN, United States) and a balloon catheter with a pressure transducer at the tip (Edwards Lifesciences, CA, United States) in all three hepatic venous branches. Zero measurement was needed before transjugular catheterization. The FHVP was measured in the R-HVPG (1-3 cm from the inferior vena cava). Then, as the balloon was inflated for total occlusion of the right, middle and left hepatic vein, the WHVP was measured venography of the occluded hepatic vein was performed by using a power injector to inject five mL of lopromide contrast medium (Ultravist 300; Bayer AG, Leverkusen, Germany) at a rate of two mL/s to confirm total occlusion of the balloon and to exclude the presence of hepatic venous-to-venous communications at the end of the WHVP measurement (Figure 1). Continuous recordings were performed until the pressure plateaued. Similarly, the above process was performed in the middle and left hepatic venous branches. R-, M- and L-HVPG were measured in fixed order. The HVPG was defined as the difference between the WHVP and FHVP. In this study, each HVPG measurement was based on three repeated measurements of WHVP and FHVP, the average of at least two measurements with a difference of < one mmHg was obtained, and the HVPG was calculated.

In the pilot cohort, the inferior vena cava pressure was measured in the inferior vena cava during HVPG measurement. The liver parenchyma and portal vein were then punctured through the inferior vena cava or the hepatic vein. After successful puncture of the portal vein, the pigtail or end-to-side hole catheter was inserted into the splenic vein or superior mesenteric vein for intravenous angiography. Before shunting, the pressure in the portal vein was measured (three measurements, averaged) and the PPG value was calculated. PPG was defined as the difference between portal venous pressure and inferior vena cava.

Statistical analysis

The primary outcome was agreement among the readings of the R-HVPG, M-HVPG, and L-HVPG. Secondary outcomes included the measurement success rate of M-HVPG and L-HVPG, and the correlation and agreement between the M-HVPG and PPG. The technical success of HVPG measurements was defined as: Successful transjugular catheterization, venography, and recording of plateaued pressure. The exploratory purpose was to determine the diagnostic value of M-HVPG and L-HVPG for determination of R-HVPG ≥ 10, 12, 16, and 20 mmHg.

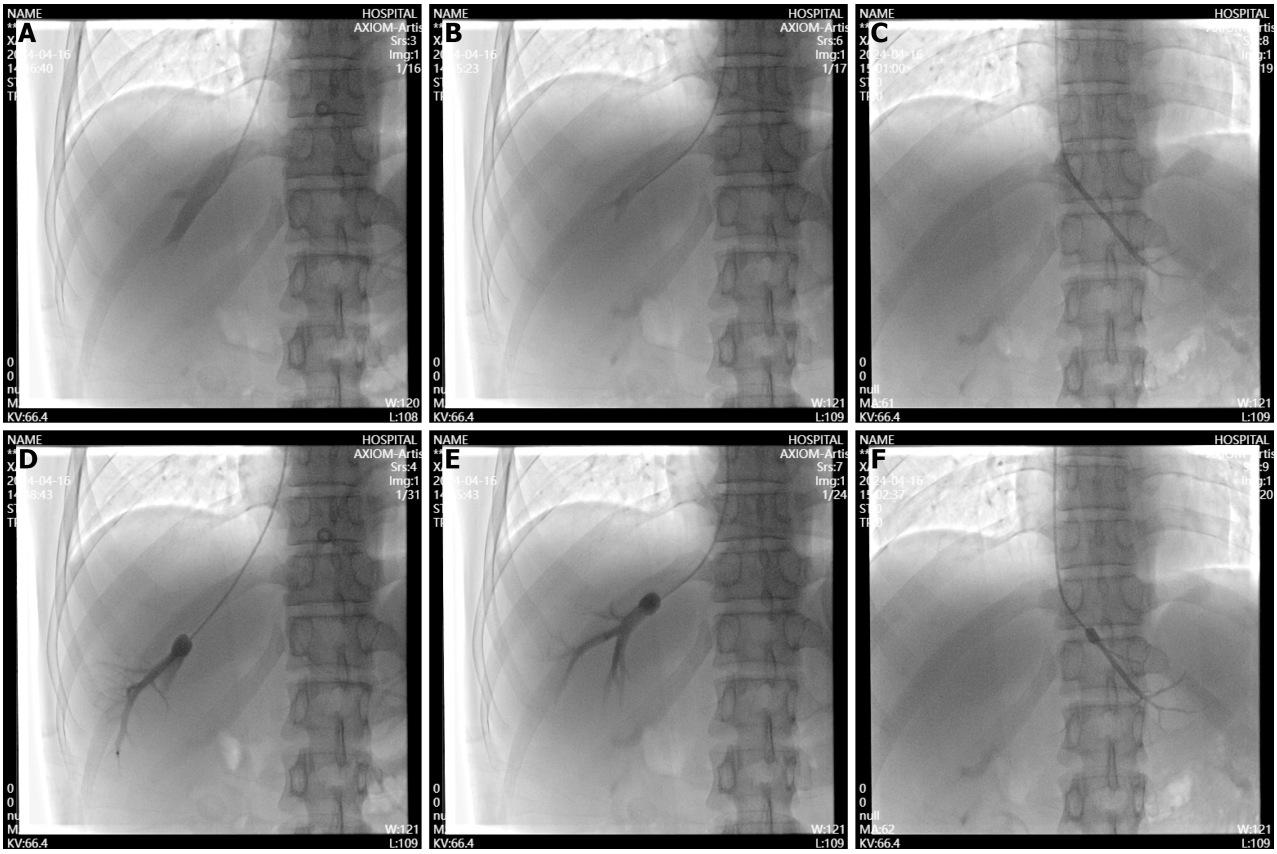


Figure 1 Hepatic venous pressure gradient measurement. A: Right free hepatic venous pressure (FHVP); B: Middle-FHVP; C: Left-FHVP; D: Right wedged hepatic venous pressure (WHVP); E: Middle-WHVP; F: Left-WHVP.

Categorical data were expressed as *n* (%), and continuous variables were expressed as mean ± SD or median (interquartile range). Intraclass correlation coefficients (ICC) and Bland-Altman plots were used to analyze the agreement. The magnitude of the ICC was interpreted according to the Landis and Koch convention: A poor agreement of 0, a slight agreement between 0 and 0.20, a fair agreement between 0.21 and 0.40, a moderate agreement between 0.41 and 0.60, a substantial agreement between 0.61 and 0.80, and an almost-perfect agreement between 0.81 and 1.00[17]. Major discrepancies between the two techniques were considered when the difference was > 5 mmHg. Diagnostic performance for detecting different cutoffs of R-HVPG was assessed by the area under the receiver operating characteristic curve (AUC), sensitivity, and specificity, and 95% confidence interval (CI) was computed using the method of Delong (1988). The cutoff values of the M-HVPG and L-HVPG were defined as the maximal sum of sensitivity and specificity. The AUC estimates the probability of the criteria to correctly distinguish between two randomly selected patients, one of whom was randomly selected from all those with the condition of interest and the other was randomly selected from all those without the condition of interest. Statistical significance was set at $P < 0.05$. All statistical calculations were performed using R language (version 4.4.3, R Core Team, 2025).

RESULTS

Study population

The flow diagram of the study population is shown in Figure 2. In sub-study 1, 88 patients with HBV- and alcohol-related cirrhosis underwent HVPG measurements in different hepatic venous branches. All patients finished successful venipuncture and catheterization. Of them, 15 patients with collateral shunts in the R-HVPG were excluded (successful cannulation but unreliable measurement due to collateral shunting). Two of them with cavernous transformation of portal veins failed to perform HVPG measurements in all three hepatic venous branches, and two patients failed in left hepatic veins (besides the right) due to incomplete occlusion. In addition, there were one patient failing only in the middle and two only in the left hepatic venous all due to collateral shunting, respectively. The M-HVPG had a better technical success rate than the R-HVPG and L-HVPG (96.6% *vs* 83.0% *vs* 93.2%, $P < 0.05$). Finally, 73 patients (mean age 53.58 years; male, 83.6%) were included in analysis. These included 43 (58.9%) patients with HBV, 20 (27.4%) with alcohol-related liver disease (ALD), and 10 (13.7%) with HBV + ALD. The number of Child-Pugh classes A, B, and C was 52 (71.2%), 18 (24.6%), and 3 (4.1%), respectively. There were 59 decompensated patients. Of them, 54 patients occurred variceal bleeding, 11 ascites needing treatment and one hepatic encephalopathy. The baseline characteristics of the enrolled patients were summarized in Table 1.

Table 1 Baseline characteristics of patients in sub-study 1

	All (*n* = 73)	HBV (*n* = 43)	ALD (*n* = 20)	HBV + ALD (*n* = 10)
Age (year), mean ± SD	53.58 ± 9.10	55.50 ± 9.00	51.10 ± 9.51	50.60 ± 10.24
Male	61 (83.6)	32 (74.4)	19 (95.0)	10 (100.0)
BMI (kg/m^2)	22.84 (3.28)	22.12 (3.14)	23.48 (3.36)	23.57 (3.04)
Decompensation, *n* (%)	59 (80.8)	37 (86.0)	13 (65.0)	9 (90.0)
Variceal bleeding	54 (74.0)	36 (83.7)	9 (45.0)	9 (90.0)
Ascites needing treatment	11 (15.1)	4 (9.3)	5 (25.0)	2 (20.0)
Hepatic encephalopathy	1 (1.4)	0 (0)	1 (5.0)	0 (0)
CSPH, *n* (%)	63 (86.3)	35 (81.4)	18 (90.0)	10 (100.0)
Etiology, *n* (%)				
HBV	43 (58.9)	NA	NA	NA
ALD	20 (27.4)	NA	NA	NA
HBV and ALD	10 (13.7)	NA	NA	NA
Child-Pugh class, *n* (%)				
Class A	52 (71.2)	35 (81.4)	11 (55.0)	6 (60.0)
Class B	18 (24.6)	7 (16.3)	7 (35.0)	4 (40.0)
Class C	3 (4.1)	1 (2.3)	2 (10.0)	0 (0)
Laboratory test, median (IQR)				
PLT (10^9/L)	54.00 (11.00)	72.00 (52.50)	87.00 (54.75)	72.00 (33.00)
TBIL (μmol/L)	19.60 (19.25)	15.40 (11.10)	27.90 (27.35)	22.35 (22.90)
Hb (10^9/L)	101.00 (43.00)	109.00 (46.00)	101.00 (50.00)	76.50 (47.50)
ALT (U/L)	25.00 (20.00)	24.00 (15.00)	29.00 (22.25)	25.00 (13.00)
AST (U/L)	34.00 (24.50)	30.00 (13.50)	62.00 (73.75)	40.00 (20.00)
GGT (U/L)	41.50 (67.50)	35.00 (31.25)	132.00 (194.00)	27.00 (57.00)
Alb (g/L)	34.55 (6.50)	37.20 (9.00)	33.00 (5.70)	33.20 (3.90)
Cr (μmol/L)	68.00 (21.00)	72.00 (23.50)	61.00 (15.25)	65.50 (18.00)
PT (seconds)	15.90 (2.50)	15.85 (2.70)	15.95 (3.10)	16.50 (2.10)
INR	1.27 (0.26)	1.26 (0.27)	1.30 (0.32)	1.34 (0.23)

HBV: Hepatitis B virus; ALD: Alcoholic-related liver disease; BMI: Body mass index; CSPH: Clinically significant portal hypertension; IQR: Interquartile range; PLT: Platelet counts; TBIL: Total bilirubin; Hb: Hemoglobin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; Alb: Albumin; Cr: Creatinine; PT: Prothrombin time; INR: International normalized ratio.

In sub-study 2, we enrolled 16 patients (mean age 50.50 years; male, 81.3%) underwent M-HVPG and PPG measurements. There were 13 (81.3%) patients with HBV and 3 (18.7%) with ALD. The number of Child-Pugh classes A, B, and C was 9 (56.3%), 6 (37.5%), and 1 (6.2%), respectively (Table 2).

Measurement of R-HVPG, M-HVPG and L-HVPG

The mean R-HVPG, M-HVPG and L-HVPG were 16.43 (5.34), 16.98 (5.69) and 15.29 (5.22), respectively, with no statistically significant difference (R-HVPG and M-HVPG, P = 0.35; R-HVPG and L-HVPG, P = 0.13; M-HVPG and L-HVPG, P = 0.07) (Table 3, Figure 3A). Major discrepancies were found in 16 patients (21.9%) between the R-HVPG and M-HVPG, 14 (19.2%) between the R-HVPG and L-HVPG, and 14 (19.2%) between the M-HVPG and L-HVPG.

In patients with HBV, the mean R-HVPG was similar to that of the M-HVPG [15.60 (5.13) *vs* 16.65 (5.55), P = 0.61]. The results were same in R-HVPG and L-HVPG [15.60 (5.13) *vs* 14.53 (5.21), P = 0.91], M-HVPG and L-HVPG [16.65 (5.55) *vs* 14.53 (5.21), P = 0.69] (Table 3, Figure 3B). In the patients with ALD, the mean R-HVPG, M-HVPG and L-HVPG were 17.65 (6.25), 18.10 (5.99) and 15.74 (4.47), respectively, with no statistically significant difference (R-HVPG and M-HVPG, P = 0.86; R-HVPG-L-HVPG, P = 0.16; M-HVPG and L-HVPG, P = 0.22) (Table 3, Figure 3C). In the patients with HBV + ALD, R-HVPG, M-HVPG and L-HVPG were 17.60 (3.89), 16.30 (5.98) and 17.78 (6.34), respectively, with no statistically significant difference (R-HVPG and M-HVPG, P = 0.22; R-HVPG and L-HVPG, P = 0.17; M-HVPG and LHVPG, P = 0.86)

Table 2 Baseline characteristics of patients in sub-study 2

	All (*n* = 16)
Age (year), mean ± SD	50.50 ± 10.73
Male, *n* (%)	13 (81.3)
HVPG, mean ± SD	23.44 ± 4.29
PPG, mean ± SD	23.63 ± 4.22
Etiology, *n* (%)	
HBV	13 (81.3)
ALD	3 (18.7)
Child-Pugh class, *n* (%)	
Class A	9 (56.3)
Class B	6 (37.5)
Class C	1 (6.2)
Laboratory test, median (IQR)	
PLT (10^9/L)	78.50 (90.5)
TBIL (μmol/L)	26.00 (37.90)
Alb (g/L)	33.90 (8.80)
PT (seconds)	16.65 (1.50)

HVPG: Hepatic venous pressure gradient; PPG: Portal pressure gradient; HBV: Hepatitis B virus; ALD: Alcoholic-related liver disease; IQR: Interquartile range; PLT: Platelet counts; TBIL: Total bilirubin; Alb: Albumin; PT: Prothrombin time.

Table 3 Measurement of right hepatic venous pressure gradient, middle hepatic venous pressure gradient and left hepatic venous pressure gradient, mean ± SD

	R-HVPG	M-HVPG	L-HVPG
All	16.43 ± 5.34	16.98 ± 5.69	15.29 ± 5.22
HBV	15.60 ± 5.13	16.65 ± 5.55	14.53 ± 5.21
ALD	17.65 ± 6.25	18.10 ± 5.99	15.74 ± 4.47
HBV + ALD	17.60 ± 3.89	16.30 ± 5.98	17.78 ± 6.34

HVPG: Hepatic venous pressure gradient; HBV: Hepatitis B virus; ALD: Alcoholic-related liver disease.

(Table 3, Figure 3D).

Agreement of R-HVPG, M-HVPG and L-HVPG

When analyzing the agreement between the R-HVPG and M-HVPG, an ICC of 0.70 (95%CI: 0.56-0.80) was obtained, suggesting a substantial agreement. The ICC was 0.56 (95%CI: 0.37-0.70) with a moderate agreement between R-HVPG and L-HVPG and 0.47 (95%CI: 0.27-0.63) with a moderate agreement between M-HVPG and L-HVPG (Table 4, Figure 3A).

In the patients with HBV, the ICC was 0.70 (0.51-0.82) with a substantial agreement between R-HVPG and M-HVPG, 0.56 (0.31-0.74) with a moderate agreement between R-HVPG and L-HVPG, 0.55 (0.31-0.72) with a moderate agreement between M-HVPG and L-HVPG (Table 4, Figure 3B). In the patients with ALD, the ICC was 0.63 (0.26-0.84) with a substantial agreement between R-HVPG and M-HVPG, 0.65 (0.33-0.83) with a substantial agreement between R-HVPG and L-HVPG, 0.42 (0.01-0.71) with a moderate agreement between M-HVPG and L-HVPG (Table 4, Figure 3C). In the patients with HBV + ALD, the ICC was 0.83 (0.63-0.93) with an almost-perfect between R-HVPG and M-HVPG, 0.22 (0-0.71) with a fair agreement between R-HVPG and L-HVPG, 0.26 (0-0.75) with a fair agreement between M-HVPG and L-HVPG (Table 4, Figure 3D).

Correlation and agreement between M-HVPG and PPG

In sub-study 2, the mean M-HVPG and PPG were 23.44 (4.29) and 23.63 (4.22), respectively. An ICC between M-HVPG

Table 4 Agreement of right hepatic venous pressure gradient, middle hepatic venous pressure gradient and left hepatic venous pressure gradient

	R-HVPG-M-HVPG	R-HVPG-L-HVPG	M-HVPG-L-HVPG
All	0.70 (0.56-0.80)	0.56 (0.37-0.70)	0.47 (0.27-0.63)
HBV	0.70 (0.51-0.82)	0.56 (0.31-0.74)	0.55 (0.31-0.72)
ALD	0.63 (0.26-0.84)	0.65 (0.33-0.83)	0.42 (0.01-0.71)
HBV + ALD	0.83 (0.63-0.93)	0.22 (0-0.71)	0.26 (0-0.75)

L-HVPG: Left hepatic venous pressure gradient; M-HVPG: Middle hepatic venous pressure gradient; R-HVPG: Right hepatic venous pressure gradient; HBV: Hepatitis B virus; ALD: Alcoholic-related liver disease.

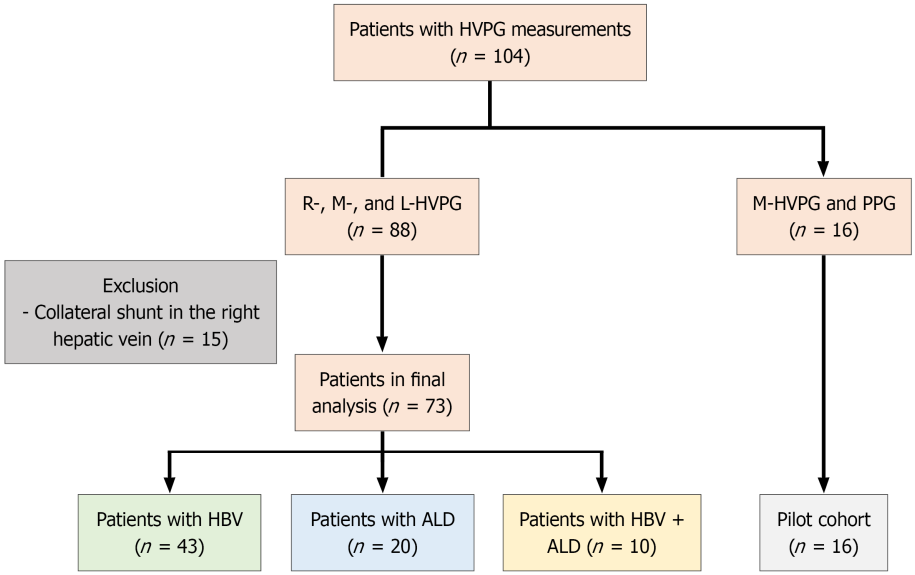


Figure 2 Flow diagram of study population. HVPG: Hepatic venous pressure gradient; PPG: Portal pressure gradient; HBV: Hepatitis B virus; ALD: Alcohol-related liver disease.

and PPG of 0.80 (95%CI: 0.52-0.92) was obtained, suggesting a substantial agreement, with one case (6.3%) outside the 1.96 SD according to the Bland-Altman plot. The ICC for absolute agreement (0.799) and consistency (0.801) remained the same, suggesting the absence of systematic error (Figure 4). Major discrepancies between the M-HVPG and PPG measurements were found in only 1/16 patients (6.3%). Diagnosis performance of M-HVPG, L-HVPG and their average for identifying R-HVPG ≥ 10, 12, and 16.20 mmHg.

The average of M-HVPG and L-HVPG showed the best performance than single M-HVPG and L-HVPG on determining R-HVPG ≥ 10 mmHg, with a cutoff of 12.75, AUC of 0.85 (95%CI: 0.67-1.00), specificity of 0.89 (95%CI: 0.67-1.00) and sensitivity of 0.86 (95%CI: 0.76-0.95); R-HVPG ≥ 12 mmHg, with a cutoff of 14.25, AUC of 0.83 (95%CI: 0.71-0.94), specificity of 0.71 (95%CI: 0.47-0.88) and sensitivity of 0.82 (95%CI: 0.70-0.92); R-HVPG ≥ 16 mmHg, with a cutoff of 17.75, AUC of 0.89 (95%CI: 0.81-0.97), specificity of 0.89 (95%CI: 0.79-0.97) and sensitivity of 0.76 (95%CI: 0.59-0.90); R-HVPG ≥ 20 mmHg, with a cutoff of 17.75, AUC of 0.90 (95%CI: 0.82-0.99), specificity of 0.74 (95%CI: 0.63-0.85) and sensitivity of 0.92 (95%CI: 0.77-1.00) (Supplementary Table 1, Supplementary Figure 1A). The AUCs of M-HVPG and L-HVPG were as follows: R-HVPG ≥ 10 mmHg, 0.78 (95%CI: 0.60-0.95) and 0.82 (95%CI: 0.62-1.00); R-HVPG ≥ 12 mmHg, 0.78 (95%CI: 0.65-0.91) and 0.79 (95%CI: 0.66-0.92); R-HVPG ≥ 16 mmHg, 0.87 (95%CI: 0.79-0.96) and 0.82 (95%CI: 0.71-0.92); R-HVPG ≥ 20 mmHg, 0.86 (95%CI: 0.75-0.98) and 0.83 (95%CI: 0.73-0.93), respectively (Supplementary Table 1, Supplementary Figure 1A).

We performed a subgroup analysis to explore the value of the M-HVPG and L-HVPG in detecting R-HVPG ≥ 10, 12, 16, and 20 mmHg in patients with HBV- and alcohol-related cirrhosis. In HBV patients, the AUCs of M-HVPG, L-HVPG and their average were as follows: R-HVPG ≥ 10 mmHg, 0.76 (95%CI: 0.41-1.00), 0.59 (95%CI: 0.08-1.00) and 0.84 (95%CI: 0.56-1.00); R-HVPG ≥ 12 mmHg, 0.91 (95%CI: 0.81-1.00), 0.90 (95%CI: 0.80-1.00) and 0.89 (95%CI: 0.78-1.00); R-HVPG ≥ 16 mmHg, 0.85 (95%CI: 0.73-0.97), 0.79 (95%CI: 0.64-0.94) and 0.88 (95%CI: 0.79-0.98); R-HVPG ≥ 20 mmHg, 0.92 (95%CI: 0.84-1.00), 0.75 (95%CI: 0.54-0.96) and 0.94 (0.86-1.00) (Supplementary Table 2, Supplementary Figure 1B). In ALD patients, the AUCs of M-HVPG, L-HVPG and their average were as follows: R-HVPG ≥ 10 mmHg, 0.78 (95%CI: 0.56-0.94), 0.96 (95%CI: 0.86-1.00) and 0.97 (95%CI: 0.90-1.00); R-HVPG ≥ 12 mmHg, 0.79 (95%CI: 0.51-1.00), 0.82 (95%CI: 0.59-1.00) and 0.79 (95%CI: 0.55-1.00); R-HVPG ≥ 16 mmHg, 0.84 (95%CI: 0.65-1.00), 0.80 (95%CI: 0.59-1.00) and 0.86 (95%CI:

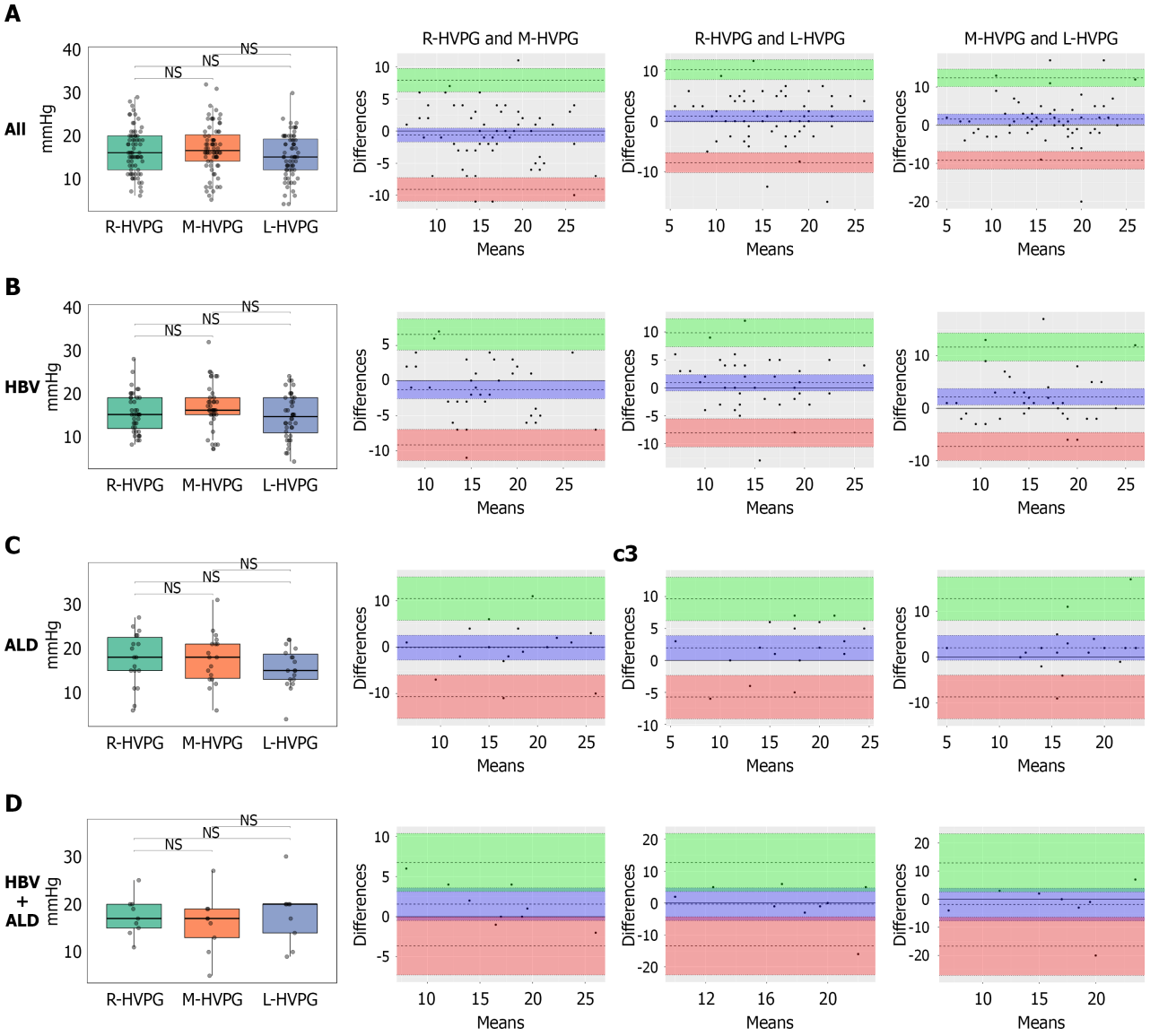


Figure 3 Bland-Altman plots and box plots of right hepatic venous pressure gradient, middle hepatic venous pressure gradient and left hepatic venous pressure gradient. A: Hepatic venous pressure gradient (HVPG) in all patients; B: HVPG in hepatitis B virus; C: HVPG in alcohol-related liver disease; D: HVPG in hepatitis B virus + alcohol-related liver disease. L-HVPG: Left hepatic venous pressure gradient; M-HVPG: Middle hepatic venous pressure gradient; R-HVPG: Right hepatic venous pressure gradient; HBV: Hepatitis B virus; ALD: Alcohol-related liver disease; NS: Not significant.

0.69-1.00); R-HVPG ≥ 20 mmHg, 0.85 (95%CI: 0.70-1.00), 0.85 (95%CI: 0.68-1.00) and 0.91 (0.77-1.00) (Supplementary Table 3, Supplementary Figure 1C).

DISCUSSION

Measurement of HVPG has become the cornerstone for managing advanced chronic liver disease. Following the Baveno VII consensus and landmark trials such as PREDESCI, HVPG-guided stratification should be encouraged in clinical trials investigating novel therapies[1,18-20]. Its prognostic value extends to predicting variceal hemorrhage, hepatocellular carcinoma risk, and post-resection outcomes[21,22]. However, the widespread application of this "gold standard" relies heavily on the technical feasibility of the procedure. While strict cut-offs (*e.g.*, 10, 12, and 16 mmHg) are well-established [23-28], they depend on obtaining accurate pressure readings, which can be challenging in real-world clinical settings.

Classically, the R-HVPG is the preferred access site for HVPG measurements because of its larger caliber and accessible trajectory. However, relying solely on the R-HVPG is not always feasible. Technical failure occurs in a significant proportion of patients owing to anatomical constraints, such as ostial stenosis or lobe atrophy. Furthermore, hemodynamic distortions, specifically intrahepatic venovenous shunts or arterialization of the hepatic vasculature, can compromise the accuracy of R-HVPG readings, leading to underestimation of the true portal pressure[29,30]. These irregularities often stem from the heterogeneous regional perfusion and structural remodeling inherent to cirrhotic livers. Consequently,

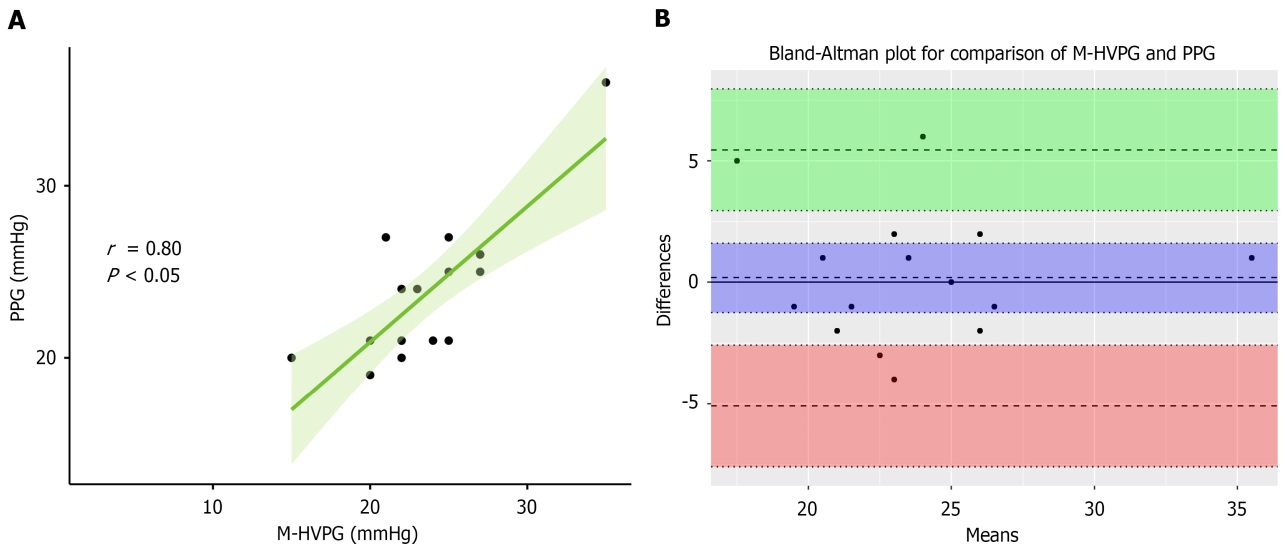


Figure 4 Middle-hepatic venous pressure gradient and portal pressure gradient measurement in the pilot cohort. A: Correlation between middle-hepatic venous pressure gradient and portal pressure gradient; B: Agreement between middle-hepatic venous pressure gradient and portal pressure gradient according to Bland-Altman plots. M-HVPG: Middle hepatic venous pressure gradient; PPG: Portal pressure gradient.

when R-HVPG catheterization is technically unsuccessful or yields unreliable traces, alternative access routes *via* the middle or left hepatic veins are necessary. However, the evidence confirming the interchangeability of measurements across these veins remains insufficient.

To date, literature addressing this issue is limited. A study by Keiding and Vilstrup[29] highlighted significant segmental variation, noting that relying on a single vein could misclassify risk in 13% of cases. More recently, Wang *et al*[31] reported poor correlation between HVPG and direct PPG across all three veins, although they noted that M-HVPG performed marginally better than the others. Unlike these previous reports, our study is the first prospective investigation to systematically compare HVPG measurements across all three hepatic veins within the same patient cohort, while also validating M-HVPG against direct PPG. This design allows for a more definitive assessment of whether the M-HVPG and L-HVPG are valid alternatives for risk stratification when the R-HVPG approach fails.

Our results demonstrated that M-HVPG was a superior alternative to R-HVPG compared with L-HVPG. The M-HVPG showed the highest technical success rate. That might benefit from the broader drainage territory in the middle hepatic vein and less affected by right posterior lobe atrophy or shunts. Furthermore, the agreement between the R-HVPG and M-HVPG was stronger than that between the R-HVPG and L-HVPG. While combining M-HVPG and L-HVPG measurements yielded the highest precision, the M-HVPG alone proved sufficiently robust for clinical use. The finding contradicted one previous study that suggested significant segmental variation. Crucially, in our pilot validation cohort, we observed substantial agreement between the M-HVPG and direct PPG, with major discrepancies occurring in only 6.3% of cases. This strongly suggests that the M-HVPG accurately reflects the actual portal pressure, overcoming the concerns of the poor correlation raised in prior studies.

Subgroup analysis stratified by etiology demonstrated that the consistency of HVPG measurements across the three hepatic veins remained irrelevant to the underlying liver disease. We observed no statistically significant differences in mean pressures among the R-HVPG, M-HVPG, and L-HVPG in patients with HBV, ALD, or HBV + ALD ($P > 0.05$). This suggests that the hemodynamic uniformity of the hepatic venous system was generally preserved across these major cirrhotic causes, validating the interchangeability of measurements in diverse patient populations. Given that HBV infection constituted the predominant etiology (58.9%), we further evaluated the diagnostic performance of the M-HVPG in this specific cohort. The M-HVPG demonstrated excellent diagnostic accuracy for identifying the key prognostic thresholds defined by the standard R-HVPG. The predictive value was particularly strong for severe portal hypertension (≥ 12 mmHg) and high-risk stages (≥ 20 mmHg), with AUCs exceeding 0.90. Notably, M-HVPG consistently outperformed L-HVPG in predicting these critical cut-offs (*e.g.*, AUC 0.92 *vs* 0.75 for ≥ 20 mmHg). These findings strongly support the clinical utility of the M-HVPG as a reliable surrogate for risk stratification in HBV-related cirrhosis when R-HVPG assessment is technically challenging.

Our study had several limitations inherent to its observational and cross-sectional design. First, the recruitment of patients from a single tertiary center who underwent complete hemodynamic evaluation might have introduced selection bias, potentially excluding those with subclinical portal hypertension. However, this bias was partially mitigated by our institution's strict protocol involving routine liver catheterization for cirrhotic workup. Second, the overall sample size was relatively small, particularly for the ALD subgroup. The cohort size was further constrained by the exclusion of patients with intrahepatic shunts, which precluded accurate HVPG measurement. Third, R-, M- and L-HVPG were measured in fixed order. The effect of fixed order for procedural duration, respiratory variation, contrast injection, or hemodynamic drift couldn't be assessed. Then, radiation dose and contrast volume were not recorded. It potentially led to more complications. Last but not the least, the etiological distribution was skewed, with a predominance of HBV and ALD. Consequently, our findings may not be generalizable to cirrhosis caused by other etiologies. Future multicenter

studies with larger cohorts and longitudinal follow-ups are needed to validate these findings and confirm their clinical utility.

CONCLUSION

In conclusion, our study demonstrated that the M-HVPG achieved the highest technical success rate, which supports the priority of technical feasibility of HVPG proposed in Baveno VII consensus. We observed no significant differences among the R-HVPG, M-HVPG, and L-HVPG and superior agreement with the R-HVPG compared to the L-HVPG, a pattern consistent in both HBV- and alcohol-related cirrhosis. Furthermore, the strong correlation between the M-HVPG and PPG suggests that the M-HVPG potentially reflected accurate clinical portal venous pressure. Consequently, we recommend that M-HVPG may be considered a preferred alternative when R-HVPG is unfeasible or unreliable, pending larger multicenter validation. Our results have important implications for individualizing hemodynamic assessments in patients with cirrhosis.

REFERENCES

1 **de Franchis R**, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. Baveno VII - Renewing consensus in portal hypertension. *J Hepatol* 2022; **76**: 959-974 [*RCA*] [PMID: 35120736 DOI: 10.1016/j.jhep.2021.12.022] [FullText] [Full Text(PDF)]

2 **Zipprich A**, Winkler M, Seufferlein T, Dollinger MM. Comparison of balloon vs. straight catheter for the measurement of portal hypertension. *Aliment Pharmacol Ther* 2010; **32**: 1351-1356 [*RCA*] [PMID: 21050237 DOI: 10.1111/j.1365-2036.2010.04484.x] [FullText]

3 **Bosch J**, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 573-582 [*RCA*] [PMID: 19724251 DOI: 10.1038/nrgastro.2009.149] [FullText]

4 **Kaplan DE**, Ripoll C, Thiele M, Fortune BE, Simonetto DA, Garcia-Tsao G, Bosch J. AASLD Practice Guidance on risk stratification and management of portal hypertension and varices in cirrhosis. *Hepatology* 2024; **79**: 1180-1211 [*RCA*] [PMID: 37870298 DOI: 10.1097/HEP.0000000000000647] [FullText]

5 **Garcia-Tsao G**, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. *Hepatology* 2017; **65**: 310-335 [*RCA*] [PMID: 27786365 DOI: 10.1002/hep.28906] [FullText]

6 **Ripoll C**, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS, Bosch J; Portal Hypertension Collaborative Group. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481-488 [*RCA*] [PMID: 17681169 DOI: 10.1053/j.gastro.2007.05.024] [FullText]

7 **Bosch J**, García-Pagán JC. Prevention of variceal rebleeding. *Lancet* 2003; **361**: 952-954 [*RCA*] [PMID: 12648985 DOI: 10.1016/S0140-6736(03)12778-X] [FullText]

8 **Merkel C**, Bolognesi M, Sacerdoti D, Bombonato G, Bellini B, Bighin R, Gatta A. The hemodynamic response to medical treatment of portal hypertension as a predictor of clinical effectiveness in the primary prophylaxis of variceal bleeding in cirrhosis. *Hepatology* 2000; **32**: 930-934 [*RCA*] [PMID: 11050041 DOI: 10.1053/jhep.2000.19322] [FullText]

9 **Abraldes JG**, Villanueva C, Bañares R, Aracil C, Catalina MV, Garci A-Pagán JC, Bosch J; Spanish Cooperative Group for Portal Hypertension and Variceal Bleeding. Hepatic venous pressure gradient and prognosis in patients with acute variceal bleeding treated with pharmacologic and endoscopic therapy. *J Hepatol* 2008; **48**: 229-236 [*RCA*] [PMID: 18093686 DOI: 10.1016/j.jhep.2007.10.008] [FullText]

10 **Moitinho E**, Escorsell A, Bandi JC, Salmerón JM, García-Pagán JC, Rodés J, Bosch J. Prognostic value of early measurements of portal pressure in acute variceal bleeding. *Gastroenterology* 1999; **117**: 626-631 [*RCA*] [PMID: 10464138 DOI: 10.1016/s0016-5085(99)70455-5] [FullText]

11 **Suk KT**. Hepatic venous pressure gradient: clinical use in chronic liver disease. *Clin Mol Hepatol* 2014; **20**: 6-14 [*RCA*] [PMID: 24757653 DOI: 10.3350/cmh.2014.20.1.6] [FullText] [Full Text(PDF)]

12 **Rodrigues SG**, Delgado MG, Stirnimann G, Berzigotti A, Bosch J. Hepatic Venous Pressure Gradient: Measurement and Pitfalls. *Clin Liver Dis* 2024; **28**: 383-400 [*RCA*] [PMID: 38945633 DOI: 10.1016/j.cld.2024.03.009] [FullText]

13 **Ma J**, Gong X, Luo J, Gu J, Yan Z, Zhang W, Li C, Zhang Z, Yang M. Impact of Intrahepatic Venovenous Shunt on Hepatic Venous Pressure Gradient Measurement. *J Vasc Interv Radiol* 2020; **31**: 2081-2088 [*RCA*] [PMID: 33261743 DOI: 10.1016/j.jvir.2020.08.027] [FullText]

14 **Imai Y**, Koizumi Y, Hiasa Y, Hirooka M, Tokumoto Y, Yoshida O, Chikamori F. Standard technique in Japan for measuring hepatic venous pressure gradient. *J Gastroenterol* 2025; **60**: 24-31 [*RCA*] [PMID: 39652102 DOI: 10.1007/s00535-024-02182-z] [FullText] [Full Text(PDF)]

15 **Chinese Portal Hypertension Alliance (CHESS)**; Minimally Invasive Intervention Collaborative Group, Chinese Society of Gastroenterology. [Consensus on clinical application of hepatic venous pressure gradient in China (2023 edition)]. *Zhonghua Yi Xue Za Zhi* 2023; **103**: 3885-3895 [*RCA*] [PMID: 38129165 DOI: 10.3760/cma.j.cn112137-20230831-00342] [FullText]

16 **Sørensen M**, Larsen LP, Villadsen GE, Aagaard NK, Grønbæk H, Keiding S, Vilstrup H. β-Blockers Improve Presinusoidal Portal Hypertension. *Dig Dis Sci* 2018; **63**: 3153-3157 [*RCA*] [PMID: 30003386 DOI: 10.1007/s10620-018-5186-1] [FullText] [Full Text(PDF)]

17 **Landis JR**, Koch GG. The Measurement of Observer Agreement for Categorical Data. *Biometrics* 1977; **33**: 159-174 [*RCA*] [DOI: 10.2307/2529310] [FullText]

18 **Reiniš J**, Petrenko O, Simbrunner B, Hofer BS, Schepis F, Scoppettuolo M, Saltini D, Indulti F, Guasconi T, Albillos A, Téllez L, Villanueva C, Brujats A, Garcia-Pagan JC, Perez-Campuzano V, Hernández-Gea V, Rautou PE, Moga L, Vanwolleghem T, Kwanten WJ, Francque S, Trebicka J, Gu W, Ferstl PG, Gluud LL, Bendtsen F, Møller S, Kubicek S, Mandorfer M, Reiberger T. Assessment of portal hypertension severity using machine learning models in patients with compensated cirrhosis. *J Hepatol* 2023; **78**: 390-400 [*RCA*] [PMID: 36152767 DOI: 10.1016/j.jhep.2022.09.012] [FullText]

19 **Villanueva C**, Albillos A, Genescà J, Garcia-Pagan JC, Calleja JL, Aracil C, Bañares R, Morillas RM, Poca M, Peñas B, Augustin S, Abraldes JG, Alvarado E, Torres F, Bosch J. β blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension

(PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2019; **393**: 1597-1608 [*RCA*] [PMID: 30910320 DOI: 10.1016/S0140-6736(18)31875-0] [FullText]

20 **Villanueva C**, Torres F, Sarin SK, Shah HA, Tripathi D, Brujats A, Rodrigues SG, Bhardwaj A, Azam Z, Hayes PC, Jindal A, Abid S, Alvarado E, Bosch J; Carvedilol-IPD-MA-group and the Baveno Cooperation: an EASL Consortium. Carvedilol reduces the risk of decompensation and mortality in patients with compensated cirrhosis in a competing-risk meta-analysis. *J Hepatol* 2022; **77**: 1014-1025 [*RCA*] [PMID: 35661713 DOI: 10.1016/j.jhep.2022.05.021] [FullText]

21 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [*RCA*] [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013] [FullText]

22 **Ripoll C**, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS; Portal Hypertension Collaborative Group. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009; **50**: 923-928 [*RCA*] [PMID: 19303163 DOI: 10.1016/j.jhep.2009.01.014] [FullText] [Full Text(PDF)]

23 **Burroughs AK**, Groszmann R, Bosch J, Grace N, Garcia-Tsao G, Patch D, Garcia-Pagan JC, Dagher L. Assessment of therapeutic benefit of antiviral therapy in chronic hepatitis C: is hepatic venous pressure gradient a better end point? *Gut* 2002; **50**: 425-427 [*RCA*] [PMID: 11839726 DOI: 10.1136/gut.50.3.425] [FullText] [Full Text(PDF)]

24 **Vinel JP**, Cassigneul J, Levade M, Voigt JJ, Pascal JP. Assessment of short-term prognosis after variceal bleeding in patients with alcoholic cirrhosis by early measurement of portohepatic gradient. *Hepatology* 1986; **6**: 116-117 [*RCA*] [PMID: 3484713 DOI: 10.1002/hep.1840060122] [FullText]

25 **Garcia-Tsao G**, Groszmann RJ, Fisher RL, Conn HO, Atterbury CE, Glickman M. Portal pressure, presence of gastroesophageal varices and variceal bleeding. *Hepatology* 1985; **5**: 419-424 [*RCA*] [PMID: 3873388 DOI: 10.1002/hep.1840050313] [FullText]

26 **Suk KT**, Kim HC, Namkung S, Han SH, Choi KC, Park SH, Sung HT, Kim CH, Kim SH, Ham YL, Kang HM, Kim DJ. Diagnostic accuracy of hepatic venous pressure gradient measurement in the prediction of stage 1 compensated liver cirrhosis in patients with chronic hepatitis B. *Eur J Gastroenterol Hepatol* 2013; **25**: 1170-1176 [*RCA*] [PMID: 23603784 DOI: 10.1097/MEG.0b013e3283613139] [FullText]

27 **Garcia-Tsao G**, Friedman S, Iredale J, Pinzani M. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. *Hepatology* 2010; **51**: 1445-1449 [*RCA*] [PMID: 20077563 DOI: 10.1002/hep.23478] [FullText] [Full Text(PDF)]

28 **Ripoll C**. Hepatic venous pressure gradient and outcomes in cirrhosis. *J Clin Gastroenterol* 2007; **41** Suppl 3: S330-S335 [*RCA*] [PMID: 17975485 DOI: 10.1097/MCG.0b013e318150d0f4] [FullText]

29 **Keiding S**, Vilstrup H. Intrahepatic heterogeneity of hepatic venous pressure gradient in human cirrhosis. *Scand J Gastroenterol* 2002; **37**: 960-964 [*RCA*] [PMID: 12229973 DOI: 10.1080/003655202760230946] [FullText]

30 **Maharaj B**, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, Pudifin DJ. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* 1986; **1**: 523-525 [*RCA*] [PMID: 2869260 DOI: 10.1016/s0140-6736(86)90883-4] [FullText]

31 **Wang HY**, Song QK, Yue ZD, Wang L, Fan ZH, Wu YF, Dong CB, Zhang Y, Meng MM, Zhang K, Jiang L, Ding HG, Zhang YN, Yang YP, Liu FQ. Correlation of pressure gradient in three hepatic veins with portal pressure gradient. *World J Clin Cases* 2022; **10**: 4460-4469 [*RCA*] [PMID: 35663094 DOI: 10.12998/wjcc.v10.i14.4460] [FullText] [Full Text(PDF)]

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Observational Study

Prevalence and risk factors for metabolic dysfunction-associated steatotic liver disease and fibrosis in recently diagnosed type 2 diabetes

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Abstract

BACKGROUND

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease and the leading cause of liver-related mortality. Long-standing type 2 diabetes mellitus (T2DM) is a major risk factor for liver steatosis and fibrosis; however, whether recently diagnosed T2DM carries the same risk remains unclear. Few studies have addressed this question in Hispanic populations, who are known to exhibit faster progression of liver disease.

AIM

To assess the prevalence of MASLD and liver fibrosis in patients with recent-onset T2DM and to identify associated risk factors.

METHODS

This was a cross-sectional study including individuals with T2DM diagnosed within the past 5 years and without disabling micro or macrovascular complications. Clinical, biochemical, liver stiffness measurement (LSM), controlled attenuation parameter (CAP), and FibroScan-AST (FAST) score were evaluated.

RESULTS

One thousand one hundred and thirty-seven patients were included, 60.9% were women, median age was 55 years (inter quartile range: 49-63) and 78.2% were overweight or obese. Overall prevalence of MASLD (using the > 288 dB/m cutoff) was 41.4%, and 10% had clinically significant liver fibrosis (LSM ≥ 8 kPa). Moreover, 3% of the population had cirrhosis. Multivariate analysis showed higher body mass index (BMI), glycated hemoglobin, triglycerides and alanine aminotransferase (ALT) were independently associated with steatosis, while higher BMI, CAP, gamma-glutamyl transferase, ALT and aspartate aminotransferase were associated with fibrosis. According to the FAST score, 4.8% of patients had at-risk metabolic dysfunction-associated steatohepatitis.

CONCLUSION

There is a high burden of MASLD and clinically significant fibrosis among adults with recently diagnosed T2DM. These findings strongly support the systematic evaluation for MASLD and liver fibrosis in this population from the time of diabetes diagnosis.

Key Words: Metabolic dysfunction-associated steatotic liver disease; Type 2 diabetes mellitus; Steatotic liver disease; Liver fibrosis

Core Tip: There is scarce data specifically evaluating the prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) and liver fibrosis in patients with recent-onset type 2 diabetes (< 5 years since diagnosis) and without micro or macrovascular complications. The Hispanic population is regarded as high-risk for metabolic diseases, including MASLD. We found a high prevalence of MASLD, affecting 41.4% of the study population, with 10% presenting clinically significant liver fibrosis (liver stiffness measurement ≥ 8 kPa) and 3% having cirrhosis. Our findings support incorporating liver health screening into the routine evaluation of patients with type 2 diabetes mellitus from diagnosis, even in those with good glycemic control and without chronic complications.

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INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of chronic liver disease and one of the leading causes of liver-related mortality, with an estimated overall prevalence of 30% in the adult population[1, 2]. A 2022 meta-analysis reported an increase in global prevalence from 25.5% in 2005 to 37.8% in 2016, with an estimated incidence of 46.9 cases per 1000 person/years[3]. MASLD prevalence appears to differ across ethnic groups; in United States studies, Hispanic individuals exhibit higher rates (around 23%) than White (14%) and Black (13%) populations; however, specific data on Hispanic populations outside the United States is still limited[1].

MASLD is strongly associated with metabolic disorders, including dyslipidemia, central obesity, hypertension, obstructive sleep apnea-hypopnea syndrome, chronic kidney disease, cardiovascular disease, insulin resistance, and especially diabetes. Diabetes is a major risk factor for both liver steatosis and fibrosis. Notably, metabolic dysfunction-associated steatohepatitis (MASH) prevalence in this population exceeds 33%, with approximately 17% of patients presenting advanced liver fibrosis[4,5].

Liver fibrosis in MASLD is the strongest predictor of adverse clinical outcomes, including liver-related and extrahepatic mortality. The risk of liver-related death rises with each fibrosis stage (F1 to F4) and is the primary determinant of outcomes in MASLD patients[6-9].

Although the risk of liver steatosis and fibrosis in diabetes is well-documented, data on the Mexican population remains limited. This is particularly relevant given the high prevalence of risk polymorphisms in Hispanics. Moreover, most studies have not assessed MASLD prevalence in newly diagnosed type 2 diabetes mellitus (T2DM) patients. This is crucial, as the likelihood of advanced fibrosis increases with diabetes duration. Early-stage T2DM presents an opportunity for intensive treatment, which can yield long-term benefits by modifying liver outcomes, slowing disease progression, and ultimately improving clinical outcomes by reducing the risk of cirrhosis, its complications, and both liver-related and non-liver-related mortality[10].

Therefore, the aim of this study was to determine the prevalence and risk factors associated with liver steatosis, fibrosis, and at-risk MASH using controlled attenuation parameter (CAP) and vibration-controlled transient elastography (VCTE) in patients with recently diagnosed T2DM.

MATERIALS AND METHODS

Study design

This is a cross-sectional study carried out at a tertiary hospital in Mexico City from January 2022 to January 2024. The study followed the principles outlined in the Declaration of Helsinki and was approved by the Research Ethics Committee of the institution (No. 2985).

Selection of patients

Patients older than 18 years with a recent diagnosis (less than 5 years) of T2DM, without disabling complications (blindness, renal failure, stroke, limb amputations, ischemic heart disease), who attended our specialized diabetes care clinic, were included. Patients with a history of liver diseases, including viral hepatitis, autoimmune liver diseases, and alcohol-related liver disease, were excluded from the analysis.

Specialized diabetes care clinic-center of comprehensive care for the patient with diabetes

The center of comprehensive care for the patient with diabetes clinic is a program aimed at improving T2DM management and preventing complications. It serves patients aged 18 to 70, diagnosed within the past 5 years. The clinic offers a multidisciplinary approach, providing self-care tools, preventing complications, and supporting long-term diabetes management. Patients receive care from endocrinologists, diabetes educators, nutritionists, psychologists, psychiatrists, dental specialists, physical activity instructors, ophthalmologists, and foot care specialists. Treatment is tailored by the attending physician without restrictions on antidiabetic drugs.

During the participants' assessment, psychologists conducted a structured interview to evaluate the quantity and frequency of alcohol consumption. Moderate alcohol consumption was defined as 140 g/week in women and 210 g/week in men.

VCTE

All patients included in the study underwent VCTE performed by one of three highly experienced physicians (each with more than 1500 studies). Evaluations were conducted using a FibroScan® Expert 630 device (Echosens, Paris, France) and met the quality criteria required for valid liver stiffness measurement (LSM) and CAP measurements, with interquartile range (IQR) < 20%[11,12].

To evaluate the presence of steatosis, we used a CAP cutoff of ≥ 288 dB/m, as recommended by the American Association for the Study of Liver Diseases (AASLD) guidelines[12]. We further categorized the patients according to the risk for liver fibrosis as low (< 8 kPa), intermediate (8-12 kPa), and high-risk (> 12 kPa)[2]. Accordingly, we defined clinically significant liver fibrosis as a LSM ≥ 8 kPa. Specifically, a LSM ≥ 13.6 kPa cutoff was used to define F4 (LSM compatible with cirrhosis).

In addition to VCTE, the FibroScan-AST (FAST) score was calculated as previously reported[13]. This score incorporates values of LSM, CAP, and serum aspartate aminotransferase (AST) concentrations, with the aim of non-invasively

identifying patients with suspected MASH who are at risk of progression. Two cut-off points were used in prior studies: ≤ 0.35 to rule out its presence (with 90% sensitivity) and ≥ 0.67 to rule it in (with 90% specificity)[13,14]. An adjusted cut-off point of ≥ 0.5 was also included for patients with glycated hemoglobin (HbA1c) ≥ 6.5% in the analysis, due to the higher proportion of patients at-risk MASH when HbA1c ≥ 6.5%[15]. The rationale for this adjustment is that patients with T2DM have increased oxidative and endoplasmic reticulum stress, intestinal dysbiosis, and adipose tissue dysfunction, promoting liver steatosis and fibrosis[16].

In the present study, information from studies under the definition of NAFLD was used, corresponding to the recent consensus definition of MASLD.

Biochemical variables

Laboratory tests were performed after an 8-hour fasting period. Samples were immediately centrifuged, and blood chemistry, alanine aminotransferase (ALT), AST, gamma-glutamyl transferase (GGT), lipid profile, and HbA1c levels were determined on the same day, following the quality standards set by our central laboratory, accredited by the College of American Pathologists.

Statistical analysis

The sample size was calculated based on a prevalence of liver steatosis of 35% in patients without diabetes and 40% in patients with diabetes. Using alpha and beta errors of 5% and 80%, respectively, the required sample size was 742 patients. After accounting for a 10% loss, the final sample size was adjusted to 817 patients. However, a total of 1337 patients were included in this study.

Kolmogorov-Smirnov test was used to evaluate data distribution. To describe the characteristics of the population, means ± SD and medians (IQR) were used for quantitative data, while proportions and relative frequencies were used for categorical variables. To compare groups based on the presence of steatosis and liver fibrosis, either the student *t*-test or Mann-Whitney *U* test was used, according to the distribution of the data. For categorical variables, the χ^2 test or Fisher's exact test was used as appropriate. Logistic regression analysis was performed to establish the factors independently associated with the presence of steatosis and liver fibrosis. Variables were selected a priori, based on clinical and biological plausibility. To evaluate the validity of the multivariable models (Supplementary Table 1) collinearity among covariables was assessed using variance inflation factors, model performance was evaluated using the number of events per variable, discrimination using χ^2 statistic with 95% confidence interval, and calibration slope. To obtain estimates corrected for optimism, internal validation was performed using 1000 bootstrap resamples. A P value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 25 (IBM, Armonk NY, United States) and STATA version 12.1 (Stata Corp LLC, College Station, TX, United States).

RESULTS

General characteristics of the study population

A total of 1337 patients with a recent diagnosis of T2DM were included in the analysis. The majority of the patients were women (n = 814, 60.9%), with a median age of 55 years, and only 21.8% of the population had normal body mass index (BMI) (< 25 kg/m^2).

Alcohol consumption within the population

Regarding alcohol consumption, 74.3% of the study population denied any alcohol intake. Only six patients (0.5%) had a history of drinking more than three days per week. The median (p25-p75) alcohol intake in the population was 0 (0-7.2) g/week. A total of 50 patients (3.9%) reported consuming alcohol at least once per week, with a median (p25-p75) alcohol consumption of 28.6 (15.9-48.4) g/week. Nine patients (7 men and two women) had moderate alcohol consumption, corresponding to 0.6% of the population, and were classified as MASLD and increased alcohol intake. Therefore, most of the cohort corresponded to MASLD.

Prevalence and risk factors associated with the presence of liver steatosis

The prevalence of MASLD was 41.4%, (554 patients), Table 1 presents the characteristics of the study population based on the absence or presence of MASLD. Patients with steatosis were younger and had a higher BMI; notably, they also had higher LSM and FAST scores.

Patients with liver steatosis also exhibited significant differences in blood chemistry values, including higher levels of triglycerides, ALT, AST, GGT, and HbA1c, as well as lower serum high-density cholesterol (HDL) levels and higher total and low-density cholesterol (LDL) cholesterol levels.

Prevalence and risk factors associated with the presence of clinically significant liver fibrosis

Table 2 shows the characteristics of the study population based on the fibrosis risk assessment. The prevalence of fibrosis was 10%, with 133 patients having LSM 8 kPa indicating clinically significant liver fibrosis (F2). Remarkably, 40 patients (3%) met the criteria for cirrhosis (F4). None of these patients had a prior diagnosis of liver disease before this assessment.

Patients with clinically significant fibrosis had higher BMI and CAP values than those without fibrosis. Additionally, they had higher ALT, AST, and GGT concentrations and lower total cholesterol levels. LDL cholesterol, HDL cholesterol, triglycerides, and hemoglobin A1c levels showed no significant differences.

Table 1 Characteristics of the study population according to presence or absence of liver steatosis, *n* (%)

	Total (*n* = 1337)	No steatosis (*n* = 783)	Steatosis (*n* = 554)	*P* value
Sex (female)	814 (60.9)	468 (59.8)	346 (62.5)	0.322
Age (years)	55 (49-63)	56 (49-63)	54 (48-61)	0.003
Classification according to BMI				
BMI (kg/m^2), median (p25-p75)	28.3 (25.4-31.5)	27.1 (24.5-30.1)	30.4 (27.2-33.3)	< 0.001
Normal	276 (21.8)	217 (29.3)	59 (11.2)	< 0.001
Overweight	510 (40.2)	325 (43.9)	185 (35.0)	
Obesity class I	346 (27.3)	153 (20.7)	193 (36.6)	
Obesity class II	99 (7.8)	35 (4.7)	64 (12.1)	
Obesity class III	37 (2.9)	10 (1.4)	27 (5.1)	
Transient elastography				
kPa	4.8 (3.8-6.0)	4.5 (3.5-5.6)	5.2 (4.2-6.6)	< 0.001
CAP (dB/m)	276 (234-317)	242 (214-266)	324 (305-346)	< 0.001
FAST score	0.07 (0.03-0.18)	0.05 (0.02-0.10)	0.15 (0.06-0.29)	< 0.001
Low risk	984 (88.0)	628 (94.3)	356 (78.8)	< 0.001
Moderate risk	109 (9.7)	32 (4.8)	77 (17.0)	
High risk	25 (2.2)	6 (0.9)	19 (4.2)	
FAST-HbA1c	54 (4.8)	11 (1.7)	43 (9.5)	< 0.001
Biochemical parameters				
Total cholesterol, mg/dL	165 (137-193)	163 (134-190)	168 (140-197)	0.036
LDL, mg/dL	98 (74-120)	96 (73-117)	100 (77-124)	0.022
HDL, mg/dL	42 (36-51)	43 (37-52)	40 (36-48)	0.000
TGC, mg/dL	139(104-194)	127 (97-177)	157 (117-224)	0.000
Creatinine, mg/dL	0.76(0.65-0.90)	0.77 (0.67-0.90)	0.73 (0.63-0.89)	0.010
ALT, U/L	23.5(17.0-34.0)	20.7 (15.7-30.3)	28.6 (19.4-41.9)	0.000
AST, U/L	20.7 (16.6-26.9)	19.4 (16.0-25.0)	22.3 (17.7-29.2)	0.000
GGT, U/L	25.9(18.4-40.4)	22.7 (17.2-33.5)	31.8 (22.2-49.2)	0.000
HbA1c, %	6.8 (6.0-8.5)	6.6 (6.0-8.4)	7.0 (6.1-8.7)	0.018

The results are expressed as median (p25-p75). Low risk (< 0.35), moderate risk (0.35-0.66), high risk (0.67). BMI: Body mass index; FAST: FibroScan-AST; LDL: Low-density cholesterol; HDL: High-density cholesterol; TGC: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; CAP: Controlled attenuation parameter; HbA1c: Glycated hemoglobin.

Lean MASLD

Supplementary Table 2 shows the characteristics of patients with lean MASLD (10.6% of patients with MASLD) compared with MASLD, apart from the expected difference in BMI, there were no significant differences. Female gender was predominantly seen in both categories, kPa and FAST score were similar, and there was a non-significant trend towards higher CAP in MASLD compared with lean MASLD, but this seems as a non-clinically significant trend.

Apart from that, there were only trends towards significance in age and total cholesterol, both being higher in the lean MASLD group.

Non-invasive evaluation according to HbA1C levels

Table 3 presents the characteristics of the study population based on glycemic control categories. The population was categorized into adequate (HbA1c ≤ 7%) and inadequate (HbA1c > 7%) glycemic control groups. Most patients (53.3%) had adequate glycemic control. Patients with inadequate glycemic control had higher liver stiffness and CAP values. As expected, they also had higher cholesterol and triglyceride levels, lower HDL, and elevated GGT levels.

To further investigate the impact of glycemic control on TE-related parameters, an analysis using different HbA1c cutoffs is presented in Supplementary Table 3. Notable findings include a higher proportion of male patients among

Table 2 Characteristics of the study population according to the risk of liver fibrosis, *n* (%)

	< 8 kPa (*n* = 1204)	8-12 kPa (*n* = 77)	> 12 kPa (*n* = 56)	*P* value
Sex (female)	729 (60.5)	52 (67.5)	33 (58.9)	0.455
Age (years)	55 (49-63)	56 (48.5-62)	58 (52-64)	0.167
BMI (kg/m^2)	27.9 (25.2-31.0)	31.8 (28.2-36.2)	31.4 (27.8-35.0)	< 0.001[a,b]
BMI classification				
Normal	274 (22.8)	9 (11.7)	8 (14.3)	< 0.001
Overweight	510 (42.4)	20 (26)	14 (25)	
Obesity	317 (26.3)	25 (32.5)	20 (35.7)	
Obesity class II	78 (6.5)	16 (20.8)	8 (14.3)	
Obesity class III	25 (2.1)	7 (9.1)	6 (10.7)	
Transient elastography				
kPa	4.6 (3.7-5.6)	9 (8.5-10.3)	16.5 (13.4-21.8)	< 0.001[a,b,c]
CAP (dB/m)	273 (231-314)	303 (266-330)	298 (257 – 349)	< 0.001[a,b]
FAST score[1]	0.06 (0.03-0.14)	0.41 (0.21-0.59)	0.56 (0.41-0.77)	< 0.001[a,b,c]
Low risk	947 (94)	31 (47.7)	(13)	
Moderate risk	59 (5.9)	29 (44.6)	(45.7)	
High risk	1 (0.1)	5 (7.7)	19 (41.3)	
FAST-HbA1c	8 (0.7)	20 (26)	26 (46.4)	< 0.001
Biochemical parameters				
Total cholesterol, mg/dL	166 (138-195)	161 (131-180)	152 (128-177)	0.028[b]
LDL, mg/dL	98 (75-121)	101 (68-116)	91 (68-104)	0.124
HDL, mg/dL	42 (36-51)	39 (35-47)	42 (35-48)	0.085
TGC, mg/dL	140 (103-195)	122 (102-189)	135 (114-181)	0.666
Creatinine mg/dL	0.77 (0.65-0.90)	0.73 (0.62-0.84)	0.73 (0.59-0.88)	0.107
ALT, U/L	22.4 (16.5-32.4)	39.9 (26.3-65.3)	40.2 (30.4-74.1)	< 0.001[a,b]
AST, U/L	19.7 (16.2-25.2)	30.5 (22.2-51.4)	33.5 (28.3-57.3)	< 0.001[a,b]
GGT, U/L	24.8 (17.9-35.8)	42.3 (28.9-64.6)	85.9 (51.1-144.8)	< 0.001[a,b,c]
HbA1c, %	6.7 (6-8.5)	7.1 (6.3 – 8.9)	7.2 (6.2-9.4)	0.074

[1]Available in 1118 patients.
[a]*P* < 0.05 *vs* group 1 and 2.
[b]*P* < 0.05 *vs* group 1 and 3.
[c]*P* < 0.05 *vs* group 2 and 3.
Group 1 (< 8 kPa), group 2 (8-12 kPa) and group 3 (> 12 kPa). The results are expressed as median (p25-p75). BMI: Body mass index; FAST: FibroScan-AST; LDL: Low density cholesterol; HDL: High density cholesterol; TGC: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; CAP: Controlled attenuation parameter; HbA1c: Glycated hemoglobin.

those with elevated HbA1c levels. Additionally, liver stiffness showed a gradual, though insignificant, increase, while CAP levels and high-risk FAST scores were higher in these groups.

In terms of liver chemistry, GGT levels exhibited a stepwise increase as HbA1c levels rose. Although differences in transaminase levels were observed across groups, no specific trend associated with HbA1c levels was identified.

Multivariate analysis

Independent risk factors for the presence of liver steatosis: Table 4 presents the results of a logistic regression analysis evaluating risk factors independently associated with liver steatosis. In the Univariate analysis, age, BMI class, HbA1c ≥ 6.5%, triglycerides, LDL-C, and abnormal transaminases were associated with the presence of liver steatosis.

The multivariate analysis identified BMI class, triglycerides, HbA1c ≥ 6.5%, and elevated ALT (as defined by guideline cutoffs) as the main factors statistically associated with the presence of liver steatosis.

Table 3 Characteristics of the study population according to control of type 2 diabetes mellitus according to glycated hemoglobin values, *n* (%)

	≤ 7% HbA1c (*n* = 649)	> 7% HbA1c (*n* = 508)	*P* value
Sex (female)	420 (64.7)	271 (53.3)	< 0.001
Age (years)	56 (49-63)	54 (47-62)	0.060
BMI (kg/m^2)	28.1 (25.3-31.5)	28.4 (25.4-31.5)	0.621
BMI classification			
Normal	135 (21.8)	102 (21.3)	0.496
Overweight	252 (40.8)	192 (40.0)	
Obesity class I	169 (27.3)	130 (27.1)	
Obesity class II	51 (8.3)	39 (8.1)	
Obesity class III	11 (1.8)	17 (3.5)	
Transient elastography			
kPa	4.6 (3.7-5.9)	4.9 (3.9-6.2)	0.015
CAP (dB/m)	271 (231-314)	282 (237-320)	0.040
FAST score	0.08 (0.03-0.17)	0.07(0.03-0.19)	0.530
Low risk	530 (87.9)	430 (88.3)	0.077
Moderate risk	64 (10.6)	41 (8.4)	
High risk	9 (1.5)	16 (3.3)	
Biochemical parameters			
Total cholesterol, mg/dL	163 (137-186)	171 (138-200)	0.002
LDL, mg/dL	97 (75-117)	99 (75-125)	0.086
HDL, mg/dL	43 (37-51)	40 (35-49)	< 0.001
TGC, mg/dL	129 (98-175)	159 (115-233)	< 0.001
Creatinine, mg/dL	0.76 (0.66-0.90)	0.76 (0.64-0.89)	0.297
ALT, U/L	22.7 (16.7-33.5)	24.1 (17.2-34.8)	0.091
AST, U/L	20.9 (17.2-27.3)	20.0 (15.7-26.6)	0.027
GGT, U/L	23.8 (17.5-35.6)	28.9 (20.4-45.5)	< 0.001
HbA1c, %	6.1(5.7-6.5)	8.9 (7.7-10.5)	< 0.001

The results are expressed as median (p25-p75). 1157 patients had HbA1c determined at the time of elastography. BMI: Body mass index; FAST: FibroScan-AST; LDL: Low density cholesterol; HDL: High density cholesterol; TGC: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; CAP: Controlled attenuation parameter; HbA1c: Glycated hemoglobin.

Independent risk factors for the presence of clinically significant liver fibrosis: To identify independent risk factors associated with the presence of clinically significant liver fibrosis (LSM ≥ 8kPa), a logistic regression analysis was conducted, as shown in Table 5. The final model identified BMI class, the presence of liver steatosis (as defined by CAP), GGT levels, and elevated ALT and AST (according to guideline cutoffs) as the main predictors of liver fibrosis in the cohort. Among these, elevated transaminases showed the highest odds ratios.

Supplementary Table 4 shows a sensitivity analysis of clinically significant fibrosis using alternative liver stiffness thresholds.

Prevalence of altered transaminases and GGT levels in the population

We evaluated the prevalence of altered transaminase levels based on guideline-recommended thresholds: 33 U/L for ALT in men and 25 U/L in women[17]. Elevated ALT levels were found in 32.6% of men, 41% of women, and 37.6%of the total population. AST levels exceeded the reference range (13-39 U/L) in 9.5% of the population, while GGT levels were above the local laboratory reference range (6-64 U/L) in 9.1%.

Overall, 40.6% of patients had abnormal ALT, AST, or GGT levels. Among them, 9.8% had abnormalities in two markers, while 3.0% had abnormalities in all three.

Table 4 Logistic regression to evaluate the risk factors associated with presence of liver steatosis

Univariate	OR	95%CI	*β*	*P* value
Age (years)	0.988	0.977-0.999	-0.12	0.027
BMI class	1.890	1.665-2.146	0.637	< 0.001
Sex	0.960	0.763-1.208	-0.041	0.727
HbA1c ≥ 6.5%	1.547	1.205-1.986	0.436	< 0.001
Triglycerides (mg/dL)	1.003	1.002-1.005	0.003	< 0.001
LDL cholesterol (mg/dL)	1.004	1.000-1.007	0.004	0.034
High AST	2.304	1.542-3.442	0.835	< 0.001
High ALT	2.634	2.044-3.395	0.969	< 0.001
Multivariate				
BMI class	1.763	1.522-2.042	0.567	< 0.001
Triglycerides, mg/dL	1.002	1.001-1.004	0.002	< 0.001
LDL cholesterol	1.004	1.000-1.008	0.004	0.065
HbA1c ≥ 6.5%	1.541	1.153-2.060	0.432	0.004
High ALT	2.269	1.678-3.068	0.819	< 0.001
High AST	1.246	0.776-2.002	0.220	0.362

Age was excluded due to poor model fit as indicated by the Hosmer-Lemeshow test.
BMI: Body mass index; FAST: FibroScan-AST; LDL: Low-density cholesterol; HDL: High-density cholesterol; TGC: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; HbA1c: Glycated hemoglobin; OR: Odds ratio; CI: Confidence interval.

Risk of MASLD with significant activity and fibrosis (at-risk MASH)

Finally, 2.2% of the total population had a FAST score ≥ 0.67, indicating a high risk of MASLD with significant activity and fibrosis (MASH). After adjusting the FAST score according to HbA1c levels (≥ 0.67 for HbA1c < 6.5%and 0.5 for HbA1c ≥ 6.5%), the prevalence increased to 4.8%. Obesity, ALT, and GGT levels were associated with at-risk MASH according to HbA1c levels. Logistic regression analysis identified obesity, ALT, and GGT as independently linked to at-risk MASH (Supplementary Table 5).

DISCUSSION

In this study, we describe the risk factors associated with the presence of steatosis, liver fibrosis, and at-risk MASH in 1337 patients recently diagnosed with T2DM (less than 5 years) from the Mexican population, which is known for its high susceptibility to metabolic diseases, including MASLD. The main findings of the study are shown in Figure 1.

Our study reported a prevalence of MASLD of 41.4%, similar to that reported in the literature[18,19], and a high prevalence of overweight/obesity, consistent with recent findings from the ENSANUT 2022 (National Survey of Health and Nutrition). One important consideration contrasting our results with previous ones is the reported prevalence. A recent meta-analysis in T2DM reported an overall NAFLD prevalence of 65.3%, using any imaging method, including magnetic resonance imaging, USG, VCTE/CAP, serum biomarkers, and computed tomography. When specifically addressing those studies with CAP, NAFLD prevalence was 45.8% (27.05%-65.85%) for CAP of 275-302 dB/m, and 80.64% (75.27%-85.08%) for CAP 222-249 dB/m (P < 0.001 for the difference between CAP groups)[20]. Using a higher CAP (≥ 288 dB/m) as suggested by the current guidelines, as well as the fact of having good metabolic control (HbA1c 6.8%), and an earlier onset of T2DM, explain the lower prevalence of MASLD. When we used a CAP cutoff of > 250 dB/m [21], MASLD prevalence increased to 66%.

As previously described, patients with liver steatosis had a higher prevalence of metabolic abnormalities, liver chemistry alterations, and elevated HbA1c levels. A key finding of our study was the high prevalence of clinically significant liver fibrosis (≥ F2), estimated at 10% in this population by VCTE. Although this percentage is lower than that reported in other studies[4,5], this could be attributed to the early stages of the disease in the patients, the absence of macro/microvascular complications, and the fact that most participants maintained good metabolic control (with an average HbA1c of 6.8% for the overall population, and more than half having values < 7%).

Regarding the duration of T2DM, although our population was well characterized to ensure early diagnosis, a potential diagnostic delay (a frequent and challenging problem in this disease) may be present. The onset of T2DM has been estimated to be 4-6 years before a clinical diagnosis[22], allowing untreated and persistent hyperglycemia to possibly

Table 5 Logistic regression to evaluate the risk factors associated with presence of liver fibrosis

Univariate	OR	95%CI	β	*P* value
Age	1.006	0.988-1.024	0.006	0.508
BMI class	1.783	1.492-2.131	0.578	< 0.001
Sex	0.809	0.551-1.187	-0.212	0.278
HbA1c ≥ 6.5%	1.664	1.074-2.580	0.509	0.023
Steatosis (CAP)	2.995	2.060-4.355	1.097	< 0.001
Triglycerides mg/dL	1.000	0.998-1.002	0.000	0.816
LDL cholesterol	0.994	0.988-1.001	-0.006	0.073
High AST	12.925	8.121-20.572	2.559	< 0.001
High ALT	6.681	4.160-10.729	1.899	< 0.001
GGT	1.026	1.019-1.032	0.025	< 0.001
Multivariate				
BMI Class	1.500	1.169-1.924	0.405	0.001
HbA1c ≥ 6.5%	1.309	0.765-2.242	0.269	0.326
Steatosis (CAP)	1.965	1.162-3.324	0.676	0.012
High AST	4.180	2.314-7.549	1.430	< 0.001
High ALT	2.193	1.184-4.061	0.785	0.013
GGT	1.021	1.013-1.028	0.020	< 0.001

BMI: Body mass index; FAST: FibroScan-AST; LDL: Low-density cholesterol; HDL: High-density cholesterol; TGC: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; HbA1c: Glycated hemoglobin; CAP: Controlled attenuation parameter; OR: Odds ratio; CI: Confidence interval.

exert a negative effect on the liver and, therefore, likely affecting the data on the prevalence of liver steatosis and fibrosis.

Remarkably, 56 patients had a high risk of advanced fibrosis, with 40 already at stage F4. Up to 4.8% of the population was categorized as at risk for MASH according to the FAST score. None of these patients had a previous liver diagnosis and were unaware of their chronic liver disease. The overall prevalence of liver fibrosis can be influenced by the different LSM cutoffs reported in the literature, with higher values inherently increasing specificity and decreasing sensitivity (a sensitivity analysis of the present study is shown in Supplementary Table 4). However, in the present work, a cutoff of 8 kPa was set as the initial value for risk of clinically significant fibrosis ($F \geq 2$), as recommended by the AASLD[2], EASL-EASD-EASO[23] and ADA[24] to provide a more widely used and pragmatic value for clinical practice. Positive predictive value (PPV) for FAST ≥ 0.67 was 0.83 and 0.69 for the derivation and validation cohorts in the original manuscript developing the FAST score[14]. On the other hand, the use of the AASLD clinical pathway for fibrosis-risk stratification in T2DM using VCTE has shown a low false-negative rate (3.3%)[25]. Specifically in T2DM, VCTE values of < 8 kPa and > 12.0 kPa have shown a PPV of 67.8%, and 77.9%, respectively[26].

As with patients with steatosis, those with clinically significant fibrosis had higher BMI and altered blood chemistry, particularly elevated HbA1c, AST, and GGT concentrations.

When stratifying the population by HbA1c levels (≥ 7% *vs* < 7%), we observed higher CAP values in patients with HbA1c > 7% ($P = 0.040$). Similarly, patients with HbA1c > 7% had elevated GGT levels, suggesting hepatocyte alterations induced by metabolic stress. This trend was more pronounced across HbA1c groups, with a stepwise increase in GGT levels. Interestingly, logistic regression analysis showed that GGT levels were associated with at-risk MASH.

Liver fibrosis stands as the most crucial prognostic factor for both liver-and non-liver-related outcomes, including mortality in MASLD patients. It has been reported that, on average, fibrosis progresses by one stage every 7 years; however, in patients with T2DM, this progression is faster[19], likely due to increased metabolic and oxidative stress[27]. Our study identified BMI, ALT, AST, and GGT as independent factors associated with liver fibrosis, suggesting an important role of metabolic stress as a driver of fibrosis response in this population.

These findings highlight the importance of detecting both liver steatosis and, more critically, liver fibrosis in patients with T2DM. Early identification of MASLD in these patients is crucial for the timely intensification of metabolic control. Additionally, patients with fibrosis should be considered for referral to specialized centers evaluating new targeted treatments for both steatosis and fibrosis and for liver disease staging and follow-up.

Our study highlights the importance of basic liver chemistry tests, such as ALT, AST, and GGT levels. This is crucial given the high prevalence of abnormalities, even among recently diagnosed T2DM patients (40.6% of the population), and the potential to identify patients at risk of MASH when combined with transient elastography.

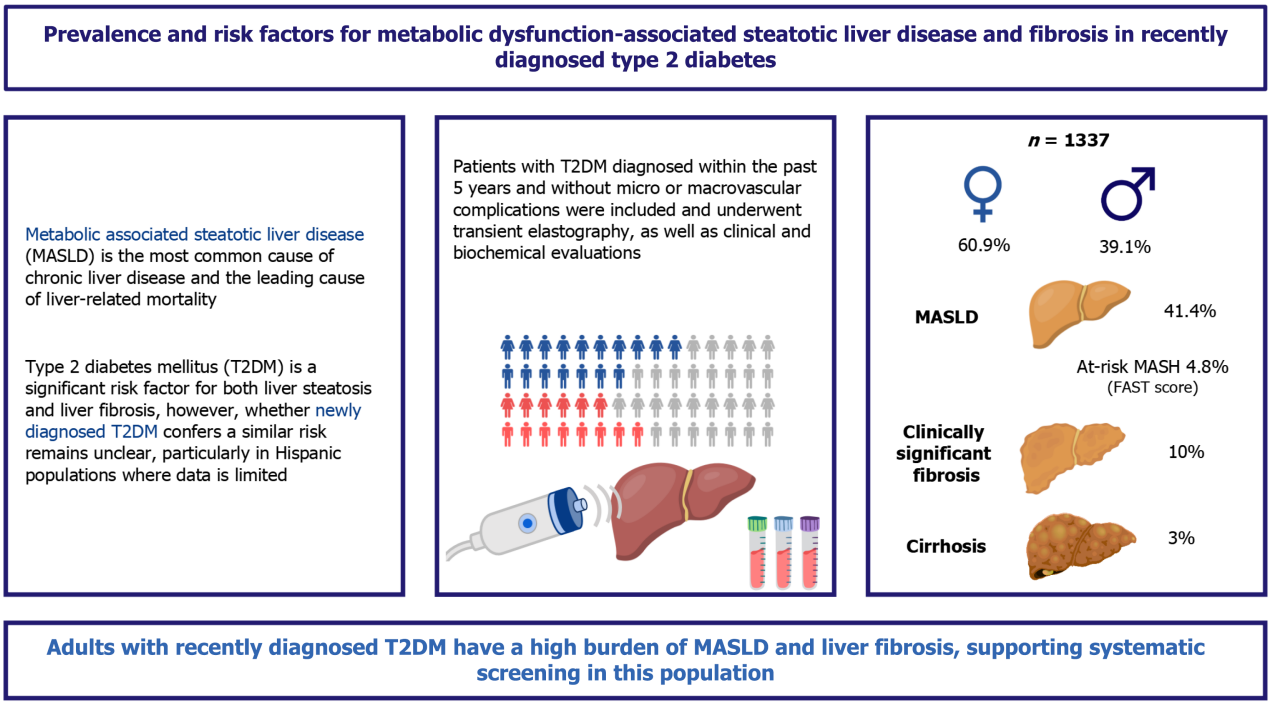


Figure 1 Main findings of the study. MASLD: Metabolic dysfunction-associated steatotic liver disease; T2DM: Type 2 diabetes mellitus.

This study has several strengths, including a robust sample size, a well-defined population of T2DM patients, appropriate methodology for VCTE examinations, and the use of established cut-off points for diagnosing liver fibrosis, steatosis, and at-risk MASH. However, it also has limitations, including the lack of recent HbA1c values for a subset of patients, the absence of liver biopsy data, and the potential for FAST score and VCTE measurements to be influenced by T2DM and obesity. Additionally, platelet data were not available as they are not part of the regular assessment at the diabetes clinic. As a result, some non-invasive risk scores could not be calculated. Although combining non-invasive risk scores with TE results or a stepwise evaluation could have been beneficial, we believe the direct transient elastography measurement provides a robust approach in this high-risk population.

Although the present study was conducted in a highly structured and specialized diabetes care center (a single tertiary referral center), the results clearly underscore the importance of screening patients with relatively good metabolic control and in the early stages of the disease. Although the generalizability of the findings in this study may be limited in primary care settings without access to VCTE, the high prevalence of MASLD with fibrosis makes liver evaluation mandatory (even with blood tests such as ALT, AST, and GGT) in patients with T2DM, even in early stages of the disease. Therefore, we recommend routinely evaluating patients with early-diagnosis T2DM using a stepwise approach with blood-based liver fibrosis scores and VCTE[28].

Beyond highlighting the high prevalence of steatosis, fibrosis, and at-risk MASH, this study underscores the importance of screening in high-risk populations, such as the Mexican population, where 18.3% of adults have T2DM. Given that 66.2% of the country's population (126 million people) are adults (INEGI), the estimated number of individuals with both MASLD and T2DM is striking, exceeding 15 million. One important consideration is that these numbers represent only adults with T2DM. Therefore, within the total population of the country, including individuals with other risk factors such as overweight, obesity, and dyslipidemia, the prevalence is likely even higher.

In addition to diagnosing and stratifying MASLD in patients, the high prevalence of MASLD in diabetes calls for including liver health evaluations in all T2DM patients. It also highlights the need to explore new interventions, both pharmacological and non-pharmacological, to address metabolic derangements, liver steatosis, and fibrosis.

CONCLUSION

The prevalence of steatosis and clinically significant liver fibrosis among patients recently diagnosed with T2DM (less than 5 years) was 41.4% and 10%, respectively. Additionally, 3% of the population had cirrhosis. Risk factors independently associated with these conditions include higher BMI, transaminase levels, GGT, triglycerides, and HbA1c. Therefore, routine assessment of these factors and liver imaging is necessary in the T2DM population to detect MASLD.

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REFERENCES

1 **Huang DQ**, Wong VWS, Rinella ME, Boursier J, Lazarus JV, Yki-Järvinen H, Loomba R. Metabolic dysfunction-associated steatotic liver disease in adults. *Nat Rev Dis Primers* 2025; **11**: 14 [*RCA*] [PMID: 40050362 DOI: 10.1038/s41572-025-00599-1] [FullText]

2 **Rinella ME**, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE, Loomba R. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology* 2023; **77**: 1797-1835 [*RCA*] [PMID: 36727674 DOI: 10.1097/HEP.0000000000000323] [FullText] [Full Text(PDF)]

3 **Riazi K**, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, Swain MG, Congly SE, Kaplan GG, Shaheen AA. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2022; **7**: 851-861 [*RCA*] [PMID: 35798021 DOI: 10.1016/S2468-1253(22)00165-0] [FullText]

4 **Lomonaco R**, Godinez Leiva E, Bril F, Shrestha S, Mansour L, Budd J, Portillo Romero J, Schmidt S, Chang KL, Samraj G, Malaty J, Huber K, Bedossa P, Kalavalapalli S, Marte J, Barb D, Poulton D, Fanous N, Cusi K. Advanced Liver Fibrosis Is Common in Patients With Type 2 Diabetes Followed in the Outpatient Setting: The Need for Systematic Screening. *Diabetes Care* 2021; **44**: 399-406 [*RCA*] [PMID: 33355256 DOI: 10.2337/dc20-1997] [FullText] [Full Text(PDF)]

5 **Younossi ZM**, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, Qiu Y, Burns L, Afendy A, Nader F. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol* 2019; **71**: 793-801 [*RCA*] [PMID: 31279902 DOI: 10.1016/j.jhep.2019.06.021] [FullText]

6 **Dulai PS**, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, Sebastiani G, Ekstedt M, Hagstrom H, Nasr P, Stal P, Wong VW, Kechagias S, Hultcrantz R, Loomba R. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology* 2017; **65**: 1557-1565 [*RCA*] [PMID: 28130788 DOI: 10.1002/hep.29085] [FullText] [Full Text(PDF)]

7 **Taylor RS**, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, Ishigami M, Toyoda H, Wai-Sun Wong V, Peleg N, Shlomai A, Sebastiani G, Seko Y, Bhala N, Younossi ZM, Anstee QM, McPherson S, Newsome PN. Association Between Fibrosis Stage and Outcomes of Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *Gastroenterology* 2020; **158**: 1611-1625.e12 [*RCA*] [PMID: 32027911 DOI: 10.1053/j.gastro.2020.01.043] [FullText]

8 **Sanyal AJ**, Van Natta ML, Clark J, Neuschwander-Tetri BA, Diehl A, Dasarathy S, Loomba R, Chalasani N, Kowdley K, Hameed B, Wilson LA, Yates KP, Belt P, Lazo M, Kleiner DE, Behling C, Tonascia J; NASH Clinical Research Network (CRN). Prospective Study of Outcomes in Adults with Nonalcoholic Fatty Liver Disease. *N Engl J Med* 2021; **385**: 1559-1569 [*RCA*] [PMID: 34670043 DOI: 10.1056/NEJMoa2029349] [FullText]

9 **Ng CH**, Lim WH, Hui Lim GE, Hao Tan DJ, Syn N, Muthiah MD, Huang DQ, Loomba R. Mortality Outcomes by Fibrosis Stage in Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2023; **21**: 931-939.e5 [*RCA*] [PMID: 35513235 DOI: 10.1016/j.cgh.2022.04.014] [FullText]

10 **Alexopoulos AS**, Crowley MJ, Wang Y, Moylan CA, Guy CD, Henao R, Piercy DL, Seymour KA, Sudan R, Portenier DD, Diehl AM, Coviello AD, Abdelmalek MF. Glycemic Control Predicts Severity of Hepatocyte Ballooning and Hepatic Fibrosis in Nonalcoholic Fatty Liver Disease. *Hepatology* 2021; **74**: 1220-1233 [*RCA*] [PMID: 33724511 DOI: 10.1002/hep.31806] [FullText] [Full Text(PDF)]

11 **Oeda S**, Tanaka K, Oshima A, Matsumoto Y, Sueoka E, Takahashi H. Diagnostic Accuracy of FibroScan and Factors Affecting Measurements. *Diagnostics (Basel)* 2020; **10**: 940 [*RCA*] [PMID: 33198092 DOI: 10.3390/diagnostics10110940] [FullText] [Full Text(PDF)]

12 **Petroff D**, Blank V, Newsome PN, Shalimar, Voican CS, Thiele M, de Lédinghen V, Baumeler S, Chan WK, Perlemuter G, Cardoso AC, Aggarwal S, Sasso M, Eddowes PJ, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Cobbold JF, Naveau S, Lupsor-Platon M, Mueller S, Krag A, Irles-Depe M, Semela D, Wong GL, Wong VW, Villela-Nogueira CA, Garg H, Chazouillères O, Wiegand J, Karlas T. Assessment of hepatic steatosis by controlled attenuation parameter using the M and XL probes: an individual patient data meta-analysis. *Lancet Gastroenterol Hepatol* 2021; **6**: 185-198 [*RCA*] [PMID: 33460567 DOI: 10.1016/S2468-1253(20)30357-5] [FullText]

13 **Newsome PN**, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, Yilmaz Y, Czernichow S, Zheng MH, Wong VW, Allison M, Tsochatzis E, Anstee QM, Sheridan DA, Eddowes PJ, Guha IN, Cobbold JF, Paradis V, Bedossa P, Miette V, Fournier-Poizat C, Sandrin L, Harrison SA. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020; **5**: 362-373 [*RCA*] [PMID: 32027858 DOI: 10.1016/S2468-1253(19)30383-8] [FullText] [Full Text(PDF)]

14 **Woreta TA**, Van Natta ML, Lazo M, Krishnan A, Neuschwander-Tetri BA, Loomba R, Mae Diehl A, Abdelmalek MF, Chalasani N, Gawrieh S, Dasarathy S, Vuppalanchi R, Siddiqui MS, Kowdley KV, McCullough A, Terrault NA, Behling C, Kleiner DE, Fishbein M, Hertel P, Wilson LA, Mitchell EP, Miriel LA, Clark JM, Tonascia J, Sanyal AJ; NASH Clinical Research Network. Validation of the accuracy of the FAST™ score for detecting patients with at-risk nonalcoholic steatohepatitis (NASH) in a North American cohort and comparison to other non-invasive algorithms. *PLoS One* 2022; **17**: e0266859 [*RCA*] [PMID: 35427375 DOI: 10.1371/journal.pone.0266859] [FullText] [Full Text (PDF)]

15 . The Liver Meeting: Boston, Massachusetts Nov 10-14, 2023. *Hepatology* 2023; **78**: S1-S2154 [*RCA*] [PMID: 37820069 DOI: 10.1097/HEP.0000000000000580] [FullText]

16 **Gancheva S**, Roden M, Castera L. Diabetes as a risk factor for MASH progression. *Diabetes Res Clin Pract* 2024; **217**: 111846 [*RCA*] [PMID: 39245423 DOI: 10.1016/j.diabres.2024.111846] [FullText]

17 **Kwo PY**, Cohen SM, Lim JK. ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. *Am J Gastroenterol* 2017; **112**: 18-35 [*RCA*] [PMID: 27995906 DOI: 10.1038/ajg.2016.517] [FullText]

18 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [*RCA*] [PMID: 15565570 DOI: 10.1002/hep.20466] [FullText]

19 **Younossi ZM**, Golabi P, Paik J, Owrangi S, Yilmaz Y, El-Kassas M, Alswat K, Alqahtani SA. Prevalence of metabolic dysfunction-associated steatotic liver disease in the Middle East and North Africa. *Liver Int* 2024; **44**: 1061-1070 [*RCA*] [PMID: 38305642 DOI: 10.1111/liv.15852] [FullText]

20 **Younossi ZM**, Golabi P, Price JK, Owrangi S, Gundu-Rao N, Satchi R, Paik JM. The Global Epidemiology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis Among Patients With Type 2 Diabetes. *Clin Gastroenterol Hepatol* 2024; **22**: 1999-2010.e8 [*RCA*] [PMID: 38521116 DOI: 10.1016/j.cgh.2024.03.006] [FullText]

21 **de Lédinghen V**, Vergniol J, Capdepont M, Chermak F, Hiriart JB, Cassinotto C, Merrouche W, Foucher J, Brigitte le B. Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. *J Hepatol* 2014; **60**: 1026-1031 [*RCA*] [PMID: 24378529 DOI: 10.1016/j.jhep.2013.12.018] [FullText]

22 **Porta M**, Curletto G, Cipullo D, Rigault de la Longrais R, Trento M, Passera P, Taulaigo AV, Di Miceli S, Cenci A, Dalmasso P, Cavallo F. Estimating the delay between onset and diagnosis of type 2 diabetes from the time course of retinopathy prevalence. *Diabetes Care* 2014; **37**: 1668-1674 [*RCA*] [PMID: 24705614 DOI: 10.2337/dc13-2101] [FullText]

23 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J Hepatol* 2024; **81**: 492-542 [*RCA*] [PMID: 38851997 DOI: 10.1016/j.jhep.2024.04.031] [FullText]

24 **Cusi K**, Abdelmalek MF, Apovian CM, Balapattabi K, Bannuru RR, Barb D, Bardsley JK, Beverly EA, Corbin KD, ElSayed NA, Isaacs S, Kanwal F, Pekas EJ, Richardson CR, Roden M, Sanyal AJ, Shubrook JH, Younossi ZM, Bajaj M. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) in People With Diabetes: The Need for Screening and Early Intervention. A Consensus Report of the American Diabetes Association. *Diabetes Care* 2025; **48**: 1057-1082 [*RCA*] [PMID: 40434108 DOI: 10.2337/dci24-0094] [FullText]

25 **Ajmera V**, Tesfai K, Sandoval E, Lopez S, Cervantes V, Madamba E, Bettencourt R, Manousou P, Richards L, Loomba R. Validation of AGA clinical care pathway and AASLD practice guidance for nonalcoholic fatty liver disease in a prospective cohort of patients with type 2 diabetes. *Hepatology* 2024; **79**: 1098-1106 [*RCA*] [PMID: 37862551 DOI: 10.1097/HEP.0000000000000635] [FullText]

26 **Boursier J**, Canivet CM, Costentin C, Lannes A, Delamarre A, Sturm N, Le Bail B, Michalak S, Oberti F, Hilleret MN, Irles-Depé M, Fouchard I, Hermabessiere P, Barthelon J, Calès P, Cariou B, de Ledinghen V, Roux M. Impact of Type 2 Diabetes on the Accuracy of Noninvasive Tests of Liver Fibrosis With Resulting Clinical Implications. *Clin Gastroenterol Hepatol* 2023; **21**: 1243-1251.e12 [*RCA*] [PMID: 35288342 DOI: 10.1016/j.cgh.2022.02.059] [FullText]

27 **Chen Z**, Tian R, She Z, Cai J, Li H. Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radic Biol Med* 2020; **152**: 116-141 [*RCA*] [PMID: 32156524 DOI: 10.1016/j.freeradbiomed.2020.02.025] [FullText]

28 **Silva-Tinoco R**, Martínez-de la Cruz EV, Galíndez-Fuentes A, Cabrera-Victoria B, Villa-Mejía E, Macías-Rodríguez RU, Avalos-Bracho A, Bernal-Ceballos MF. Integrating MASLD detection into diabetes care in primary care settings in Mexico: A cascade analysis. *Clin Med (Lond)* 2026; **26**: 100585 [*RCA*] [PMID: 42009199 DOI: 10.1016/j.clinme.2026.100585] [FullText] [Full Text(PDF)]

FOOTNOTES

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ORIGINAL ARTICLE

Observational Study

Small dense low-density lipoprotein in primary biliary cholangitis: Independent association with metabolic syndrome

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Abstract

BACKGROUND

Primary biliary cholangitis (PBC) is frequently associated with cholestasis-driven hypercholesterolemia characterized by lipoprotein X accumulation. Despite markedly elevated cholesterol levels, cardiovascular risk in PBC is not consistently increased, suggesting that conventional lipid parameters may inadequately reflect true atherogenic burden. Small dense low-density lipoprotein (sdLDL), a highly atherogenic low-density lipoprotein (LDL) subfraction closely linked to insulin resistance and metabolic syndrome (MetS), has not been systematically evaluated in PBC.

AIM

To evaluate determinants of sdLDL levels in PBC, focusing on MetS, metabolic dysfunction-associated steatotic liver disease (MASLD), liver fibrosis, and biochemical response, and assess whether sdLDL reflects metabolic dysfunction rather than cholestatic disease severity.

METHODS

In this cross-sectional study, 148 consecutive patients with PBC were recruited from a tertiary hepatology center. sdLDL was quantified using the Lipoprint® LDL System. MetS was defined according to International Diabetes Federation criteria. Hepatic steatosis and fibrosis were assessed using vibration-controlled

transient elastography. Multivariable linear and logistic regression analyses were performed to evaluate independent determinants of sdLDL.

RESULTS

MetS was present in 39.2% of patients. sdLDL levels were significantly higher in patients with MetS (P = 0.004). In multivariable analysis, MetS remained independently associated with ln-transformed sdLDL levels [B = 0.76; 95% confidence interval (CI): 0.26-1.26; P = 0.003] and with the presence of detectable sdLDL (odds ratio 2.10; 95%CI: 1.02-4.35; P = 0.045), independent of age, sex, adiposity, and lipid-lowering therapy. In contrast, MASLD, advanced fibrosis, and cholestatic activity were not independently associated with sdLDL. Notably, LDL-C levels were lower in patients with MetS, whereas sdLDL and sdLDL/largeLDL ratio were significantly increased, suggesting discordance between conventional LDL-C and qualitative lipoprotein remodeling.

CONCLUSION

In PBC, sdLDL appears to reflect superimposed metabolic dysfunction rather than cholestatic liver disease severity. Advanced lipoprotein profiling may therefore help distinguish metabolically driven atherogenic dyslipidemia from cholestatic hypercholesterolemia in contemporary PBC cohorts.

Key Words: Primary biliary cholangitis; Small dense low-density lipoprotein; Metabolic dysfunction; Lipoprotein subfractions; Atherogenic dyslipidemia

Core Tip: Hypercholesterolemia in primary biliary cholangitis (PBC) is largely driven by cholestasis and lipoprotein X, making conventional lipid parameters difficult to interpret. In this study, small dense low-density lipoprotein (LDL) was independently associated with metabolic syndrome but not with liver fibrosis, cholestatic activity, metabolic dysfunction-associated steatotic liver disease, or biochemical response. These findings suggest that small dense LDL reflects superimposed metabolic dysfunction rather than intrinsic cholestatic liver disease and may help identify a metabolically driven atherogenic phenotype in contemporary PBC cohorts.

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INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic autoimmune cholestatic liver disease characterized by progressive destruction of small intrahepatic bile ducts, leading to cholestasis and, in a subset of patients, progression to cirrhosis[1,2]. It predominantly affects women and has a stable incidence but increasing prevalence in developed countries[3]. Diagnosis is based on persistent cholestatic liver enzyme elevation, PBC-specific autoantibodies, and compatible histology[1].

Recent guidelines on PBC recommend addressing and treating several extrahepatic diseases, including osteoporosis, celiac disease, thyroid disease, and Sjögren's syndrome. However, only these specific extrahepatic diseases are mentioned, and the level of evidence is low to moderate. Furthermore, the prevalence of these extrahepatic diseases varied significantly across studies, making it difficult to assess the extent and severity of these conditions[1,4].

Ursodeoxycholic acid (UDCA) remains first-line therapy, while second-line treatment options are available for patients with incomplete biochemical response[5,6]. Response to UDCA therapy is commonly evaluated using biochemical parameters. Complete biochemical response (CBR) was defined as normalization of serum alkaline phosphatase (ALP) and bilirubin concentrations, although other validated response criteria, such as the Toronto criteria [ALP < 1.67 × upper limit of normal (ULN)], are also used in clinical practice[7]. Biochemical response is associated with improved prognosis [8].

Cholestasis profoundly alters lipid metabolism in PBC. Impaired bile acid secretion promotes the formation of lipoprotein X (LpX), an abnormal phospholipid-rich particle lacking apolipoprotein B (ApoB)[9,10]. Impaired bile acid secretion causes phospholipids and free cholesterol to accumulate in the liver and subsequently enter the circulation. These lipids then associate with albumin to form LpX, an unusual ApoB-deficient lipoprotein that remains in circulation since it is not removed *via* low-density lipoprotein (LDL) receptors. As a result, standard LDL-C measurements can overstate the actual amount of atherogenic lipoproteins in patients with PBC[9]. Hypercholesterolemia in PBC differs from classic metabolic dyslipidemia in that it is primarily caused by the accumulation of LpX. In contrast, metabolic dyslipidemia is characterized by elevated concentrations of ApoB-containing lipoproteins, hypertriglyceridemia, insulin resistance, and an increased proportion of small, dense LDL particles, which contribute significantly to the development of atherosclerosis[9,11,12]. Consequently, hypercholesterolemia occurs in up to 75%-95% of patients[13,14]. Because LpX overlaps with LDL in density, routine lipid panels may report elevated LDL-C levels, potentially mimicking a proatherogenic lipid profile[9].

Despite markedly elevated total cholesterol levels, cardiovascular risk in PBC is not consistently increased[13,15]. Experimental data suggest that LpX-containing fractions are less susceptible to oxidative modification and may attenuate LDL atherogenicity[16]. Thus, hypercholesterolemia in PBC appears qualitatively distinct from classical metabolic dyslipidemia. Importantly, conventional LDL-C measurements may inadequately reflect true atherogenic burden and qualitative lipoprotein remodeling in cholestatic PBC. However, detailed characterization of lipoprotein subfractions in this setting remains limited.

Small dense LDL (sdLDL) is a particularly atherogenic LDL subfraction. Compared with larger LDL particles, sdLDL exhibits prolonged circulation time, increased arterial wall penetration, and greater susceptibility to oxidative modification[12,17]. Elevated sdLDL levels are closely linked to insulin resistance and hypertriglyceridemia and are commonly observed in metabolic syndrome (MetS) and type 2 diabetes[11]. In prospective cohorts, sdLDL-C predicts incident coronary heart disease independently of traditional lipid parameters[11,12,18]. Whether sdLDL in PBC reflects metabolically driven atherogenic dyslipidemia or cholestatic lipid remodeling remains unknown.

The clinical phenotype of PBC is evolving. Contemporary cohorts increasingly include patients with metabolic dysfunction and hepatic steatosis. Metabolic dysfunction-associated steatotic liver disease (MASLD), previously classified as metabolic associated fatty liver disease, has been reported across autoimmune liver diseases, including PBC[19,20], while coexisting non-alcoholic fatty liver disease has been described in up to 24% of patients[21]. Nearly half of patients in observational cohorts fulfill criteria for MASLD[22]. In this context, atherogenic lipoprotein patterns may increasingly reflect superimposed metabolic dysfunction rather than cholestasis itself.

To date, determinants of sdLDL levels in PBC have not been systematically evaluated. It remains unclear whether sdLDL primarily reflects cholestatic liver disease severity or an underlying metabolic phenotype independent of hepatic factors. Clarifying this distinction may improve interpretation of dyslipidemia and refine cardiovascular risk assessment in PBC. Although the association between sdLDL-C and hepatic steatosis has recently been described in patients with MASLD[23], little is still known about the clinical significance of sdLDL in cholestatic liver diseases such as PBC, particularly given the distinct nature of lipid metabolism disorders compared to metabolic dyslipidemia. Therefore, the aim of the present study was to evaluate determinants of sdLDL levels in patients with PBC, with particular focus on MetS, MASLD, fibrosis stage, and biochemical response.

MATERIALS AND METHODS

We conducted a cross-sectional observational study including consecutive patients with PBC recruited from the Hepatology Outpatient Clinic of the 2nd Department of Internal Medicine, Louis Pasteur University Hospital and Pavol Jozef Šafárik University Faculty of Medicine in Košice, Slovakia. Patients were consecutively enrolled during a predetermined recruitment period from January to June 2024. Statistical analyses were performed after completion of patient recruitment and database verification.

All participants fulfilled diagnostic criteria for PBC according to the European Association for the Study of the Liver (EASL) guidelines[1]. Exclusion criteria included refusal to participate and presence of alternative causes of hepatic steatosis or chronic liver disease. All patients underwent comprehensive etiological evaluation, including testing for viral hepatitis B and C and assessment for other liver diseases.

Study visits included laboratory testing, vibration-controlled transient elastography (VCTE), anthropometric assessment, and structured clinical data collection. Use of UDCA, fibrates, and statins at the time of lipoprotein assessment was recorded. Information on concomitant autoimmune diseases was not collected or evaluated as part of the study protocol, therefore, it has not been included in further analyses. The study protocol was approved by the Ethics Committee of Louis Pasteur University Hospital in Košice (reference No. 2020/EK10076). Patient recruitment was delayed due to the coronavirus disease 2019 pandemic.

Definitions

MetS was diagnosed according to the International Diabetes Federation criteria[24].

Hepatic steatosis was assessed using VCTE with controlled attenuation parameter (CAP). A CAP value ≥ 248 dB/m defined hepatic steatosis, while thresholds of 248 dB/m, 268 dB/m, and 280 dB/m corresponded to steatosis grades ≥ S1, ≥ S2, and S3 according to current EASL-EASD-EASO clinical practice guidelines on the management of MASLD, respectively[25].

MASLD was defined as hepatic steatosis in the presence of at least one cardiometabolic risk factor and absence of alternative causes of steatotic liver disease[25].

Liver fibrosis was categorized according to VCTE-derived liver stiffness as mild-to-moderate fibrosis (F0-F2; < 10.7 kPa) or advanced fibrosis (F3/F4; ≥ 10.7 kPa)[26,27].

CBR was defined as normalization of ALP and total bilirubin levels.

Lipoprint subfractions analysis

Lipoprotein subfractions were analyzed using the Lipoprint® LDL System (Quantimetrix Corporation, Redondo Beach, CA, United States), a linear polyacrylamide gel electrophoresis method separating lipoproteins according to particle size.

Fasting venous blood samples were obtained after ≥ 12 hours of fasting. Serum samples were processed according to manufacturer recommendations. Briefly, 25 μL of serum was mixed with 200 μL of loading gel containing lipophilic dye, photopolymerized, and subjected to electrophoresis at 3 mA per gel tube (maximum 500 V) for approximately 60 minutes.

Fractions were separated into very low-density lipoprotein (VLDL), intermediate-density lipoproteins (midbands A-C), LDL-1 to LDL-7, and high-density lipoprotein (HDL) based on relative electrophoretic mobility. Bands were scanned and quantified using Lipoware software. Cholesterol content of each subfraction was calculated from relative band area and total cholesterol concentration.

sdLDL was defined as the sum of LDL-3 through LDL-7 subfractions[28].

Exposure and outcome definitions

The primary exposure of interest was MetS, defined according to the above-mentioned established clinical criteria. Additional exposure variables included the presence of MASLD, advanced liver fibrosis, CBR, and lipid-lowering therapy. Current use of statins and fibrates at the time of lipoprotein subfraction assessment was recorded and included as covariates in multivariable analyses.

The primary outcome was serum sdLDL concentration. Ln-transformed sdLDL was used as the continuous dependent variable in linear regression analyses. As a secondary outcome, detectable sdLDL (sdLDL > 0) was analyzed as a binary variable using logistic regression models to explore its association with metabolic and liver-related factors.

Additional exploratory analyses included assessment of conventional and advanced lipoprotein-related parameters, including LDL-C, ApoB, lipoprotein(a) [Lp(a)], ApoB/apolipoprotein A-I (ApoA-I) ratio, large LDL subfractions, and sdLDL/LargeLDL ratio. Receiver operating characteristic (ROC) analyses were performed to evaluate the discriminatory performance of sdLDL, LDL-C, large LDL, and sdLDL/LargeLDL ratio for identification of MetS.

Statistical analysis

Continuous variables were expressed as mean ± SD or median with interquartile range (IQR), as appropriate, based on data distribution. Normally distributed variables were analyzed using independent-samples *t*-test and presented as mean ± SD, whereas non-normally distributed variables were analyzed using Mann-Whitney *U* test and presented as median (IQR). Categorical variables were presented as absolute numbers and percentages. Between-group comparisons were performed using the independent-samples *t*-test or Mann-Whitney *U* test, as appropriate. No formal sample size calculation was performed because this was an exploratory observational study.

The primary multivariable analyses were prespecified to evaluate the association between MetS and sdLDL, given the central role of MetS in atherogenic dyslipidemia.

Because sdLDL values demonstrated a right-skewed distribution and included zero values, a small constant (0.01 mmol/L) was added before logarithmic transformation. Ln-transformed sdLDL was calculated as ln (sdLDL + 0.01) and used in linear regression analyses. In addition, a substantial proportion of participants had undetectable sdLDL concentrations (sdLDL = 0), resulting in a partially zero-inflated distribution. Therefore, a complementary logistic regression analysis using detectable sdLDL (sdLDL > 0) as a binary outcome was performed to assess the robustness of the observed associations. Multivariable linear regression models were constructed using ln-transformed sdLDL as the dependent variable. The primary model included MetS, age, sex, and body mass index (BMI) as covariates, while a second model additionally adjusted for statin and fibrate therapy. Since MetS was included in the multivariate regression models as a binary variable defined according to standard diagnostic criteria, its individual components (waist circumference, blood pressure, triglycerides, HDL-C, and fasting glucose) were not included in the models separately, in order to avoid multicollinearity. To assess robustness of the findings, a sensitivity analysis was performed replacing BMI with waist circumference.

Additional exploratory analyses evaluated associations between MetS and lipoprotein-related parameters, including LDL-C, ApoB, Lp(a), large LDL subfractions, and sdLDL/LargeLDL ratio. ROC analyses were performed as exploratory analyses to compare the ability of lipoprotein-related parameters to identify the metabolic phenotype.

Multicollinearity was assessed using variance inflation factors (VIF) and condition index. Logistic regression analyses were performed to evaluate factors associated with detectable sdLDL (sdLDL > 0). Odds ratio (OR) with 95% confidence intervals (CI) were calculated. Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test. Additional lipoprotein-related comparisons and ROC analyses were considered exploratory and were therefore interpreted cautiously without formal correction for multiple testing.

All statistical analyses were conducted using SPSS software (IBM Corp., Armonk, NY, United States). A two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of 148 patients with PBC were included in the analysis. The cohort was predominantly female (92.6%), with a mean age of 64.2 ± 10.7 years.

MetS was present in 39.2% of patients, while MASLD was identified in 42.6%. Advanced fibrosis (F3/F4 by transient elastography) was observed in 21.6% of the cohort, and CBR was achieved in 55.4%.

At the time of lipoprotein assessment, 19.6% of patients were receiving statins and 23.0% fibrates. The mean sdLDL concentration in the overall cohort was 0.09 ± 0.17 mmol/L. Because sdLDL values demonstrated a right-skewed distribution and included zero values, ln-transformed sdLDL values calculated as ln (sdLDL + 0.01) were used in subsequent regression analyses. Detailed demographic, clinical, biochemical, and lipoprotein-related characteristics are summarized in Table 1.

Table 1 Baseline characteristics of the study population, *n* (%)

Number of patients	*n* = 148
Demographic and anthropometric characteristics	
Age (years)	64.15 ± 10.73 (33-88)
BMI	26.76 ± 4.47 (16.51-40.16)
Waist circumference (cm)	89.36 ± 11.27 (60-121)
Gender	Female *n* = 137 (92.60); Male *n* = 11 (7.40)
Cardiometabolic comorbidities	
Coronary artery disease	4 (2.70)
Acute coronary syndrome	3 (2.00)
History of stroke	7 (4.70)
Peripheral artery disease	2 (1.40)
Treatment	
Statin therapy	29 (19.60)
Fibrate therapy	34 (23.00)
UDCA therapy	148 (100.00)
Liver disease characteristics	
Complete biochemical response	82 (55.40)
Advanced liver fibrosis F3/F4	32 (21.60)
Metabolic syndrome	58 (39.20)
MASLD	63 (42.60)
CAP (dB/m)	244.87 ± 58.07
TE (kPa)	8.92 ± 8.16
AST × ULN (%)	101.52 ± 60.85
ALT × ULN (%)	87.43 ± 65.42
GGT × ULN (%)	212.10 ± 372.79
ALP × ULN (%)	109.12 ± 84.10
Total bilirubin (μmol/L)	13.71 ± 7.75
Conjugated bilirubin (μmol/L)	3.20 ± 2.75
Metabolic and lipoprotein parameters	
Glucose (mmol/L)	5.83 ± 1.87
C-peptide (ng/mL)	2.08 ± 1.2
HOMA-IR	6.37 ± 4.47
Total cholesterol (mmol/L)	5.32 ± 1.26
HDL-C (mmol/L)	1.67 ± 0.44
LDL-C (mmol/L)	3.27 ± 0.92
Triglycerides (mmol/L)	1.30 ± 0.89
Platelets × 10^9/L	245.29 ± 97.35
sdLDL (mmol/L)	0.09 ± 0.17
largeLDL (mmol/L)	1.29 ± 0.49
sdLDL/LargeLDL	0.08 ± 0.18
ApoB (mg/dL)	43.91 ± 16.87
Lp(a) (mg/dL)	10.75 ± 15.34

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ApoB: Apolipoprotein B; AST: Aspartate aminotransferase; BMI: Body mass index; CAP: Controlled attenuation parameter; F3/F4: Advanced liver fibrosis stages 3 and 4; GGT: Gamma-glutamyl transferase; HDL: High-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein; Lp(a): Lipoprotein a; MASLD: Metabolic dysfunction-associated steatotic liver disease; sdLDL: Small dense low-density lipoprotein; TE: Transient elastography; ULN: Upper limit of normal; UDCA: Ursodeoxycholic acid.

Univariable analyses

In univariable analyses, sdLDL levels were significantly higher in patients with MetS compared with those without MetS [median 0.07 (IQR 0.00-0.14) *vs* 0.00 (IQR 0.00-0.07); $P = 0.004$] (Figure 1).

In contrast, no significant differences in sdLDL concentrations were observed according to MASLD status ($P = 0.324$) or presence of advanced fibrosis ($P = 0.155$), suggesting that sdLDL levels were more closely associated with metabolic phenotype than liver disease severity.

Patients with CBR exhibited higher sdLDL levels compared with patients without CBR [median 0.06 (IQR 0.00-0.13) *vs* 0.00 (IQR 0.00-0.07); $P = 0.017$].

Univariable comparisons of sdLDL according to metabolic and liver-related factors are summarized in Table 2.

Multivariable linear regression analyses

In multivariable linear regression analyses using ln-transformed sdLDL as the dependent variable, MetS was independently associated with higher ln(sdLDL) levels ($B = 0.72$, 95%CI: 0.23-1.21; standardized $\beta = 0.26$; $P = 0.005$), whereas age, sex, and BMI were not significantly associated with ln(sdLDL).

After additional adjustment for lipid-lowering therapy, the association between MetS and ln(sdLDL) remained statistically significant ($B = 0.76$, 95%CI: 0.26-1.26; standardized $\beta = 0.27$; $P = 0.003$). Neither statin nor fibrate therapy was independently associated with ln(sdLDL). Although fibrates may influence LDL particle remodeling through triglyceride reduction and PPAR-α activation, fibrate therapy was not independently associated with sdLDL in our cohort, possibly reflecting the cross-sectional design and confounding by indication. The fully adjusted model was statistically significant but showed modest explanatory performance, explaining 9.3% of variance in ln(sdLDL) (adjusted $R^2 = 0.054$) (Table 3 and Figure 2).

No evidence of clinically relevant multicollinearity was observed (maximum VIF 1.30; minimum tolerance 0.80).

Given the size of the cohort and the numerous zero values, we performed bootstrapping. Bootstrap resampling (1000 samples) confirmed the robustness of the regression model. The association between MetS and ln-transformed sdLDL remained statistically significant (bootstrap $B = 0.760$, bias = -0.009, BCa 95%CI: 0.265-1.229).

To further explore the relationship between cholestatic activity and sdLDL, an additional model including ALP as a continuous marker of cholestasis was constructed. ALP was not independently associated with ln(sdLDL) ($B = -0.001$, $P = 0.315$), whereas MetS remained a significant predictor ($B = 0.716$, $P = 0.005$). Inclusion of ALP did not materially improve model performance.

In a sensitivity analysis adjusted only for age and sex, MetS remained independently associated with ln(sdLDL) ($B = 0.763$, $P = 0.002$), suggesting that the association was not solely attributable to anthropometric overlap.

Sensitivity analysis

In a sensitivity analysis replacing BMI with waist circumference as a marker of central adiposity, MetS remained independently associated with higher ln-transformed sdLDL levels ($B = 0.68$, 95%CI: 0.17-1.18; standardized $\beta = 0.24$; $P = 0.010$). Waist circumference itself was not independently associated with ln(sdLDL) ($B = 0.01$, 95%CI: -0.01 to 0.03; $P = 0.393$). Results of the sensitivity analysis are summarized in Table 4.

Logistic regression analysis

In a multivariable logistic regression model adjusted for age, sex, and lipid-lowering therapy (statins and fibrates), MetS was independently associated with the presence of detectable sdLDL (OR 2.10, 95%CI: 1.02-4.35; $P = 0.045$).

Age, sex, statin therapy, and fibrate therapy were not significantly associated with detectable sdLDL. The model demonstrated acceptable calibration according to the Hosmer-Lemeshow goodness-of-fit test ($\chi^2 = 11.79$, $df = 8$; $P = 0.161$).

Results of the multivariable logistic regression analysis are presented in Table 5.

Lipoprotein remodeling according to MetS status

Patients with MetS exhibited a distinct atherogenic lipoprotein phenotype compared with patients without MetS (Table 6). Despite significantly lower LDL-C levels, patients with MetS demonstrated higher sdLDL concentrations, lower large LDL subfractions, and increased sdLDL/LargeLDL and triglycerides/HDL-C ratios. HDL-C levels were also significantly lower in patients with MetS.

This paradoxical pattern suggests that conventional LDL-C measurements may inadequately reflect qualitative atherogenic lipoprotein remodeling in PBC patients with superimposed metabolic dysfunction.

Additional lipoprotein-related parameters according to MetS status

ApoB concentrations, Lp(a), and ApoB/ApoA-I ratio did not differ significantly between patients with and without MetS (Table 7).

Table 2 Univariable comparison of small dense low-density lipoprotein levels according to metabolic and liver-related factors

Variable	Group	sdLDL (mmol/L), median (IQR)	*P* value
MetS	Present	0.07 (0.00-0.14)	0.004
	Absent	0.00 (0.00-0.07)	
MASLD	Present	0.03 (0.00-0.13)	0.324
	Absent	0.03 (0.00-0.08)	
Advanced fibrosis (F3/F4)	Present	0.00 (0.00-0.08)	0.155
	Absent	0.04 (0.00-0.12)	
CBR	Present	0.06 (0.00-0.13)	0.017
	Absent	0.00 (0.00-0.07)	

CBR: Complete biochemical response; F3/F4: Fibrosis stages 3 and 4; MASLD: Metabolic dysfunction-associated steatotic liver disease; sdLDL: Small dense low-density lipoprotein; IQR: Interquartile range; MetS: Metabolic syndrome.

Table 3 Multivariable linear regression analysis of factors associated with ln-transformed small dense low-density lipoprotein levels

Variable	*B* (95%CI)	Standardized *β*	*P* value
MetS	0.76 (0.26 to 1.26)	0.27	0.003
Age	-0.004 (-0.03 to 0.02)	-0.03	0.750
Female sex	-0.14 (-1.00 to 0.72)	-0.03	0.745
BMI	0.024 (-0.03 to 0.07)	0.08	0.354
Statin therapy	-0.45 (-1.04 to 0.13)	-0.13	0.133
Fibrate therapy	-0.01 (-0.53 to 0.51)	-0.004	0.964
Model statistics: R^2 = 0.093 adjusted R^2 = 0.054 F = 2.393 P = 0.031			

BMI: Body mass index; CI: Confidence interval; MetS: Metabolic syndrome.

Table 4 Sensitivity analysis using waist circumference instead of body mass index in multivariable linear regression

Variable	*B* (95%CI)	Standardized *β*	*P* value
Metabolic syndrome	0.68 (0.17 to 1.18)	0.24	0.010
Age (years)	-0.01 (-0.03 to 0.01)	-0.07	0.421
Female sex	-0.02 (-0.83 to 0.86)	0.00	0.969
Waist circumference (cm)	0.01 (-0.01 to 0.03)	0.08	0.393

CI: Confidence interval.

Despite the absence of significant differences in ApoB-related parameters, patients with MetS demonstrated a markedly more atherogenic lipoprotein subfraction profile characterized by higher sdLDL levels and increased sdLDL/LargeLDL ratio.

Discriminatory performance of lipoprotein-related parameters for MetS

ROC analyses were performed to evaluate the discriminatory performance of lipoprotein-related parameters for identification of MetS in patients with PBC (Figure 3 and Table 8). Optimal cut-off values were derived using the maximum Youden index and are presented for descriptive purposes only. Given the exploratory nature of the ROC analyses, these thresholds should not be interpreted as clinically applicable diagnostic cut-offs.

Among the evaluated parameters, the sdLDL/LargeLDL ratio showed the highest discriminatory ability, although overall discrimination remained modest, whereas lower LDL-C and lower large LDL levels were associated with the presence of MetS. This inverse association is reflected by ROC curves falling below the diagonal reference line. Overall, sdLDL-related parameters showed modestly better discriminatory performance than conventional LDL-C measurements

Table 5 Multivariable logistic regression analysis of factors associated with detectable small dense low-density lipoprotein

Variable	OR (95%CI)	*P* value
Metabolic syndrome	2.10 (1.02-4.35)	0.045
Age (years)	0.99 (0.96-1.03)	0.731
Female sex	1.43 (0.37-5.49)	0.606
Fibrate therapy	0.92 (0.41-2.04)	0.832
Statin therapy	0.51 (0.21-1.27)	0.149

Model adjusted for age, sex, statin therapy, and fibrate therapy. Hosmer-Lemeshow goodness-of-fit test: $\chi^2 = 11.79$, $df = 8$; $P = 0.161$. OR: Odds ratio; CI: Confidence interval.

Table 6 Lipoprotein subfraction phenotype in primary biliary cholangitis patients with and without metabolic syndrome

Variable	No MetS	MetS	*P* value
LDL-C (mmol/L)	3.37 ± 0.82	3.08 ± 1.08	0.021[1]
HDL-C (mmol/L)	1.81 ± 0.38	1.44 ± 0.43	< 0.001[1]
sdLDL (mmol/L)	0.00 IQR (0.00-0.07)	0.07 IQR (0.00-0.14)	0.004[2]
largeLDL (mmol/L)	1.27 IQR (1.03-1.78)	1.09 IQR (0.87-1.49)	0.041[2]
TG (mmol/L)	1.04 IQR (0.82-1.29)	1.31 IQR (1.03-1.78)	< 0.001[2]
TG/HDL-C ratio	0.60 IQR (0.42-0.79)	1.02 IQR (0.61-1.47)	< 0.001[2]
sdLDL/LargeLDL ratio	0.00 IQR (0.00-0.05)	0.05 IQR (0.00-0.15)	< 0.001[2]

[1]Independent-samples *t*-test.
[2]Mann-Whitney *U* test.
HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol; MetS: Metabolic syndrome; sdLDL: Small dense low-density lipoprotein; IQR: Interquartile range; TG: Triglycerides.

Table 7 Additional lipoprotein-related parameters in primary biliary cholangitis patients with and without metabolic syndrome

Variable	No MetS	MetS	*P* value
Lp(a) (mg/dL)	3.76 IQR (1.56-13.99)	5.33 IQR (1.65-11.88)	0.802[1]
ApoB (mg/dL)	39.69 IQR (32.02-52.92)	41.61 IQR (31.60-52.16)	0.560[1]
ApoB/ApoA-I	0.59 IQR (0.41-0.76)	0.64 IQR (0.47-0.83)	0.062[1]

[1]Mann Whitney *U* test.
MetS: Metabolic syndrome; Lp(a): Lipoprotein a; ApoA-I: Apolipoprotein A-I; IQR: Interquartile range; ApoB: Apolipoprotein B.

Table 8 Receiver operating characteristic analysis of lipoprotein-related parameters for identification of metabolic syndrome

Variable	AUC (95%CI)	*P* value	Optimal cut-off	Sensitivity (%)	Specificity (%)	Youden index
sdLDL	0.634 (0.540-0.729)	0.006	0.075	46.6	22.2	0.243
largeLDL	0.400 (0.305-0.495)	0.048	2.290	6.9	97.8	0.047
LDL-C	0.378 (0.283-0.473)	0.013	5.360	6.9	98.9	0.058
sdLDL/LargeLDL ratio	0.650 (0.554-0.745)	0.002	0.034	62.1	70.0	0.321

LDL: Low-density lipoprotein cholesterol; sdLDL: Small dense low-density lipoprotein; AUC: Area under the curve; CI: Confidence interval.

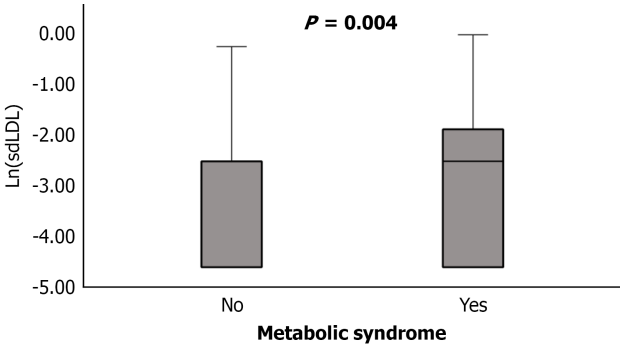


Figure 1 Distribution of ln-transformed small dense low-density lipoprotein levels according to metabolic syndrome status. Ln (small dense low-density lipoprotein) levels were significantly higher in patients with metabolic syndrome than in those without metabolic syndrome (P = 0.004, Mann-Whitney U test). Boxplots represent median values, interquartile ranges, and whiskers. sdLDL: Small dense low-density lipoprotein.

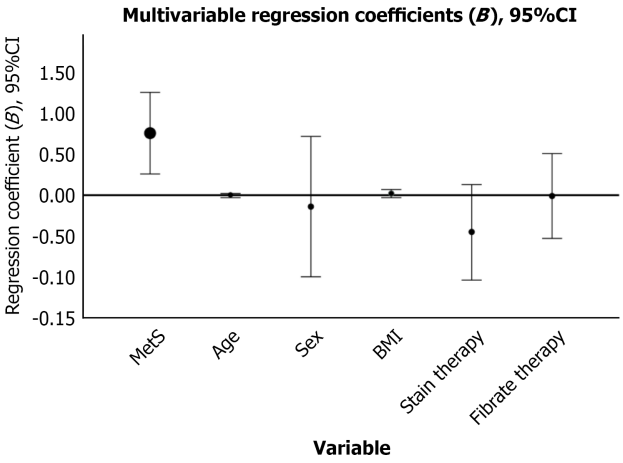


Figure 2 Multivariable linear regression analysis of factors associated with ln-transformed small dense low-density lipoprotein levels. Error bars represent 95% confidence interval. Metabolic syndrome remained independently associated with higher ln (small dense low-density lipoprotein) levels after adjustment for age, sex, body mass index, and lipid-lowering therapy. BMI: Body mass index; CI: Confidence interval.

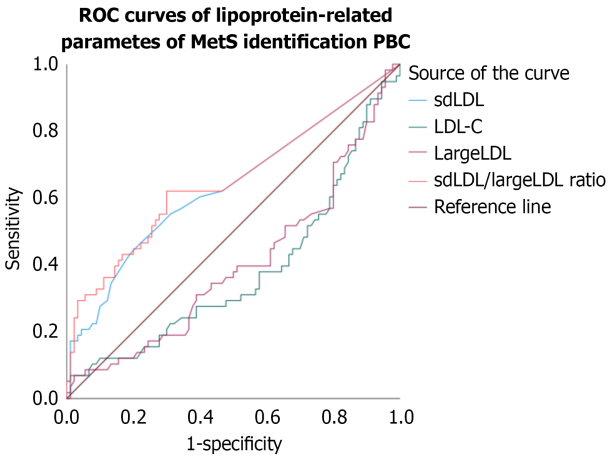


Figure 3 Receiver operating characteristic analysis of lipoprotein-related parameters for identification of metabolic syndrome. Receiver operating characteristic curves are shown for small dense low-density lipoprotein (sdLDL), low-density lipoprotein (LDL)-C, large LDL, and sdLDL/LargeLDL ratio. The sdLDL/LargeLDL ratio demonstrated the highest discriminatory performance, whereas LDL-C and large LDL showed inverse discriminatory patterns. Curves for LDL-C and large LDL fall below the diagonal reference line, indicating that lower values of these parameters were associated with metabolic syndrome. sdLDL: Small dense low-density lipoprotein; ROC: Receiver operating characteristic; MetS: Metabolic syndrome; LDL: Low-density lipoprotein; PBC: Primary biliary cholangitis.

for identification of the metabolically remodeled lipoprotein phenotype.

DISCUSSION

In this cross-sectional cohort of patients with PBC, sdLDL levels were independently associated with MetS, whereas no significant associations were observed with liver fibrosis stage, MASLD, or markers of cholestatic activity. Importantly, the association between MetS and sdLDL remained stable after adjustment for age, sex, adiposity, and lipid-lowering therapy. These findings suggest that sdLDL in contemporary PBC cohorts primarily reflects superimposed metabolic dysfunction rather than cholestatic liver injury itself.

Hypercholesterolemia is a well-recognized feature of PBC and affects the majority of patients during the course of disease[29]. At first glance, markedly elevated total cholesterol and LDL-C levels may suggest a classical atherogenic lipid profile. However, lipid alterations in cholestatic liver disease fundamentally differ from those observed in metabolic dyslipidemia. Impaired bile secretion promotes accumulation of phospholipids and free cholesterol in plasma and leads to formation of LpX, an abnormal phospholipid-rich lipoprotein particle largely lacking ApoB[30]. Unlike native LDL particles, LpX is not cleared through LDL receptors and demonstrates distinct structural and functional properties[31]. Mechanistically, LpX formation is linked to impaired biliary phospholipid transport and altered lipid remodeling, including reduced lecithin-cholesterol acyltransferase activity[32]. Importantly, experimental and clinical observations suggest that LpX-containing fractions may be less susceptible to oxidative modification and may attenuate LDL atherogenicity[33]. Consistent with this concept, systematic reviews and cohort studies have not demonstrated a clear increase in cardiovascular morbidity or mortality in PBC despite frequently elevated cholesterol concentrations[34]. Contemporary evidence therefore suggests that cardiovascular risk in PBC is driven predominantly by traditional metabolic risk factors rather than cholestasis-associated hypercholesterolemia alone[29]. Because the majority of patients received contemporary guideline-based therapy including UDCA, our findings likely reflect lipid remodeling patterns in treated modern PBC cohorts rather than untreated advanced cholestatic disease.

In contrast to this cholestasis-driven lipid phenotype, our findings indicate that sdLDL is linked predominantly to metabolic dysregulation. Formation of sdLDL is tightly associated with insulin resistance and hypertriglyceridemia. As described by Ginsberg and others, insulin-resistant states promote hepatic overproduction of large triglyceride-rich VLDL1 particles, which subsequently undergo cholesteryl ester transfer protein-mediated lipid exchange with LDL particles[11]. Triglyceride-enriched LDL particles then become preferred substrates for hepatic lipase, resulting in formation of smaller and denser LDL particles characteristic of the sdLDL phenotype[35]. Importantly, this remodeling cascade depends on a triglyceride-rich metabolic environment and does not arise from impaired bile excretion or cholestatic lipid retention. Therefore, the absence of association between sdLDL and cholestatic markers in our cohort is mechanistically consistent with current understanding of lipid metabolism.

Beyond their formation, sdLDL particles possess several properties that increase their atherogenic potential. Compared with large buoyant LDL particles, sdLDL demonstrates reduced affinity for LDL receptors, prolonged plasma residence time, greater susceptibility to oxidative modification, and enhanced penetration into the arterial intima[35]. These properties explain why sdLDL is increasingly regarded as a qualitative marker of atherogenic dyslipidemia rather than merely a quantitative LDL-C abnormality.

One of the most notable findings of our study was the paradoxical relationship between conventional LDL-C and metabolically driven lipoprotein remodeling. Patients with MetS exhibited significantly lower LDL-C and large LDL levels despite simultaneously demonstrating higher sdLDL concentrations and increased sdLDL/LargeLDL ratios. Similarly, LDL-C and large LDL showed inverse discriminatory performance in ROC analyses, whereas sdLDL-related parameters demonstrated modest but significant discriminatory ability for identification of MetS. However, the observed AUC values indicate limited diagnostic accuracy and should be interpreted primarily as evidence of biological association rather than clinical classification performance. Collectively, these findings support the concept that conventional LDL-C measurements may inadequately reflect qualitative atherogenic lipoprotein remodeling in metabolically affected PBC patients.

Although MetS was the most consistent independent correlate of ln(sdLDL), the explanatory power of the model was modest. Therefore, our findings should not be interpreted as indicating that MetS quantitatively determines sdLDL levels in PBC. Rather, MetS appears to identify a clinical phenotype characterized by qualitative lipoprotein remodeling toward smaller and denser LDL particles. MetS is defined as a cluster of metabolic abnormalities. It cannot be ruled out that sdLDL levels are associated with individual components of MetS. However, to avoid collinearity, we did not include individual metabolic abnormalities in our analyses, instead, we used MetS as a single variable. This interpretation is supported by the concurrent pattern of lower LDL-C and large LDL levels, higher sdLDL levels, increased sdLDL/LargeLDL ratio, and inverse discriminatory performance of LDL-C and large LDL in ROC analyses.

Interestingly, ApoB concentrations did not differ significantly according to MetS status despite substantial differences in sdLDL levels and lipoprotein remodeling indices. This observation may suggest that qualitative alterations in LDL particle composition occur even in the absence of major differences in total ApoB-containing lipoprotein burden. In this context, sdLDL-related parameters may capture aspects of metabolically driven lipoprotein remodeling not fully reflected by conventional ApoB measurements.

Our findings further reinforce the concept that lipid abnormalities in PBC are heterogeneous. While cholestasis is characterized predominantly by LpX accumulation and a distinct hypercholesterolemic phenotype, sdLDL appears to reflect a superimposed metabolic phenotype rather than an intrinsic feature of cholestatic liver disease. This mechanistic separation may partly explain why cardiovascular risk in classical PBC without metabolic comorbidity is not consistently

increased, whereas patients with concomitant metabolic dysfunction may exhibit a more atherogenic lipoprotein profile. These findings do not imply that sdLDL measurement should replace standard cardiovascular risk assessment. However, advanced lipoprotein profiling may provide complementary information and help distinguish metabolically driven atherogenic dyslipidemia from cholestasis-associated hypercholesterolemia in patients with PBC (Figure 4). To our knowledge, this is the first study evaluating sdLDL as a marker of metabolic dysfunction in patients with PBC.

Interestingly, sdLDL levels were associated with MetS but not with MASLD. Although MASLD was present in a substantial proportion of patients, sdLDL concentrations did not significantly differ according to steatosis status. This discrepancy likely reflects conceptual differences between MASLD and MetS. According to current consensus definitions, MASLD requires hepatic steatosis together with at least one cardiometabolic risk factor[36]. However, this definition encompasses a heterogeneous population ranging from mild metabolic disturbance to overt insulin resistance. In contrast, MetS represents a more advanced systemic metabolic phenotype characterized by central obesity, insulin resistance, hypertriglyceridemia, and low HDL-C levels.

Importantly, hepatic steatosis assessed using CAP primarily quantifies liver fat accumulation and may not directly capture systemic insulin resistance or the qualitative lipoprotein remodeling required for sdLDL generation. Previous studies have shown that CAP correlates with hepatic steatosis but demonstrates weaker associations with systemic metabolic dysregulation and atherogenic lipoprotein phenotype[37,38]. Our findings therefore suggest that in PBC, sdLDL reflects systemic metabolic dysfunction rather than steatosis alone.

In univariable analyses, patients who achieved CBR demonstrated higher sdLDL concentrations. Although this finding may initially appear paradoxical, one possible explanation is that improvement of cholestasis reduces circulating LpX levels, thereby unmasking metabolically driven ApoB-containing lipoprotein remodeling. In this scenario, the lipid profile may shift from a cholestasis-dominated phenotype toward a more classical metabolic dyslipidemia in which sdLDL becomes more apparent. However, this association did not persist after multivariable adjustment, suggesting that the observed difference is likely confounded by metabolic factors rather than representing an independent effect of biochemical response. Therefore, this observation should be interpreted cautiously and viewed primarily as a hypothesis-generating finding requiring further validation in longitudinal studies.

Limitations

The present study should be interpreted in light of several limitations. First, its cross-sectional design precludes causal inference, and the observed association between MetS and sdLDL therefore reflects correlation rather than temporal sequence.

Second, this was a single-center study conducted at a tertiary referral center, which may limit generalizability. Although the cohort reflects real-world hepatology practice, external validation in independent populations is warranted. Despite the single-center design, the study included a comprehensively phenotyped PBC cohort with standardized Lipoprint lipoprotein profiling and VCTE assessment. In addition, the sample size was moderate and the proportion of male patients was low, consistent with the well-established female predominance of PBC observed in European epidemiological cohorts, but limiting adequately powered sex-specific analyses.

Third, cardiovascular outcomes were not assessed. Although sdLDL is widely regarded as a marker of atherogenic dyslipidemia, we did not evaluate incident cardiovascular events or surrogate markers of atherosclerosis. Therefore, the clinical implications of elevated sdLDL concentrations in this specific population remain mechanistic rather than outcome-driven.

Fourth, sdLDL was measured at a single time point. Lipoprotein subfractions may vary according to metabolic status, therapeutic interventions, and disease activity, and longitudinal studies are needed to evaluate temporal stability and prognostic significance. In addition, sdLDL values demonstrated a partially zero-inflated distribution, which may limit conventional linear modeling approaches despite complementary logistic analyses yielding consistent results.

Fifth, direct quantification of LpX was not performed. Therefore, its contribution to observed lipid patterns remains indirect and mechanistic. In addition, coexisting autoimmune disorders commonly associated with PBC, such as Sjögren syndrome or autoimmune thyroid disease, were not systematically analyzed and their potential influence on lipid metabolism cannot be excluded.

In addition, information on concomitant autoimmune diseases was not collected in our study, therefore, their potential effect on lipid metabolism was not evaluated and cannot be excluded.

Finally, the explanatory power of the multivariable model was modest (adjusted $R^2 = 0.054$), indicating that a substantial proportion of variability in sdLDL remains unexplained. However, sdLDL metabolism is biologically complex and influenced by multiple interacting metabolic, genetic, and environmental factors. Dietary habits and physical activity were not systematically assessed and residual lifestyle-related confounding cannot be excluded.

CONCLUSION

In conclusion, sdLDL levels in patients with PBC were independently associated with MetS while associations with cholestatic activity, liver fibrosis, MASLD, and biochemical response were not independently sustained after multivariable adjustment. These findings support the concept that sdLDL reflects superimposed metabolic dysfunction rather than intrinsic cholestatic lipid alterations in contemporary PBC cohorts. sdLDL-related parameters may help characterize a subgroup of PBC patients with metabolically driven qualitative lipoprotein remodeling beyond conventional LDL-C measurements.

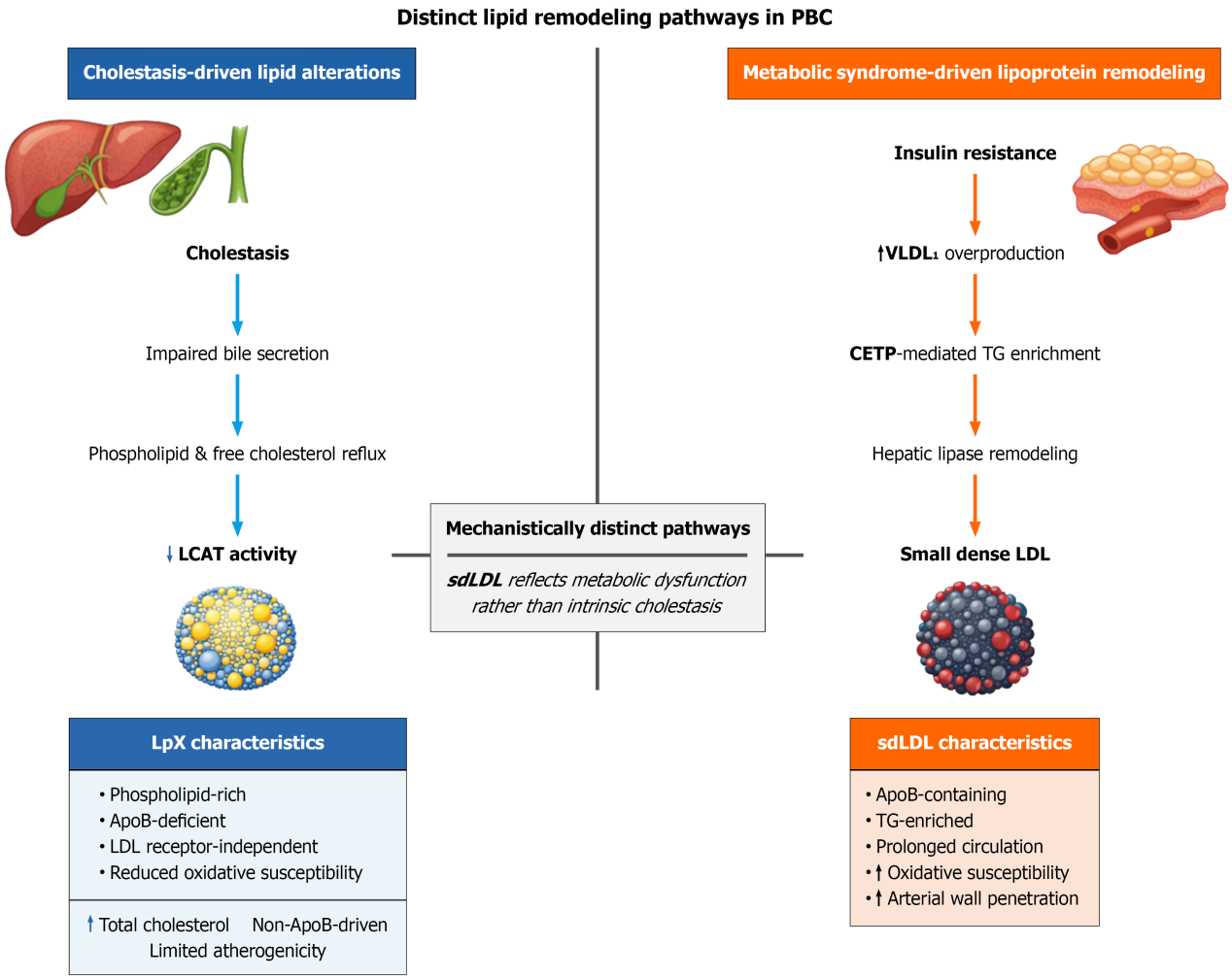


Figure 4 Mechanistically distinct lipid remodeling pathways in primary biliary cholangitis. Left panel: Cholestasis impairs bile acid secretion and disrupts biliary phospholipid transport, leading to reflux of phospholipids and free cholesterol into the circulation and reduced lecithin-cholesterol acyltransferase activity. These alterations promote the formation of lipoprotein X, a phospholipid-rich, apolipoprotein B (ApoB)-deficient particle that contributes to hypercholesterolemia but does not reflect classical ApoB-driven atherogenic dyslipidemia. Right panel: In contrast, insulin resistance associated with metabolic syndrome promotes hepatic overproduction of triglyceride-rich very low-density lipoprotein 1 particles. Subsequent cholesteryl ester transfer protein-mediated lipid exchange and hepatic lipase-dependent remodeling generate small dense low-density lipoprotein (sdLDL), an ApoB-containing lipoprotein subfraction characterized by prolonged circulation time, increased oxidative susceptibility, and enhanced arterial wall penetration. These pathways are mechanistically distinct and support the concept that sdLDL levels in primary biliary cholangitis primarily reflect superimposed metabolic dysfunction rather than intrinsic cholestatic activity. Created by the authors for illustrative purposes (OpenAI 1.3.2026). LCAT: Lecithin-cholesterol acyltransferase; LpX: Lipoprotein X; PBC: Primary biliary cholangitis; sdLDL: Small dense low-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; ApoB: Apolipoprotein B; CETP: Cholesteryl ester transfer protein; TG: Triglycerides.

REFERENCES

1 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. *J Hepatol* 2017; **67**: 145-172 [*RCA*] [PMID: 28427765 DOI: 10.1016/j.jhep.2017.03.022] [FullText]

2 **Lindor KD**, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2021 practice guidance update from the American Association for the Study of Liver Diseases. *Hepatology* 2022; **75**: 1012-1013 [*RCA*] [PMID: 34431119 DOI: 10.1002/hep.32117] [FullText]

3 **Gazda J**, Drazilova S, Janicko M, Jarcuska P. The Epidemiology of Primary Biliary Cholangitis in European Countries: A Systematic Review and Meta-Analysis. *Can J Gastroenterol Hepatol* 2021; **2021**: 9151525 [*RCA*] [PMID: 34239845 DOI: 10.1155/2021/9151525] [FullText] [Full Text(PDF)]

4 **Hirschfield GM**, Chazouillères O, Cortez-Pinto H, Macedo G, de Lédinghen V, Adekunle F, Carbone M. A consensus integrated care pathway for patients with primary biliary cholangitis: a guideline-based approach to clinical care of patients. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 929-939 [*RCA*] [PMID: 34233565 DOI: 10.1080/17474124.2021.1945919] [FullText]

5 **Drazilova S**, Babinska I, Gazda J, Halanova M, Janicko M, Kucinsky B, Safcak D, Martinkova D, Tarbajova L, Cekanova A, Jarcuska P; Eastern Slovakia PBC Group. Epidemiology and clinical course of primary biliary cholangitis in Eastern Slovakia. *Int J Public Health* 2020; **65**: 683-691 [*RCA*] [PMID: 32500239 DOI: 10.1007/s00038-020-01391-6] [FullText]

6 **Sylvia D**, Tomas K, Marian M, Martin J, Dagmar S, Peter J. The treatment of primary biliary cholangitis: from shadow to light. *Ther Adv Gastroenterol* 2024; **17**: 17562848241265782 [*RCA*] [PMID: 39081664 DOI: 10.1177/17562848241265782] [FullText] [Full Text(PDF)]

7 **Gazda J**, Drazilova S, Gazda M, Janicko M, Koky T, Macej M, Carbone M, Jarcuska P. Treatment response to ursodeoxycholic acid in

primary biliary cholangitis: A systematic review and meta-analysis. *Dig Liver Dis* 2023; **55**: 1318-1327 [*RCA*] [PMID: 36593158 DOI: 10.1016/j.dld.2022.12.010] [FullText]

8 **Gazda J**, Drazilova S, Janicko M, Grgurevic I, Filipec Kanizaj T, Koller T, Bodorovska B, Bozin T, Mijic M, Rob Z, Mikolasevic I, Madir A, Kucinsky B, Jarcuska P. Prognostic Factors in Primary Biliary Cholangitis: A Retrospective Study of Joint Slovak and Croatian Cohort of 249 Patients. *J Pers Med* 2021; **11**: 495 [*RCA*] [PMID: 34205918 DOI: 10.3390/jpm11060495] [FullText] [Full Text(PDF)]

9 **Kalra DK**, Shotwell M, Garg A, Duell PB, Wilson DP, Martin SS, Soffer DE, Rosenson RS, Shapiro MD, Ahmad Z, Underberg J, Sperling L, Jortani SA, Remaley A. Lipoprotein X - Pathophysiology, diagnosis, and management. *J Clin Lipidol* 2025; **19**: 759-774 [*RCA*] [PMID: 40555623 DOI: 10.1016/j.jacl.2025.05.015] [FullText]

10 **Kattah L**, Gómez A, Gutiérrez S, Puerto K, Moreno-Pallares ED, Jaramillo A, Mendivil CO. Hypercholesterolemia Due to Lipoprotein X: Case Report and Thematic Review. *Clin Med Insights Endocrinol Diabetes* 2019; **12**: 1179551419878687 [*RCA*] [PMID: 31632171 DOI: 10.1177/1179551419878687] [FullText] [Full Text(PDF)]

11 **Hirano T**. Clinical significance of small dense low-density lipoprotein cholesterol measurement in type 2 diabetes. *J Diabetes Investig* 2025; **16**: 370-383 [*RCA*] [PMID: 39778086 DOI: 10.1111/jdi.14398] [FullText]

12 **Hoogeveen RC**, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, Couper D, Virani SS, Kathiresan S, Boerwinkle E, Ballantyne CM. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. *Arterioscler Thromb Vasc Biol* 2014; **34**: 1069-1077 [*RCA*] [PMID: 24558110 DOI: 10.1161/ATVBAHA.114.303284] [FullText]

13 **Loaeza-Del Castillo AM**, Gaytán-Santillán A, López-Tello A, Merit MV, Milke-García MDP, Flores-Rodríguez J, Alvirde-García U. Patterns of serum lipids derangements and cardiovascular risk assessment in patients with primary biliary cholangitis. *Ann Hepatol* 2019; **18**: 879-882 [*RCA*] [PMID: 31506216 DOI: 10.1016/j.aohep.2019.07.006] [FullText]

14 **Reshetnyak VI**, Maev IV. Features of Lipid Metabolism Disorders in Primary Biliary Cholangitis. *Biomedicines* 2022; **10**: 3046 [*RCA*] [PMID: 36551803 DOI: 10.3390/biomedicines10123046] [FullText] [Full Text(PDF)]

15 **Suraweera D**, Fanous C, Jimenez M, Tong MJ, Saab S. Risk of Cardiovascular Events in Patients with Primary Biliary Cholangitis - Systematic Review. *J Clin Transl Hepatol* 2018; **6**: 119-126 [*RCA*] [PMID: 29951355 DOI: 10.14218/JCTH.2017.00064] [FullText] [Full Text (PDF)]

16 **Chang PY**, Lu SC, Su TC, Chou SF, Huang WH, Morrisett JD, Chen CH, Liau CS, Lee YT. Lipoprotein-X reduces LDL atherogenicity in primary biliary cirrhosis by preventing LDL oxidation. *J Lipid Res* 2004; **45**: 2116-2122 [*RCA*] [PMID: 15314101 DOI: 10.1194/jlr.M400229-JLR200] [FullText]

17 **Bekbossynova M**, Saliev T, Ivanova-Razumova T, Andossova S, Kali A, Myrzakhmetova G. Small dense LDL: An underestimated driver of atherosclerosis (Review). *Mol Med Rep* 2025; **32**: 328 [*RCA*] [PMID: 40999979 DOI: 10.3892/mmr.2025.13693] [FullText] [Full Text(PDF)]

18 **Ikezaki H**, Lim E, Cupples LA, Liu CT, Asztalos BF, Schaefer EJ. Small Dense Low-Density Lipoprotein Cholesterol Is the Most Atherogenic Lipoprotein Parameter in the Prospective Framingham Offspring Study. *J Am Heart Assoc* 2021; **10**: e019140 [*RCA*] [PMID: 33586462 DOI: 10.1161/JAHA.120.019140] [FullText] [Full Text(PDF)]

19 **Wang J**, Zhao X, Zhao Y, Jin R, Li Y, Wang J, Liu Y, Wu Z, Guo X, Tao L. Discordance of Small Dense LDL Cholesterol Beyond LDL Cholesterol or Non-HDL Cholesterol and Carotid Plaque. *JACC Asia* 2025; **5**: 1012-1028 [*RCA*] [PMID: 40632051 DOI: 10.1016/j.jacasi.2025.04.015] [FullText] [Full Text(PDF)]

20 **Danielsson O**, Vesterinen T, Arola J, Åberg F, Nissinen MJ. Coexistence of metabolic-associated fatty liver disease and autoimmune or toxic liver disease. *Eur J Gastroenterol Hepatol* 2024; **36**: 961-969 [*RCA*] [PMID: 38829946 DOI: 10.1097/MEG.0000000000002785] [FullText] [Full Text(PDF)]

21 **Ren W**, Wang Z, Liu X, Liu X, Ma D, Jin Q, Wang J, Feng J, Rao H, Wang X, Huang R. Concurrent nonalcoholic fatty liver disease may decrease liver fibrosis severity in patients with primary biliary cholangitis. *BMC Gastroenterol* 2025; **25**: 541 [*RCA*] [PMID: 40739215 DOI: 10.1186/s12876-025-04145-x] [FullText] [Full Text(PDF)]

22 **Koky T**, Drazilova S, Komarova S, Macej M, Toporcerova D, Janicko M, Spakova I, Rabajdova M, Marekova M, Jarcuska P. Adipokine profiles reflect metabolic dysfunction but not fibrosis in patients with primary biliary cholangitis. *World J Hepatol* 2026; **18**: 113685 [*RCA*] [PMID: 41809476 DOI: 10.4254/wjh.v18.i2.113685] [FullText] [Full Text(PDF)]

23 **Jiang S**, Zhang F, Yang H, Han X, Mao J, Zheng G, Fan Y. Estimated sdLDL-C as a biomarker of hepatic steatosis severity in MASLD: a retrospective study. *BMC Gastroenterol* 2025; **25**: 168 [*RCA*] [PMID: 40082781 DOI: 10.1186/s12876-025-03759-5] [FullText] [Full Text (PDF)]

24 **Alberti KG**, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; **366**: 1059-1062 [*RCA*] [PMID: 16182882 DOI: 10.1016/S0140-6736(05)67402-8] [FullText]

25 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J Hepatol* 2024; **81**: 492-542 [*RCA*] [PMID: 38851997 DOI: 10.1016/j.jhep.2024.04.031] [FullText]

26 **Corpechot C**, Heurgue A, Tanne F, Potier P, Hanslik B, Decraecker M, de Lédinghen V, Ganne-Carrié N, Bureau C, Bourlière M. Non-invasive diagnosis and follow-up of primary biliary cholangitis. *Clin Res Hepatol Gastroenterol* 2022; **46**: 101770 [*RCA*] [PMID: 34332135 DOI: 10.1016/j.clinre.2021.101770] [FullText]

27 **Bueti F**, Pitrone C, Filomia R, Franzè M, Caccamo G, Maimone S, Cacciola I, Raimondo G, Saitta C. Liver steatosis assessed by CAP in patients with primary biliary cholangitis. *Dig Liver Dis* 2024; **56**: S29-S30 [DOI: 10.1016/j.dld.2024.01.044] [FullText]

28 **Hoefner DM**, Hodel SD, O'Brien JF, Branum EL, Sun D, Meissner I, McConnell JP. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. *Clin Chem* 2001; **47**: 266-274 [*RCA*] [PMID: 11159775] [FullText]

29 **Wah-Suarez MI**, Danford CJ, Patwardhan VR, Jiang ZG, Bonder A. Hyperlipidaemia in primary biliary cholangitis: treatment, safety and efficacy. *Frontline Gastroenterol* 2019; **10**: 401-408 [*RCA*] [PMID: 31656566 DOI: 10.1136/flgastro-2018-101124] [FullText]

30 **Rao N**, Jain A, Goyale A, Persaud JW, Al-musalhi K, Nair DR. Lipoprotein X in autoimmune liver disease causing interference in routine and specialist biochemical investigations. *Clin Lipidol* 2017; **12**: 8-13 [DOI: 10.1080/17584299.2017.1308670] [FullText]

31 **Heimerl S**, Boettcher A, Kaul H, Liebisch G. Lipid profiling of lipoprotein X: Implications for dyslipidemia in cholestasis. *Biochim Biophys Acta* 2016; **1861**: 681-687 [*RCA*] [PMID: 27112638 DOI: 10.1016/j.bbalip.2016.04.016] [FullText]

32 **Fellin R**, Manzato E. Lipoprotein-X fifty years after its original discovery. *Nutr Metab Cardiovasc Dis* 2019; **29**: 4-8 [*RCA*] [PMID: 30503707 DOI: 10.1016/j.numecd.2018.09.006] [FullText]

33 **Neely RDG**, Boot CS. Laboratory investigation of lipoprotein X. *Clin Lipidol* 2017; **12**: 43-44 [DOI: 10.1080/17584299.2017.1337952] [Full Text]

34 **Sorokin A**, Brown JL, Thompson PD. Primary biliary cirrhosis, hyperlipidemia, and atherosclerotic risk: a systematic review. *Atherosclerosis* 2007; **194**: 293-299 [*RCA*] [PMID: 17240380 DOI: 10.1016/j.atherosclerosis.2006.11.036] [FullText]

35 **Jin X**, Yang S, Lu J, Wu M. Small, Dense Low-Density Lipoprotein-Cholesterol and Atherosclerosis: Relationship and Therapeutic Strategies. *Front Cardiovasc Med* 2021; **8**: 804214 [*RCA*] [PMID: 35224026 DOI: 10.3389/fcvm.2021.804214] [FullText] [Full Text(PDF)]

36 **Rinella ME**, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, Arrese M, Bataller R, Beuers U, Boursier J, Bugianesi E, Byrne CD, Castro Narro GE, Chowdhury A, Cortez-Pinto H, Cryer DR, Cusi K, El-Kassas M, Klein S, Eskridge W, Fan J, Gawrieh S, Guy CD, Harrison SA, Kim SU, Koot BG, Korenjak M, Kowdley KV, Lacaille F, Loomba R, Mitchell-Thain R, Morgan TR, Powell EE, Roden M, Romero-Gómez M, Silva M, Singh SP, Sookoian SC, Spearman CW, Tiniakos D, Valenti L, Vos MB, Wong VW, Xanthakos S, Yilmaz Y, Younossi Z, Hobbs A, Villota-Rivas M, Newsome PN; NAFLD Nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* 2023; **78**: 1966-1986 [*RCA*] [PMID: 37363821 DOI: 10.1097/HEP.0000000000000520] [FullText] [Full Text(PDF)]

37 **Cao YT**, Xiang LL, Qi F, Zhang YJ, Chen Y, Zhou XQ. Accuracy of controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) for assessing steatosis and fibrosis in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *EClinicalMedicine* 2022; **51**: 101547 [*RCA*] [PMID: 35844772 DOI: 10.1016/j.eclinm.2022.101547] [FullText] [Full Text(PDF)]

38 **Sharif A**, Abbas Z, Ahmed S, Ali Samjo S, Baqai K. Effect of Non-alcoholic Fatty Liver Disease on Transaminase Levels and Transient Elastography in Patients with Chronic Hepatitis B. *Cureus* 2019; **11**: e5995 [*RCA*] [PMID: 31807383 DOI: 10.7759/cureus.5995] [FullText] [Full Text(PDF)]

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ORIGINAL ARTICLE

Basic Study

Suppression of the STAT3/MYC/MCL-1 pathway by the fixed Jianpi Huoxue Formula relieves early biochemical abnormalities in liver fibrosis

Long Huang, Cheng-Gui Wu, Teng-Fei Zhu, Qing-Lin Li, Qing-Sheng Yu, Qi Zhang, Yi Shen

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Abstract

BACKGROUND

Liver fibrosis (LF), a core pathological process driving chronic liver disease, has no ideal clinical intervention approaches. A fixed Jianpi Huoxue Formula (FJHF), a traditional Chinese medicine (TCM) prescription derived from the Sijunzi Decoction and Taohong Siwu Decoction, has shown clinical benefits against LF.

AIM

To investigate the mechanism by which FJHF attenuates LF *via* STAT3/MYC/MCL-1 pathway regulation.

METHODS

Three strategies were integrated: (1) Clinical single-cell RNA sequencing (scRNA-seq) was performed on liver tissues from three untreated cirrhosis patients and three FJHF-treated patients to characterize hepatic cell heterogeneity and FJHF-responsive gene expression; (2) A randomized controlled trial was conducted in 40 cirrhosis patients with hypersplenism (20 each in the Jianpi Huoxue Formula and control groups) to assess a hepatocellular injury indicator alanine aminotransferase (ALT), hepatic reserve function indicators [total bilirubin (TBIL) and prothrombin time], and fibrosis indicators [hyaluronic acid (HA), laminin, type III

procollagen N-terminal peptide (PIIINP), and type IV collagen (CIV)]; and (3) *In vitro* experiments were performed in human hepatic stellate cells (HSCs) LX-2 using cell counting kit-8, transmission electron microscopy, western blotting, and quantitative real-time PCR to verify the regulatory role of FJHF in the STAT3/MYC/MCL-1 pathway.

RESULTS

FJHF modulated the proportion of HSC populations in a small-sample scRNA-seq cohort. According to clinical data, FJHF treatment significantly decreased ALT ($P < 0.01$), TBIL ($P < 0.05$), and the fibrosis markers HA, PIIINP, and CIV ($P < 0.05$) compared to the control group. *In vitro*, 20% FJHF-containing serum suppressed HSC activation, as indicated by reduced α-SMA, collagen I, and TIMP1 levels and elevated MMP1 levels. Notably, FJHF inhibits the STAT3/MYC/MCL-1 pathway to ameliorate LF.

CONCLUSION

Short-term FJHF intervention improves early biochemical abnormalities in LF by inhibiting the STAT3/MYC/MCL-1 pathway and suppressing HSC activation. This work provides a scientific basis for the application of FJHF and identifies the STAT3/MYC/MCL-1 axis as a potential target for TCM intervention in early LF metabolic abnormalities.

Key Words: Liver fibrosis; STAT3/MYC/MCL-1 pathway; Network pharmacology; Single-cell RNA sequencing; Hepatic stellate cells

Core Tip: This study comprehensively explored the pharmacological mechanism of a fixed Jianpi Huoxue Formula (FJHF). We integrated single-cell transcriptomics, clinical observations, and *in vitro* experiments to confirm that FJHF inhibits the STAT3/MYC/MCL-1 pathway, suppresses hepatic stellate cell activation, and ameliorates early biochemical abnormalities in liver fibrosis.

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INTRODUCTION

Chronic liver disease (CLD) represents a significant global health issue, with cirrhosis accounting for the majority of CLD-related mortality. In 2016, cirrhosis accounted for 2.2% of global deaths, ranking as the 11th leading cause of mortality and the 15th leading cause of morbidity worldwide; however, specific antifibrotic therapies remain largely unavailable[1,2]. Despite advances in modern medical management of CLD, effective pharmacotherapy for liver fibrosis (LF) remains limited[3]. In contrast, traditional Chinese medicine (TCM) has demonstrated unique clinical benefits in alleviating LF[4].

The fixed Jianpi Huoxue Formula (FJHF) used in this study was specially developed with fixed herbal components and dosage, modified from the Sijunzi and Taohong Siwu decoctions. FJHF, developed by Professor Yusheng Yu, consists of ten herbs: *Panax ginseng* (Renshen), *Poria cocos* (Fuling), *Atractylodes macrocephala* (Baizhu), *Glycyrrhiza uralensis* (Gancao), *Angelica sinensis* (Danggui), *Ligusticum chuanxiong* (Chuanxiong), *Paeonia lactiflora* (Baishao), *Rehmannia glutinosa* (Shudihuang), *Prunus persica kernel* (Taoren), and *Carthamus tinctorius* (Honghua). All the herbal names were verified using the MPNS database (https://mpns.science.kew.org/) (Table 1). Clinically, FJHF has been proven to alleviate hepatocellular injury and enhance the hepatic reserve function, thereby attenuating fibrosis. Previous studies have validated the antifibrotic effects of several components or herb pairs in FJHF. For instance, ginseng regulates the TGF-β1/Smad pathway[5], the Taoren-Honghua pair modulates oxidative stress *via* the PI3K/Akt pathway[6], Chuanxiong extract alleviates LF *via* the CTCF/c-MYC/H19 pathway[7], and the Danggui-Chuanxiong pair inhibits collagen deposition and inflammation[8].

The STAT3 signaling pathway plays a pivotal role in LF pathogenesis. Phosphorylated STAT3 directly targets downstream molecules, including MYC and MCL-1, which are strongly involved in LF progression[9-11]. STAT3 activation drives hepatic stellate cell (HSC) activation and aggravates LF, whereas its suppression effectively improves early metabolic abnormalities in hepatic fibrosis. Although the individual herbal components of FJHF are known to act on multiple molecular pathways, the integrated mechanism of this multicomponent formula remains insufficiently understood. Therefore, we hypothesized that FJHF inhibited the STAT3/MYC/MCL-1 pathway to suppress HSC activation, thereby alleviating LF. To test this hypothesis, we combined clinical single-cell RNA sequencing (scRNA-seq), a randomized controlled trial, network pharmacology analysis, and *in vitro* cellular experiments to elucidate the mechanism by which FJHF improves early metabolic disorders associated with LF, providing both theoretical rationale and experimental evidence to support its clinical application.

Table 1 Details of the herbs in the Jianpi Huoxue Formula

Chinese name	Latin name	Part(s) used	Amount (g)	Source (manufacturer)	Batch number	Processing specification (per ChP 2020)
Renshen	*Panax Ginseng C. A. Mey.*	Roots and rhizomesroots	10	Anguo Kangtai Pharmaceutical Co., Ltd.	20230115	Unprocessed (selected for intact roots, no insect damage)
Fulin	*Poria Cocos (Schw.) Wolf.*	Sclerotium	10	Anhui Huayuan Chinese Herbal Pieces Co., Ltd.	20230208	Decorticated (removed outer bark, cut into 1-2 cm thick slices)
Baizhu	*Atractylodes Macrocephala Koidz.*	Roots and rhizomesroots	10	Bozhou Huichuntang Chinese Herbal Pieces Co., Ltd.	20230312	Stir-fried (stir-fried with wheat bran until slightly yellow, to enhance spleen-invigorating effect)
Gancao	*licorice*	Roots and rhizomesroots	10	Ningxia Qiyuan Chinese Herbal Pieces Co., Ltd.	20230120	Honey-roasted (stir-fried with mature honey until evenly coated, to strengthen qi-tonifying effect)
Danggui	*Angelicae Sinensis Radix*	Roots	10	Gansu Minxian Zhongtian Pharmaceutical Co., Ltd.	20230225	Unprocessed (cut into 2-4 mm thin slices, with strong aromatic odor)
Chuanxiong	*Chuanxiong Rhizoma*	Roots and rhizomesroots	10	Sichuan Chuanxiong Pharmaceutical Co., Ltd.	20230305	Unprocessed (cut into irregular thin slices, brownish-yellow cross-section)
Baishao	*Paeoniae Radix Alba*	Roots	10	Anhui Bozhou Baicao Chinese Herbal Pieces Co., Ltd.	20230130	Boiled (boiled in water until the cross-section is white, peeled, cut into slices, dried; raw Baishao, to nourish blood and soothe liver)
Shudihuang	*Rehmanniae Radix Praeparata*	Roots	10	Henan Huaiqing Pharmaceutical Co., Ltd.	20230218	Steamed (steamed with rice wine, then sun-dried repeatedly until black and glossy, to tonify kidney-yin)
Taoren	*Persicae Semen*	Seed	10	Shaanxi Weinan Chinese Herbal Pieces Co., Ltd.	20230320	Blanched (blanched in boiling water to remove seed coat, dried, crushed slightly)
Honghua	*Carthami Flos*	Flower	10	Xinjiang Hotan Red Flower Co., Ltd.	20230205	Unprocessed (selected for bright red florets, no impurities)

MATERIALS AND METHODS

General information

In this randomized controlled trial, patients with cirrhosis and hypersplenism were consecutively recruited at the First Affiliated Hospital of Anhui University of Chinese Medicine from January 2023 to December 2024. Finally, 40 patients were included in the statistical analysis (Figure 1). A statistician, independent of recruitment, generated a 1: 1 random sequence (1-40) *via* SPSS 21.0. Inclusion criteria: Definite cirrhosis diagnosis per the EASL Clinical Practice Guidelines[12], confirmed by: (1) Clinical evidence of portal hypertension (splenomegaly, jaundice, ascites, varices, or encephalopathy); (2) Biochemical markers: Albumin < 35 g/L, bilirubin > 1.2 mg/dL; and (3) Imaging: Liver stiffness (LS) ≥ 17.5 kPa by FibroTouch or nodular hepatic morphology on contrast-enhanced computed tomography/magnetic resonance imaging.

Exclusion criteria were: (1) Incomplete clinical data (missing more than two key parameters); (2) Refusal to provide informed consent; and (3) Early hospital discharge.

Patients were assigned to the control (n = 20, odd numbers) or Jianpi Huoxue Formula (JHF) (n = 20, even numbers) group using sealed opaque envelopes stored at the clinical research center, ensuring allocation concealment until baseline data collection. The participants in the control group received conventional Western medicine for liver protection, jaundice reduction, and other supportive symptomatic therapies. The JHF group received conventional western medicine combined with FJHF, and the formula was administered twice a day orally, with a continuous 21-day medication period as one treatment course. Blood samples for hepatocellular injury [serum alanine aminotransferase (ALT)], hepatic reserve function [total bilirubin (TBIL) and prothrombin time (PT)], and fibrosis markers [hyaluronic acid (HA), laminin (LN), type III procollagen N-terminal peptide (PIIINP), and type IV collagen (CIV)] were anonymized and tested by a third-party laboratory (Anhui Provincial Clinical Laboratory Center). Clinical data of patients, including demographic characteristics, hepatocellular injury indicator (ALT), hepatic reserve function parameters (TBIL, PT), and LF marker results (HA, LN, PIIINP, and CIV), were collected by two nurses. Following the end of treatment, three patients were randomly selected from both the control and JHF groups among the 40 enrolled patients who underwent liver biopsy, and their liver tissue samples were analyzed *via* scRNA-seq.

The sample size was determined based on primary outcome measures, including serum ALT and LF markers (HA, PIIINP, and CIV). Assuming a mean difference of 10-15 U/L in ALT and 20-30 ng/mL in fibrosis markers between the two groups, with a type I error (α) of 0.05, a power (1-β) of 0.80, a minimum sample size of 20 patients per group was determined to be sufficient to detect statistically significant differences. The sample size was reviewed, and the study was approved by the Institutional Review Board of the First Affiliated Hospital of Anhui University of Chinese Medicine. The total sample size was set to 40 patients (20 in each group). This study was conducted from January 2023 to December 2024. Forty cirrhotic patients with hypersplenism were enrolled, including 24 males and 16 females, with a mean age of 37.88 ± 13.16 years. The higher proportion of male patients was consistent with the epidemiological features of liver

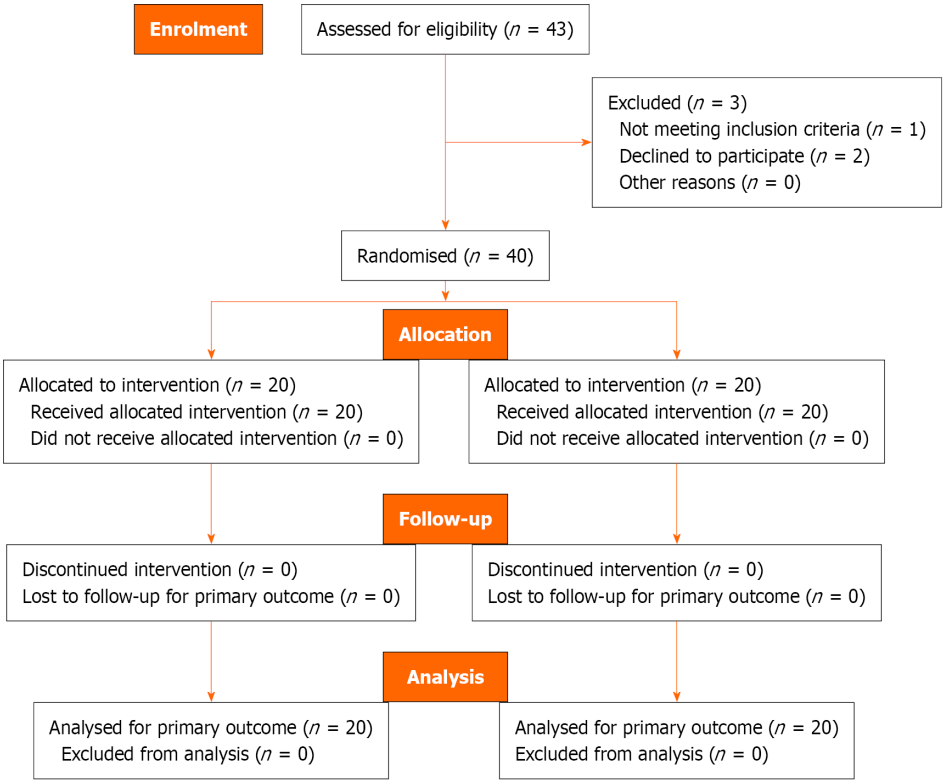


Figure 1 Flow diagram of the participant inclusion process.

cirrhosis and CLD, in which males have a higher incidence because they are more exposed to risk factors such as alcohol consumption, viral hepatitis infection, and unhealthy lifestyles.

Ethics statement

Prior to the study implementation, all participants were provided with a complete explanation of the research objectives and detailed protocols, and written informed consent was obtained from every subject or their authorized legal representative. This research was conducted in compliance with the ethical guidelines of the Declaration of Helsinki of the World Medical Association (revised in Fortaleza, Brazil, October 2013). Ethical approval for this study was granted by the Institutional Review Board of the First Affiliated Hospital of the Anhui University of Chinese Medicine (No. 2024AH-30). All patient information was anonymized and stored in a password-protected database that adheres to HIPAA requirements, with secure data management performed using the Research Electronic Data Capture platform. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Anhui University of Chinese Medicine (No. 20240030).

Preparation and quality control of the FJHF

All 10 medicinal herbs included in FJHF were obtained from the Bozhou TCM Market located in Bozhou, Anhui Province, China. All herbs were authenticated by Prof. Qing-Lin Li from the Anhui University of Chinese Medicine, in strict accordance with the requirements specified in the 2020 Edition of the Chinese Pharmacopoeia (2020 Edition, Volume I). The FJHF decoction was prepared in the GMP-qualified TCM Decoction Room of the First Affiliated Hospital of Anhui University of Chinese Medicine following a standardized manufacturing procedure to ensure consistency and reproducibility. Each dose of medicinal material was added to 500 mL of water, decocted for 30 minutes, filtered, and concentrated to a crude drug decoction at a concentration of 1.0 g/mL. The decoction was stored at 4 °C and rewarmed before administration, and the entire preparation process was completed with assistance from the institutional preparation center. This formula adopts a fixed herbal species, dosage, and preparation procedure in the present study, which is a specific modified prescription rather than a general Jianpi Huoxue therapeutic principle, and the research conclusions are only applicable to this fixed formulation.

ScRNA-seq

Liver tissue samples were collected from cirrhotic patients (n = 3) and FJHF-treated cirrhotic patients (n = 3) for scRNA-seq analysis. Tissue samples were dissociated into single-cell suspensions (QC: Viability > 80%, no aggregation) and processed for library construction using the 10 × Genomics Chromium platform, followed by mRNA release, reverse transcription to cDNA, PCR amplification, and library construction using (10 × Genomics Single Cell 3′ Reagent Kit v3.1). Validated libraries (Agilent 2100, Qubit 4.0, insert size approximately 300 bp, adapter dimer < 5%) were sequenced using Illumina NovaSeq 6000 (PE150 mode). For bioinformatics, 10 × Genomics Cell Ranger (v6.0) was used for read filtering

(Q30 > 85%), genome alignment (GRCh38/GRCm38), and UMI-based quantification. Seurat (v4.0) filtered low-quality cells and integrated samples (Canonical Correlation Analysis), performed PCA-tSNE, and conducted Louvain clustering/differential gene analysis (adjusted $P < 0.05$, log2FC > 1). SingleR (v1.0) annotated cell types, CellChat (v1.1.0), and inferred ligand-receptor interactions (all *via* R v4.5.1). We used the VlnPlot function to draw violin plots and applied the feature plot function to generate feature plots for data visualization. Experimental analyses were performed by Nanjin Personal Gene Technology Co., Ltd (Nanjing, China). Baseline characteristics of the six enrolled patients are listed in Supplementary Table 1. Given the limited sample size (n = 3 per group), our scRNA-seq results should be interpreted with caution and cannot be generalized to all patients with cirrhosis.

Network pharmacology analysis of FJHF in LF

Active compounds and their targets in FJHF were retrieved from TCMSP (https://www.91tcmsp.com/#/home) and filtered by pharmacokinetic properties (oral bioavailability ≥ 30%, drug-likeness ≥ 0.18)[13]. The corresponding target genes were obtained from UniProt (https://www.uniprot.org/) and validated using UniProt and GeneCards[14]. LF-related targets were collected from five public databases (Online Mendelian Inheritance in Man (OMIM, https://omim.org/), GeneCards (https://www.genecards.org/), DrugBank (https://go.drugbank.com/), Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB, https://www.pharmgkb.org/), Therapeutic Target Database (TTD, https://db.idrblab.net/ttd/) using the keyword "liver fibrosis". The intersection targets of FJHF and LF were used to construct drug-compound-gene (Cytoscape 3.8.0) and protein-protein interaction (PPI) (STRING 11.0, confidence = 0.9) networks[15]. Hub genes were selected using the CytoNCA plugin based on six topological indicators: Betweenness, closeness, degree, eigenvector, local average connectivity, and network centrality. Genes with values above the median of all six indices were defined as key hub targets[16]. Functional enrichment analyses, including Gene Ontology (GO), terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, were performed using the clusterProfiler package in R software (version 4.5.1)[17]. Molecular docking (AutoDockTools v1.5.6) was performed using compound structures (PubChem, https://pubchem.ncbi.nlm.nih.gov/) and hub target structures (PDB, http://www.rcsb.org/pdb/home/home.do), with the predicted binding affinity used to evaluate interactions at the computational simulation level[18]. Molecular docking was performed as a computational bioinformatics simulation to predict the potential binding affinity between the active compounds and core targets, without experimental validation. All detailed docking results are provided in the Supplementary Figure 1 section of the manuscript.

Drug-containing serum preparation and quality control

Healthy SD rats were acquired from the Animal Experiment Center of Anhui Medical University [production license No. SCXK(Anhui)20170002; quality certificate No. 202017532]. After one week of adaptive feeding, the rats were randomly divided into two groups (n = 10 per group). Rats in the experimental group were intragastrically administered the FJHF decoction at a daily dose of 10 g/kg for seven consecutive days, while control rats received an equal volume of normal saline *via* the same route. One hour after the last administration, abdominal aortic blood was collected and centrifuged at 3000 *g* for 15 minutes to separate the serum. Serum was inactivated in a water bath at a temperature of 56 °C for 30 minutes, and then filtered and sterilized through a 0.22 μm filter to prepare FJHFcontaining serum and blank serum. The sera were aliquoted and stored at -20 °C for later use. The rat dosage was converted from the clinical human equivalent dose, according to the body surface area (BSA) normalization method[19]. The theoretical conversion factor calculated by standard Km values (human Km = 37, rat Km = 6) is 37/6 = 6.17 (rat dose = human dose × 6.17). This BSA-based conversion is the most widely accepted standard for translating clinical human doses to experimental rat doses in pharmacological studies. To guarantee the stability and bioactivity of major constituents in the drug-containing serum, UPLC was employed for quantitative determination to satisfy experimental criteria.

Cell co-culture and experimental grouping

LX-2 (source: CCTCC, catalog No.: GNHu58, species: Human) cells were cultured in DMEM with 10% FBS at 37 °C in a 5% CO_2 atmosphere and sub-cultured (80%-90% confluence) *via* 0.25% trypsin digestion. LX-2 cells in the logarithmic growth phase were prepared as cell suspensions and co-seeded into 96-well culture plates. LX-2 cells were cultured in FJHF drug-containing serum for 24 hours, after which the supernatant cells were collected for detection.

To investigate the regulatory effects of FJHF on HSCs, the study sample was randomly divided into five groups: (1) Control group: LX-2 cells treated with blank serum for 24 hours[20]; (2) JHF group: LX2 cells treated with JHF drug-containing serum for 24 hours; (3) STAT3 activator group: LX-2 cells treated with the STAT3 activator colivelin (10 μM) for 24 hours[21]; (4) STAT3 inhibitor group: LX-2 cells treated with the STAT3 activator colivelin (10 μM) and STAT3 inhibitor Stattic (10 μM) for 24 hours[22]; and (5) STAT3 activator + JHF group: LX-2 cells treated with JHF drug-containing serum and the STAT3 activator colivelin (10 μM) for 24 hours.

Determination of cell viability via the cell counting kit-8 assay

LX-2 cells were seeded in 96-well plates and treated with blank serum, control serum, or FJHF-containing serum at gradient concentrations of 2%, 5%, 10%, 20%, and 40%. After 24 hours of incubation at 37 °C, the culture medium was decanted, and the cells were rinsed twice. Subsequently, 100 μL of fresh medium supplemented with 10 μL of the cell counting kit-8 (CCK-8) solution was added to each well. After 2 hours of incubation, the optical density at 450 nm was measured using a microplate reader.

Observation by transmission electron microscopy

HSCs were fixed (2.5% glutaraldehyde/osmium tetroxide), dehydrated, embedded, sectioned, stained, and observed

using transmission electron microscopy (TEM) (JEM-1400)[23].

Western blotting and the quantitative real-time PCR assay

Subsequently, 10% or 12% gels were prepared. Protein samples (20 μg) were boiled in loading buffer. Electrophoresis was performed at 80 V for 20 minutes for the stacking gel and at 120 V for 60 minutes to separate the gel. The semi-dry transfer was performed at 25 V for 30 minutes, followed by membrane activation. Membranes were blocked and incubated with primary and secondary antibodies before imaging with enhanced chemiluminescence using a gel scanner. The antibodies used for immunoblotting included GAPDH (Proteintech, 60004-1-AP), α-SMA (Proteintech, 14395-1-AP), Collagen I (Abcam, Ab138492), MMP1 (Abcam, Ab134184), TIMP1(Abcam, Ab211926), STAT3 (Abcam, ab68153), MYC (Abcam, ab32), MCL-1 (Abcam, ab32087), and a goat anti-mouse HRP-conjugated secondary antibody (Zhongshan Goldenbridge, ZB-2305). GAPDH was used as the reference band.

Total RNA was extracted from adherent cells using a reagent with chloroform and isopropanol steps, followed by washing with 75% ethanol. cDNA was synthesized from RNA. qPCR was performed in triplicate using standard cycling [24]. Relative expression was calculated *via* the $2^{-\Delta\Delta Ct}$ method.

All western blotting and quantitative real-time PCR (qRT-PCR) assays were carried out in three independent biological replicates, and each biological replicate was further examined in triplicate to guarantee the accuracy and dependability of the results.

Statistical analysis

Statistical evaluation was performed using SPSS 21.0. All experiments were performed in triplicate. Quantitative data were expressed as mean ± SD. Differences in quantitative variables between the two groups were analyzed using the independent-sample *t*-test, and comparisons among multiple groups were performed using one-way analysis of the relative protein/mRNA expression levels was conducted using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. Categorical data are presented as *n* (%) and analyzed using the χ^2 test; Fisher's exact test was used when the expected frequency was < 5 or the total sample size was small ($n < 40$). Statistical ANOVA, followed by Tukey's *post-hoc* test. A *P* value of < 0.05 was considered statistically significant. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$.

RESULTS

In total, 40 cirrhotic patients with hypersplenism were enrolled, including 24 males and 16 females, with a mean age of 37.88 ± 13.16 years.

ScRNA-seq analysis

To investigate changes in cell types during the progression of LF following FJHF treatment, scRNA-seq was performed on six liver tissue samples (three from the control group and three from the JHF treatment group). After quality control, 56550 cells, including 32151 cells in the control group and 24399 cells in the JHF group, were retained for analysis. Cells from both groups were integrated into a batch effect-free standardized dataset *via* Canonical Correlation Analysis. Dimensionality reduction was conducted using principal component analysis, and the resulting low-dimensional data were visualized *via* Uniform Manifold Approximation and Projection (Figure 2A). Fourteen major liver cell types were identified using cell type-specific marker genes, and their proportional distribution was significantly altered post-JHF treatment (Figure 2B). The top 20 differentially expressed genes in HSC subtypes were subjected to GO and KEGG analyses, which indicated significant enrichment in inflammation-related pathways and cytokinereceptor interaction signaling (Figure 2C). FJHF modulated cytokine expression in HSCs (Figure 2D and E). (PRJNA1304907, https://www.ncbi.nlm.nih.gov/sra/PRJNA1304907, the Control Group (SAMN50575320, SAMN50575321, and SAMN50575322), and the JHF Group (SAMN50575323, SAMN50575324, and SAMN50575325).

Clinical results of FJHF on hepatocellular injury, hepatic reserve function, and LF markers

No significant differences in baseline characteristics prior to treatment, including general clinical indices, baseline hepatocellular injury, hepatic reserve function, fibrosis markers, LS, and shear wave velocity, were observed between the two groups (all $P > 0.05$) (Table 2).

Following the FJHF intervention, the level of ALT (the hepatocellular injury indicator) was significantly lower in the JHF group than in the control group ($P < 0.01$). Meanwhile, the levels of PT and TBIL (hepatic reserve function indicators) also significantly decreased (both $P < 0.05$). Similarly, the concentrations of HA, PIIINP, and CIV were significantly lower in the JHF group than in the control group ($P < 0.05$). While LN levels did not differ significantly between the groups, a downward tendency was detected in the JHF group, supporting the antifibrotic potential of FJHF. Additionally, no significant between-group differences were observed in LS (12.50 ± 3.61 kPa *vs* 13.60 ± 3.93 kPa, $P = 0.367$) or shear wave velocity (1.98 ± 0.28 m/second *vs* 2.08 ± 0.29 m/second, $P = 0.315$) after treatment.

Network pharmacology analysis of FJHF in LF

Disease-related genes were screened using GeneCards, TTD, DrugBank, OMIM, and PharmGKB (Supplementary Figure 1A). In total, 223 intersecting genes were identified between 248 active compound targets and 8968 disease targets (Supplementary Figure 1B). A "herbal-compound-gene target" network (212 nodes, 415 edges) was constructed to analyze the relationships between herbs, ingredients, targets, and diseases (Supplementary Figure 1C). Inputting 223

Table 2 Baseline characteristics of the included patients

Variable	JHF group (*n* = 20)	Control group (*n* = 20)	*P* value
Sex (male/female)	10:10	14:6	0.167
Age	36.25 ± 12.53	39.5 ± 13.89	0.442
Etiology			0.673
HLD	17	15	
HBV	2	4	
Autoimmune hepatitis	1	1	
Pre-treatment			
ALT (U/L)	66.04 ± 11.72	67.09 ± 12.43	0.786
TBIL (μmol/L)	61.49 ± 18.97	64.29 ± 24.26	0.687
PT (s)	13.75 ± 1.72	13.16 ± 1.46	0.248
HA (ng/mL)	187.38 ± 67.98	171.84 ± 49.02	0.412
LN (ng/mL)	441.74 ± 455.09	404.33 ± 404.86	0.785
PIIINP (ng/mL)	157.95 ± 57.07	153.03 ± 82.53	0.828
CIV (ng/mL)	89.19 ± 30.76	65.75 ± 56.59	0.112
Liver stiffness (kPa)	14.12 ± 4.43	14.50 ± 5.02	0.802
Shear wave velocity (m/s)	2.10 ± 0.33	2.13 ± 0.39	0.760
Post-treatment			
ALT (U/L)	30.65 ± 11.63	40.00 ± 9.27	0.008
TBIL (μmol/L)	22.47 ± 9.73	31.01 ± 10.33	0.032
PT (s)	11.25 ± 1.08	12.28 ± 1.80	0.033
HA (ng/mL)	132.09 ± 66.54	171.74 ± 52.42	0.043
LN (ng/mL)	86.44 ± 27.76	167.35 ± 178.08	0.052
PIIINP (ng/mL)	15.87 ± 5.04	76.34 ± 80.59	0.002
CIV (ng/mL)	21.83 ± 12.67	39.44 ± 32.35	0.029
Liver stiffness (kPa)	12.50 ± 3.61	13.60 ± 3.93	0.367
Shear wave velocity (m/s)	1.98 ± 0.28	2.08 ± 0.29	0.315

JHF: Jianpi Huoxue Formula; HLD: Hepatolenticular degeneration; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; TBIL: Total bilirubin; PT: Prothrombin time; HA: Hyaluronic acid; PIIINP: Procollagen N-terminal peptide; CIV: Type IV collagen; LN: Laminin.

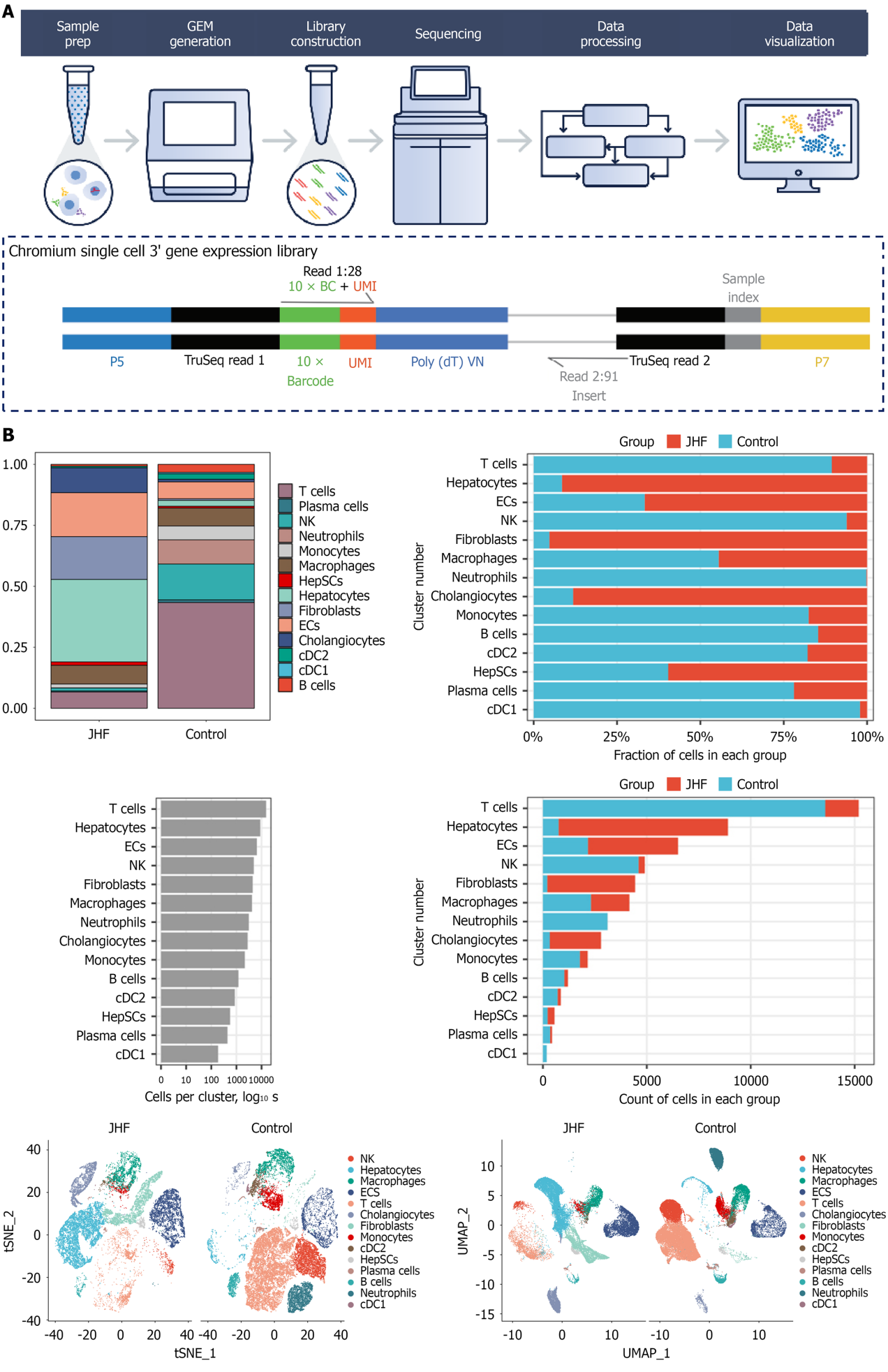
intersection genes into STRING 11.0 yielded a PPI network (222 nodes, 679 edges, interaction score ≥ 0.9). We analyzed the topological properties of the PPI network in Cytoscape using a network analysis tool based on six indicators. Nodes with values above the median across all parameters were defined as core nodes, yielding 20 hub nodes and 217 edges for the final hub gene network (Supplementary Figure 1D). The enrichment analysis revealed 2409 GO biological processes, 107 cellular components, 253 molecular functions, and 191 KEGG pathways (Supplementary Figure 1E and F). Molecular docking simulation using AutoDockTools 1.5.6 predicted that Licochalcone A luteolin has binding affinities of -6.0 and -7.3 kcal/mol for STAT3 and MCL-1, respectively (Supplementary Figure 1G and H).

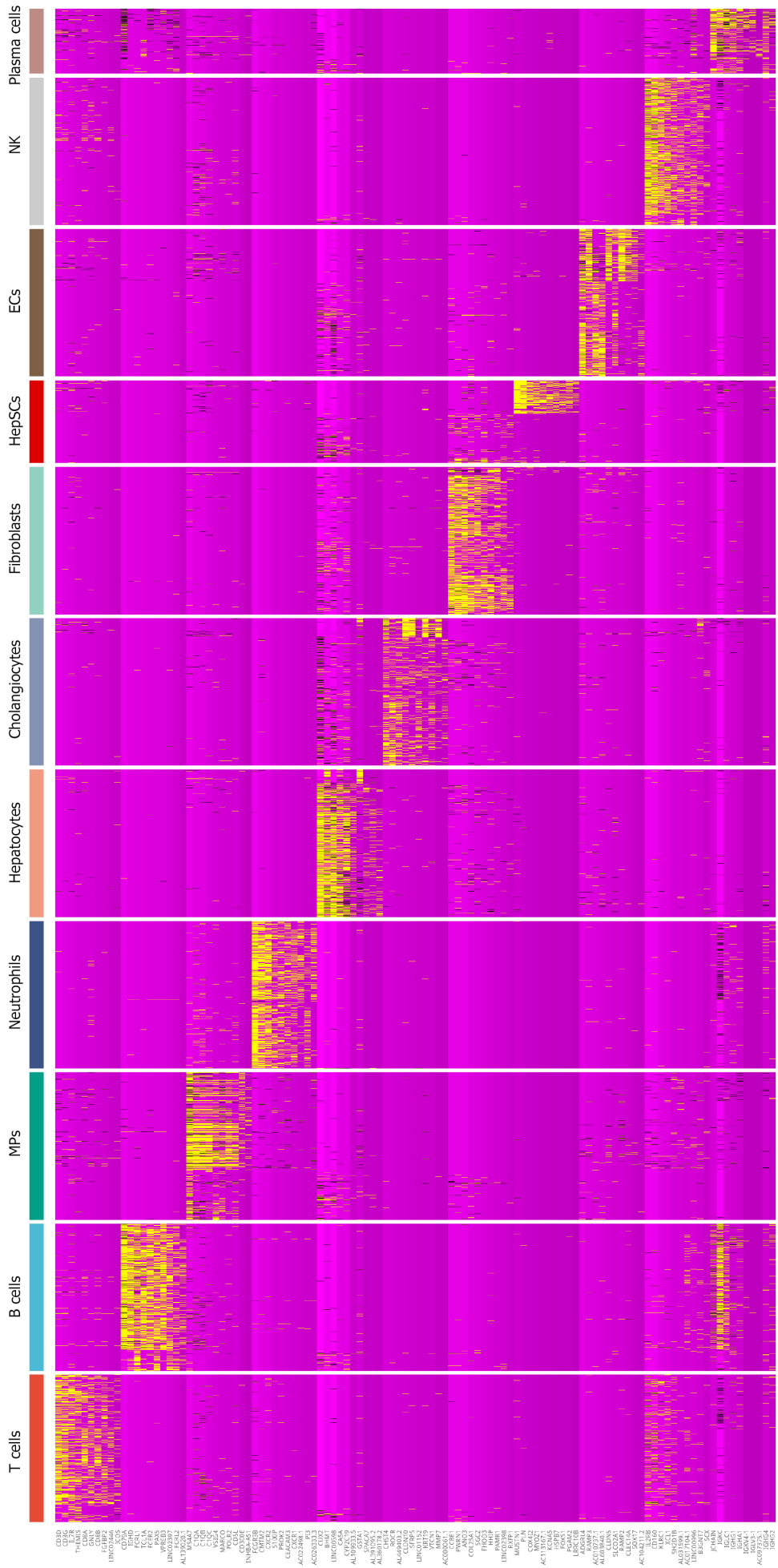
Screening of drug-containing serum concentrations

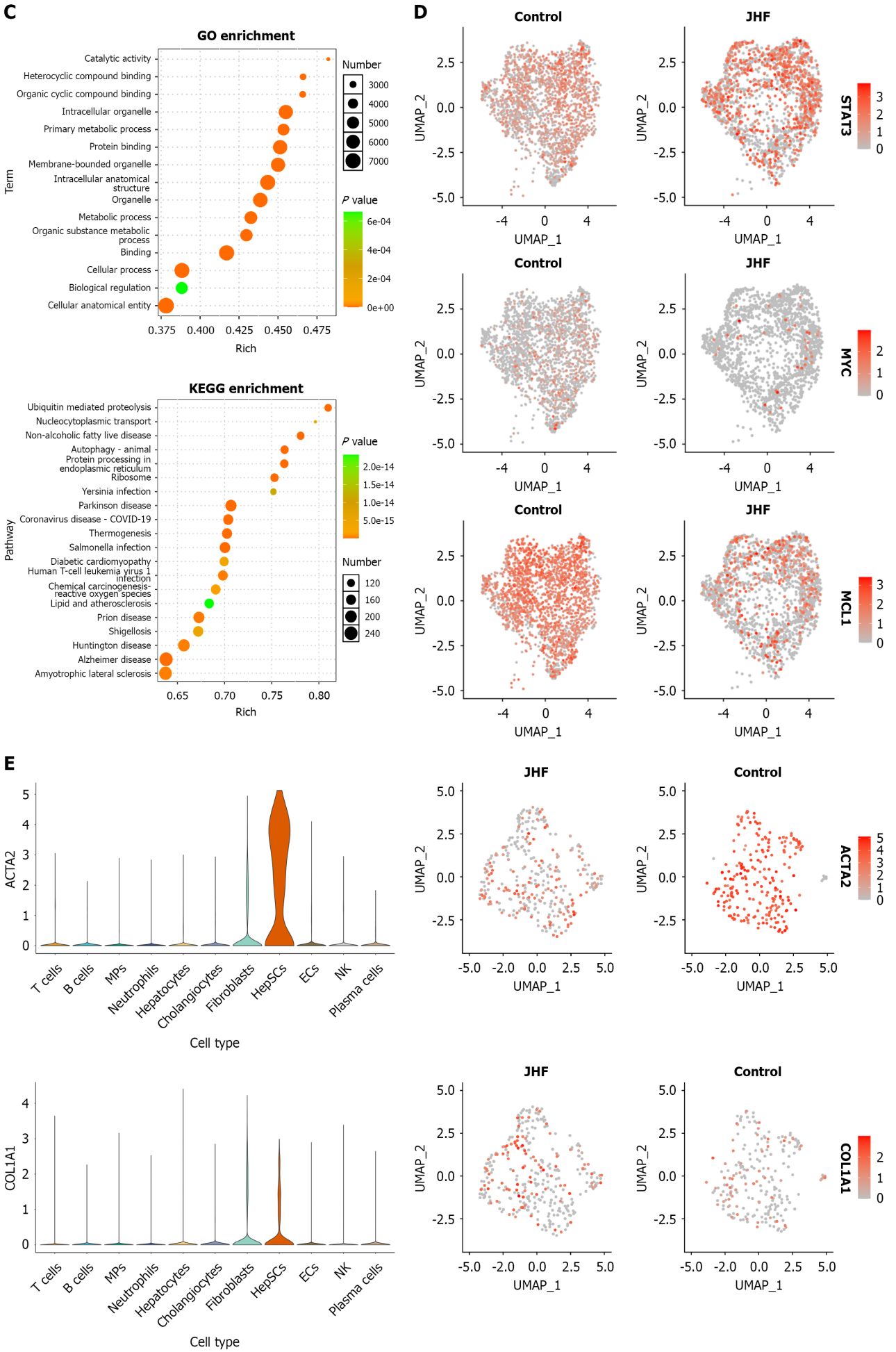
LX-2 cells were incubated with gradient concentrations of FJHF-containing serum (2%, 5%, 10%, 20%, and 40%) with blank and control sera as negative controls. After incubation, the CCK-8 reagent was added to measure the absorbance and calculate viability. As shown in Figure 3, 20% FJHF-containing serum was optimal (appropriate biological effect and no significant cytotoxicity) for subsequent experiments.

TEM observations of HSC ultrastructure

The control and JHF groups had HSCs with lipid droplets, sparse short rough endoplasmic reticulum (rER), simple Golgi-scarce mitochondria, small nuclei with indistinct nucleoli, and no collagen. The STAT3 activator group showed HSCs without lipid droplets but with densely distributed and dilated rER, developed Golgi, more mitochondria, large nuclei,


A
Sample prep
GEM generation
Library construction
Sequencing
Data processing
Data visualization
Chromium single cell 3' gene expression library
Read 1:28
10 × BC + UMI
Sample index
P5
TruSeq read 1
10 × Barcode
UMI
Poly (dT) VN
TruSeq read 2
P7
Read 2:91
Insert
B
T cells
Plasma cells
NK
Neutrophils
Monocytes
Macrophages
HepSCs
Hepatocytes
Fibroblasts
ECs
Cholangiocytes
cDC2
cDC1
B cells
JHF
Control
Group
Cluster number
Fraction of cells in each group
Cells per cluster, log10 s
Count of cells in each group
tSNE_1
tSNE_2
UMAP_1
UMAP_2


Plasma cells
NK
ECs
HepSCs
Fibroblasts
Cholangiocytes
Hepatocytes
Neutrophils
MPs
B cells
T cells


C
GO enrichment
Catalytic activity
Heterocyclic compound binding
Organic cyclic compound binding
Intracellular organelle
Primary metabolic process
Protein binding
Membrane-bounded organelle
Intracellular anatomical structure
Organelle
Metabolic process
Organic substance metabolic process
Binding
Cellular process
Biological regulation
Cellular anatomical entity
Term
0.375 0.400 0.425 0.450 0.475
Rich
Number
3000
4000
5000
6000
7000
P value
6e-04
4e-04
2e-04
0e+00
KEGG enrichment
Ubiquitin mediated proteolysis
Nucleocytoplasmic transport
Non-alcoholic fatty live disease
Autophagy - animal
Protein processing in endoplasmic reticulum
Ribosome
Yersinia infection
Parkinson disease
Coronavirus disease - COVID-19
Thermogenesis
Salmonella infection
Diabetic cardiomyopathy
Human T-cell leukemia virus 1 infection
Chemical carcinogenesis-reactive oxygen species
Lipid and atherosclerosis
Prion disease
Shigellosis
Huntington disease
Alzheimer disease
Amyotrophic lateral sclerosis
Pathway
0.65 0.70 0.75 0.80
Rich
P value
2.0e-14
1.5e-14
1.0e-14
5.0e-15
Number
120
160
200
240
D
Control
JHF
STAT3
MYC
MCL1
UMAP_1
UMAP_2
E
ACTA2
COL1A1
T cells
B cells
MPs
Neutrophils
Hepatocytes
Cholangiocytes
Fibroblasts
HepSCs
ECs
NK
Plasma cells
Cell type

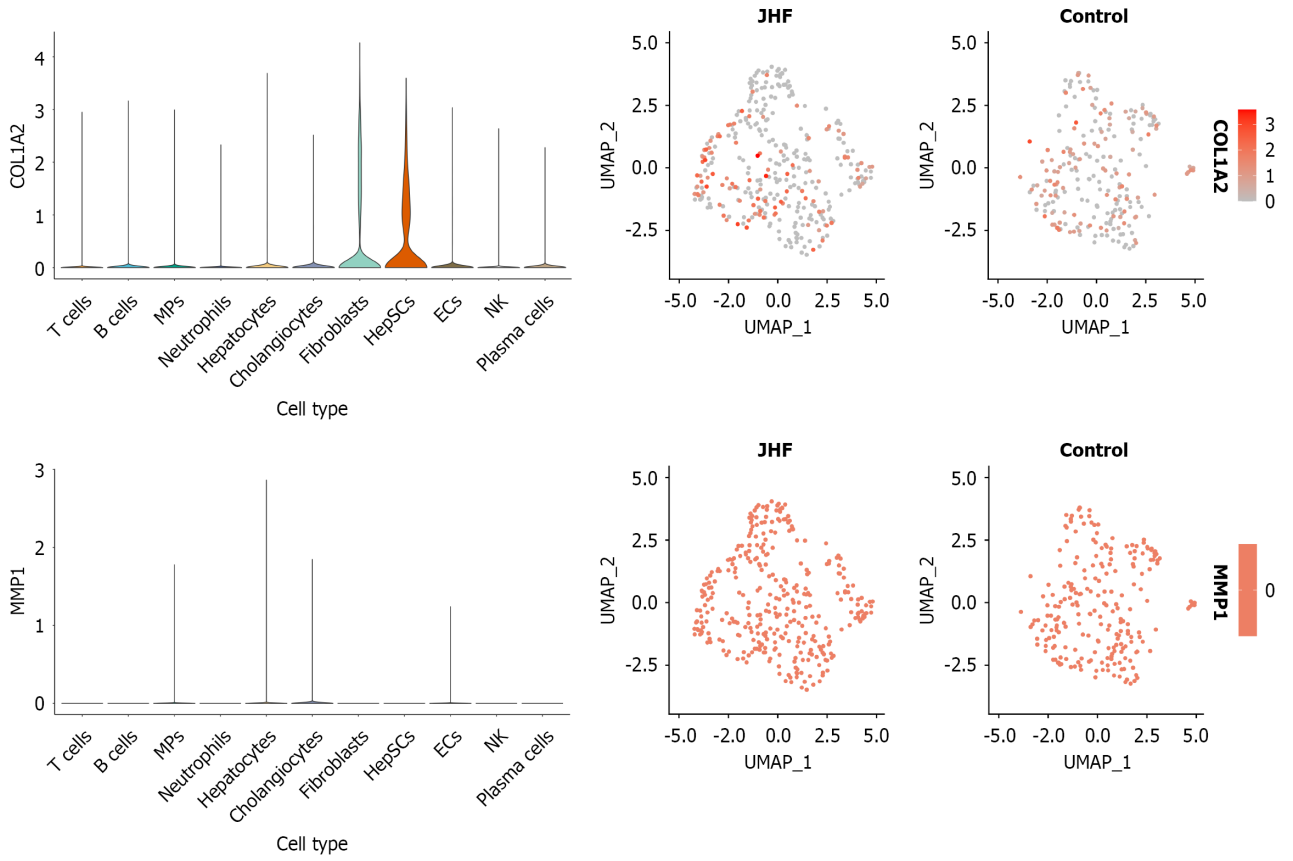


Figure 2 Single-cell RNA sequencing of liver tissues from cirrhotic patients. Six liver samples, divided into the control group (*n* = 3) and the Jianpi Huoxue Formula (JHF) group (*n* = 3), were analyzed using single-cell RNA sequencing. A: Diagram showing the experimental procedure in this study; B: Fourteen major liver cell types were identified based on cell-type-specific marker genes after JHF treatment; C: Gene Ontology and terms and Kyoto Encyclopedia of Genes and Genomes analyses of the top 20 differentially expressed genes in human hepatic stellate cells (HSCs) subtypes; D: Violin plots and Uniform Manifold Approximation and Projection (UMAP) plots showing the expression levels of STAT3, MYC, and MCL-1 across HSC subpopulations after JHF treatment; E: Violin plots and UMAP plots showing the expression levels of ACTA2, COL1A1, COL1A2, and MMP1 in the major cell population.

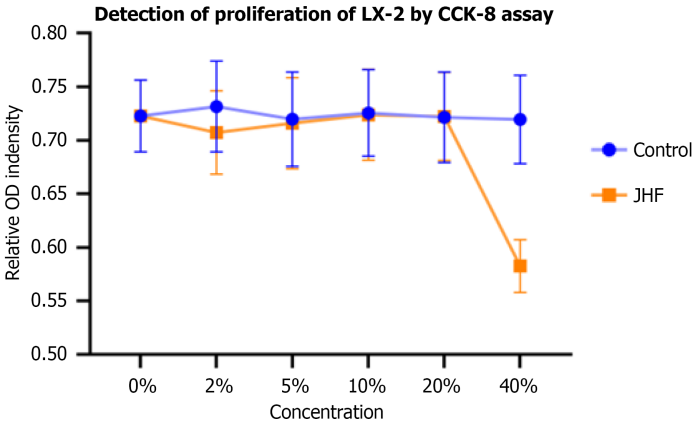


Figure 3 Screening of Jianpi Huoxue Formula drug-containing serum concentrations and detection of LX-2 cell proliferation *via* the CCK-8 assay. CCK-8: Cell counting kit-8; JHF: Jianpi Huoxue Formula.

and dense collagen deposition. The STAT3 inhibitor and STAT3 activator + JHF groups had HSCs with reduced rER, simplified Golgi, fewer mitochondria, shrunken nuclei, and no lipid droplets or collagen (Figure 4).

FJHF inhibits the activation of HSCs

To explore the effect of FJHF on collagen I, MMP1, α-SMA, and TIMP1 expression, western blotting and qRT-PCR were performed. Relative to the control group, the STAT3 activator group exhibited notably upregulated protein and mRNA expression of collagen I, α-SMA, and TIMP1, along with downregulated MMP1 expression ($P < 0.05$). The STAT3 inhi-

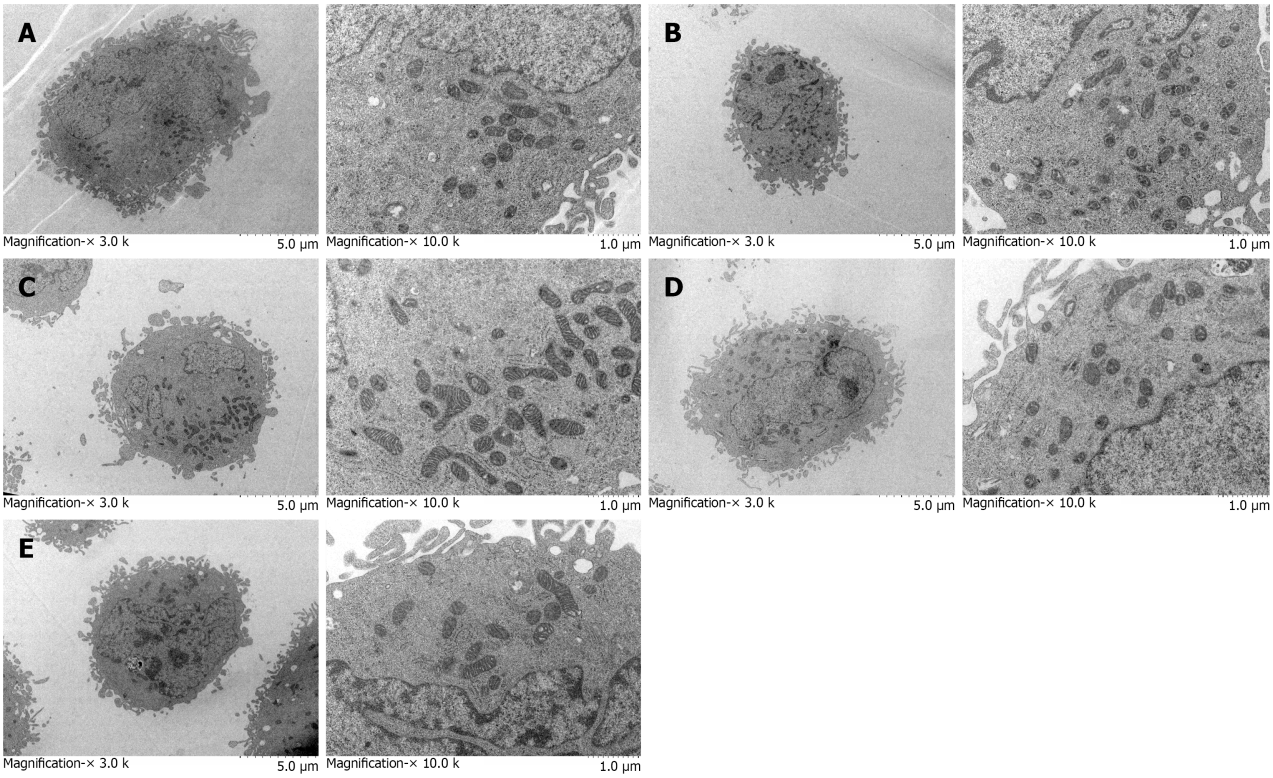


Figure 4 Transmission electron microscopy observations of human hepatic stellate cells ultrastructure. A: Transmission electron microscopy (TEM) observation results of the control group; B: TEM observation results of the Jianpi Huoxue Formula (JHF) group; C: TEM observation results of the STAT3 activator group; D: TEM observation results of the STAT3 inhibitor group; E: TEM observation results of the STAT3 activator + JHF group.

bitor and STAT3 activator + JHF groups not only showed reduced levels of collagen I, α-SMA, and TIMP1 but also increased levels of MMP1 (Figure 5).

FJHF inhibits the STAT3/MYC/MCL-1 pathway and modulates early fibrotic metabolic abnormalities

To investigate FJHF's regulatory effects on MCL-1, MYC, and STAT3 expression, western blotting and qRT-PCR analyses were performed. Compared with the control group, the STAT3 activator group showed significantly upregulated protein and mRNA levels of these factors ($P < 0.05$), whereas the STAT3 inhibitor and STAT3 activator + JHF groups significantly reversed this effect and decreased their expression ($P < 0.05$) (Figure 6).

DISCUSSION

This study integrated clinical scRNA-seq, network pharmacology, and *in vitro* experiments to elucidate the antifibrotic mechanisms of FJHF and its monomeric compounds. The principal finding of this study was that FJHF inhibits the STAT3/MYC/MCL-1 pathway to suppress HSC activation, thereby ameliorating early metabolic abnormalities in LF. Human liver scRNA-seq revealed that FJHF regulates the proportion and expression of cytokines in HSCs. A clinical study revealed that FJHF treatment could alleviate hepatocellular injury (ALT), enhance hepatic reserve function (TBIL and PT), and reduce the levels of early fibrosis markers (HA, PIIINP, and CIV) in the short term. Network pharmacology and computational molecular docking prediction indicated that the main active monomers (luteolin and licochalcone A) of FJHF predicted the binding potential toward STAT3 and MCL-1 at the simulation level. *In vitro* experiments confirmed that FJHF suppresses HSC activation and downregulates the expression of proteins and mRNAs associated with the STAT3/MYC/MCL-1 pathway. Suppression of HSC activation was evidenced by reduced expression of α-SMA, collagen I, and TIMP1. These findings were consistent with the western blotting, qRT-PCR, and TEM observations.

Collectively, these findings indicate that FJHF exerts antifibrotic effects through the modulation of the STAT3/MYC/MCL1 signaling axis. In addition to consolidating the scientific basis for the therapeutic potential of FJHF against LF, this study identified the STAT3/MYC/MCL-1 axis as a promising therapeutic target. Activation of STAT3 (phosphorylation at Tyr705) upregulates the transcription of MYC and MCL-1, thereby promoting LF[25,26]. Per our findings, FJHF markedly reduced the expression of total STAT3 and its downstream genes, MYC and MCL-1. Further detection of p-STAT3 is needed to confirm its inhibitory effect on STAT3 phosphorylation.

The therapeutic efficacy of FJHF is likely attributed to the synergistic interactions between its herb pairs. For example, the Angelica-Ligusticum pair suppresses collagen deposition, whereas the Prunus-Carthamus pair modulates oxidative stress. These herb-pair-mediated effects complement FJHF's inhibitory role in the STAT3/MYC/MCL-1 pathway, collec-

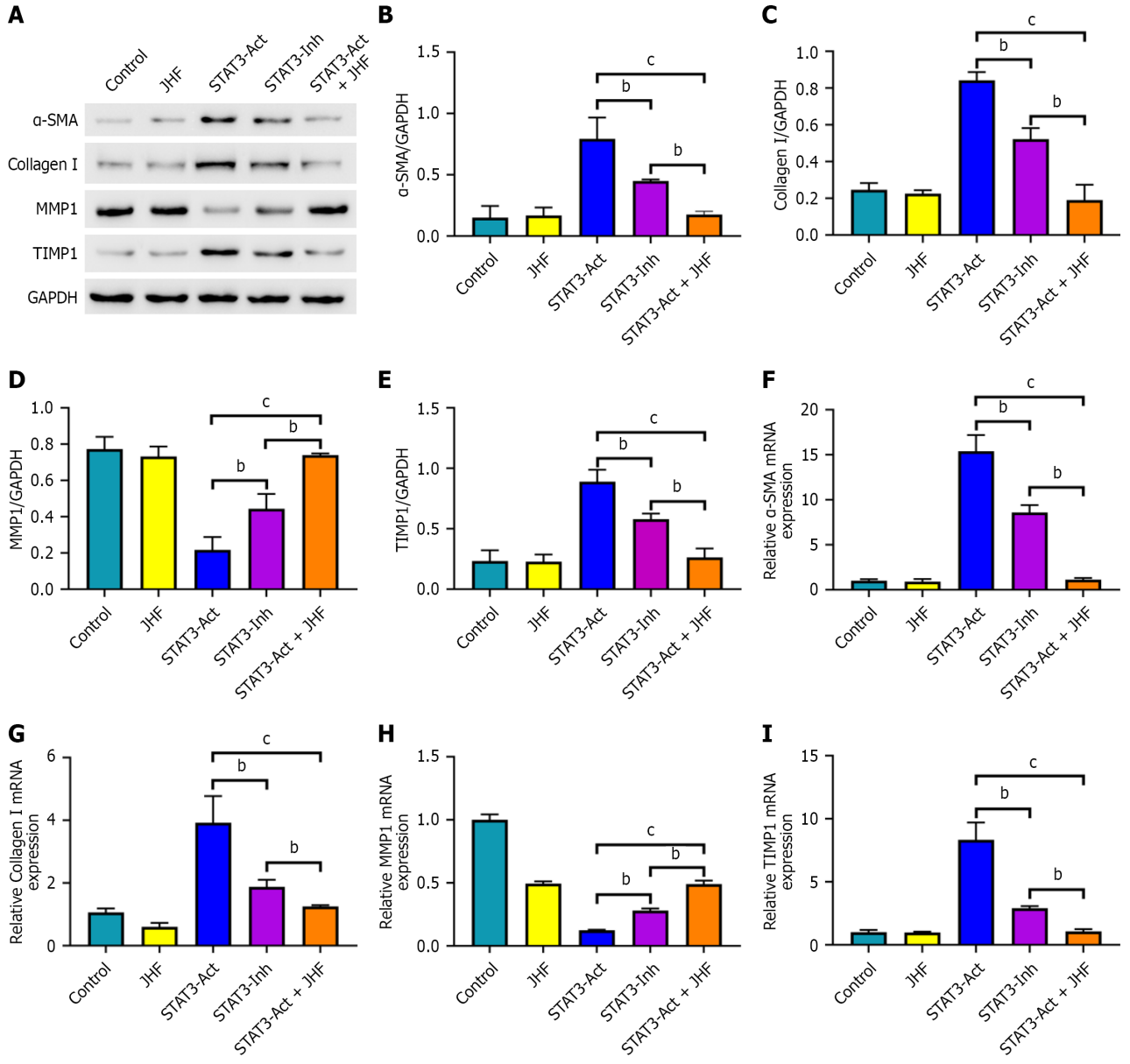


Figure 5 Western blot and quantitative real-time PCR for detecting α-SMA, Collagen I, MMP1, and TIMP1. A: Western blot results of α-SMA, Collagen I, MMP1, and TIMP1; B: A-SMA western blot result; C: Collagen I western blot result; D: MMP1 western blot result; E: TIMP1 western blot result; F: A-SMA quantitative real-time PCR (qRT-PCR) result; G: Collagen I qRT-PCR result; H: MMP1 qRT-PCR result; I: TIMP1 qRT-PCR result. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

tively reflecting the "multicomponent, multi-target" advantage of TCM over single-target western antifibrotic drugs.

A discrepancy was observed between the non-significant change in LS ($P = 0.367$) and shear wave velocity ($P = 0.315$), and the significant reductions in the levels of fibrosis markers (HA, PIIINP, and CIV) after FJHF treatment. This inconsistency may be attributed to the following factors. First, LS and shear wave velocity measurements are sensitive to hepatic inflammation and edema, and HA, PIIINP, and CIV are direct biomarkers of extracellular matrix (ECM) degradation, which more specifically reflects fibrotic activity. Second, the short treatment duration (21 days) may be insufficient to induce detectable changes in established hepatic scar tissue, whereas fibrosis markers can reflect early changes in ECM metabolism. Consistent with the clinical findings, 21-day FJHF treatment significantly reduced the levels of serum biochemical fibrosis markers, whereas the levels of LS and shear wave velocity did not differ significantly between the groups. This phenomenon suggests that FJHF primarily modulates early ECM turnover, rather than reversing mature hepatic scar formation. The short intervention duration was insufficient to induce measurable improvements in liver histology and elastography. Therefore, the current results only support short-term biochemical improvement of fibrotic markers and cannot be interpreted as a histological or elastographic reversal of LF. Future studies with longer intervention periods and stratification by inflammatory status are needed to confirm whether FJHF can reduce LS in patients with controlled hepatic inflammation.

The findings of this study provide a theoretical and scientific basis for the clinical application of FJHF in patients with cirrhosis complicated by hypersplenism. Short-term (21-day) FJHF treatment alleviated hepatocellular injury (ALT), enhanced hepatic reserve function (TBIL and PT), and reduced levels of early fibrosis markers (HA, PIIINP, and CIV).

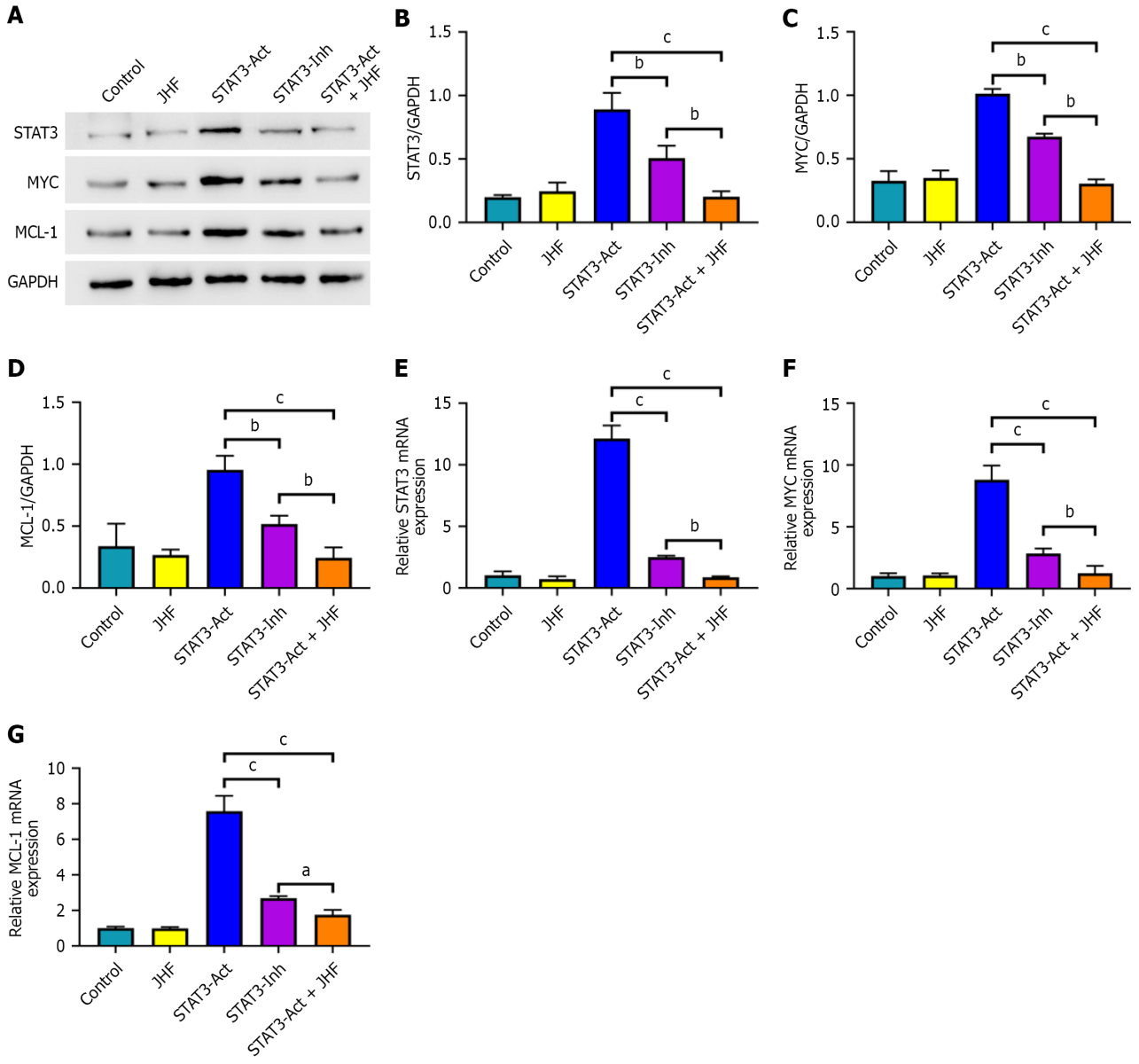


Figure 6 Western blot and quantitative real-time PCR for detecting STAT3, MYC, and MCL-1. A: Western blot results of STAT3, MYC, and MCL-1; B: STAT3 western blot result; C: MYC western blot result; D: MCL-1 western blot result; E: STAT3 quantitative real-time PCR (qRT-PCR) result; F: MYC qRT-PCR result; G: MCL-1 qRT-PCR result. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

Furthermore, the identification of the STAT3/MYC/MCL1 pathway as a key target provides potential for biomarker development, which can be employed as a non-invasive biomarker for assessing the clinical therapeutic efficacy of FJHF.

Nevertheless, this study had the following noteworthy limitations: (1) ScRNA-seq employed only six human samples (three per group). Such a minute sample cannot fully reflect the individual heterogeneity of cirrhotic patients and may introduce potential selection bias, which limits the generalizability of our single-cell transcriptomic findings; (2) This study entailed only *in vitro* cell experiments and clinical observational analysis, and there were no *in vivo* functional verification using classic LF animal models such as CCl-induced or bile duct ligation models. The absence of histological and pathological evaluation of LF in animal models weakens the *in vivo* evidence for the regulatory effect of FJHF on the STAT3/MYC/MCL-1 pathway; (3) We focused on the STAT3/MYC/MCL-1 pathway, without examining potential crosstalk with other pathways, which may also contribute to FJHF's effects; (4) The short treatment duration (21 days), despite confirming the short-term benefits of FJHF, cannot fully reflect its efficacy in retarding or reversing established LF, thus precluding the ability of the study to assess long-term safety and efficacy; (5) This study only detected the protein and mRNA expression of total STAT3, but did not examine the phosphorylation level of STAT3 (p-STAT3) or calculate the p-STAT3/total STAT3 ratio, which weakens the evidence for the activation status of the STAT3 signaling pathway. Furthermore, we did not perform STAT3 overexpression rescue experiments to further confirm the specific regulatory mechanism of FJHF on the STAT3/MYC/MCL-1 axis; and (6) The molecular docking analysis conducted in this study is merely a bioinformatics computational prediction, and it lacks *in vitro* or *in vivo* experimental verification of actual protein binding. Future research will focus on six key directions: First, to compensate for this limitation, we have supplemented detailed clinical baseline information of the six patients enrolled for scRNA-seq in the Supplementary

Table 1 to confirm the baseline balance between the two groups; second, we will adopt classic CCl-induced and bile duct ligation LF animal models to further verify the *in vivo* regulatory effect of FJHF on the STAT3/MYC/MCL-1 pathway, and systematic histological staining and fibrosis stage grading will be performed to evaluate the pathological changes in LF and confirm the *in vivo* efficacy of FJHF; third, to explore the crosstalk between the STAT3/MYC/MCL-1 pathway and other fibrotic pathways (such as the TGF-β pathway) using proteomic techniques; fourth, future work will detect p-STAT3 and total STAT3 protein expression to calculate their ratio and verify FJHF inhibition on STAT3 phosphorylation, and conduct STAT3 overexpression rescue assays in LX-2 cells to elucidate how FJHF modulates the STAT3/MYC/MCL-1 pathway and represses HSC activation; fifth, to enroll a larger cohort of cirrhotic patients, extend the treatment course to 3-6 months, and conduct follow-up assessments at 6 months, 12 months, and 24 months post-treatment; and sixth, further experimental validation is required to confirm the real molecular interaction.

CONCLUSION

In conclusion, short-term FJHF therapy improves early biochemical abnormalities of LF by inhibiting the STAT3/MYC/MCL-1 pathway and suppressing HSC activation without reversing mature hepatic scar tissue or improving LS in the short term. This study not only establishes a robust theoretical foundation for the clinical use of FJHF in patients with cirrhosis but also identifies the STAT3/MYC/MCL-1 axis as a potential target for TCM intervention in early LF metabolic abnormalities.

REFERENCES

1 **Cheemerla S**, Balakrishnan M. Global Epidemiology of Chronic Liver Disease. *Clin Liver Dis (Hoboken)* 2021; **17**: 365-370 [*RCA*] [PMID: 34136143 DOI: 10.1002/cld.1061] [FullText] [Full Text(PDF)]

2 **Younossi ZM**, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* 2023; **77**: 1335-1347 [*RCA*] [PMID: 36626630 DOI: 10.1097/HEP.0000000000000004] [FullText]

3 **Ginès P**, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. *Lancet* 2021; **398**: 1359-1376 [*RCA*] [PMID: 34543610 DOI: 10.1016/S0140-6736(21)01374-X] [FullText]

4 **Chen X**, Wang Y, Dou X, Wan J, Zhou J, Li T, Yu J, Ye F. Integrative metabolomics and proteomics reveal the effect and mechanism of Zi Qi decoction on alleviating liver fibrosis. *Sci Rep* 2024; **14**: 28943 [*RCA*] [PMID: 39578538 DOI: 10.1038/s41598-024-80616-7] [FullText]

5 **Hafez MM**, Hamed SS, El-Khadragy MF, Hassan ZK, Al Rejaie SS, Sayed-Ahmed MM, Al-Harbi NO, Al-Hosaini KA, Al-Harbi MM, Alhoshani AR, Al-Shabanah OA, Alsharari SD. Effect of ginseng extract on the TGF-β1 signaling pathway in CCl(4)-induced liver fibrosis in rats. *BMC Complement Altern Med* 2017; **17**: 45 [*RCA*] [PMID: 28086769 DOI: 10.1186/s12906-016-1507-0] [FullText] [Full Text(PDF)]

6 **Huang L**, Yu Q, Peng H, Zhen Z. The mechanism of peach kernel and safflower herb-pair for the treatment of liver fibrosis based on network pharmacology and molecular docking technology: A review. *Medicine (Baltimore)* 2023; **102**: e33593 [*RCA*] [PMID: 37083803 DOI: 10.1097/MD.0000000000033593] [FullText]

7 **Li Y**, Li F, Ding M, Ma Z, Li S, Qu J, Li X. Chuanxiong Rhizoma extracts prevent liver fibrosis *via* targeting CTCF-c-MYC-H19 pathway. *Chin Herb Med* 2024; **16**: 82-93 [*RCA*] [PMID: 38375042 DOI: 10.1016/j.chmed.2023.07.003] [FullText] [Full Text(PDF)]

8 **Wu JZ**, Li YJ, Huang GR, Xu B, Zhou F, Liu RP, Gao F, Ge JD, Cai YJ, Zheng Q, Li XJ. Mechanisms exploration of Angelicae Sinensis Radix and Ligusticum Chuanxiong Rhizoma herb-pair for liver fibrosis prevention based on network pharmacology and experimental pharmacologylogy. *Chin J Nat Med* 2021; **19**: 241-254 [*RCA*] [PMID: 33875165 DOI: 10.1016/S1875-5364(21)60026-2] [FullText]

9 **Xia T**, Zhang M, Lei W, Yang R, Fu S, Fan Z, Yang Y, Zhang T. Advances in the role of STAT3 in macrophage polarization. *Front Immunol* 2023; **14**: 1160719 [*RCA*] [PMID: 37081874 DOI: 10.3389/fimmu.2023.1160719] [FullText] [Full Text(PDF)]

10 **Jiang QY**, Lin ZL, Su ZW, Li S, Li J, Guan S, Ling Y, Zhang L. Peptide identification of hepatocyte growth-promoting factor and its function in cytoprotection and promotion of liver cell proliferation through the JAK2/STAT3/c-MYC pathway. *Eur J Pharmacol* 2022; **920**: 174832 [*RCA*] [PMID: 35183533 DOI: 10.1016/j.ejphar.2022.174832] [FullText]

11 **Mishra A**, Kumar A, Naik L, Patel S, Das M, Behura A, Nayak DK, Mishra A, Bhutia SK, Singh R, Dhiman R. Soybean lectin-triggered IL-6 secretion induces autophagy to kill intracellular mycobacteria through P2RX7 dependent activation of the JAK2/STAT3/Mcl-1 pathway. *Cytokine* 2023; **171**: 156366 [*RCA*] [PMID: 37716189 DOI: 10.1016/j.cyto.2023.156366] [FullText]

12 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [*RCA*] [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024] [FullText]

13 **Ru J**, Li P, Wang J, Zhou W, Li B, Huang C, Li P, Guo Z, Tao W, Yang Y, Xu X, Li Y, Wang Y, Yang L. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform* 2014; **6**: 13 [*RCA*] [PMID: 24735618 DOI: 10.1186/1758-2946-6-13] [FullText] [Full Text(PDF)]

14 **UniProt Consortium T**. UniProt: the universal protein knowledgebase. *Nucleic Acids Res* 2018; **46**: 2699 [*RCA*] [PMID: 29425356 DOI: 10.1093/nar/gky092] [FullText] [Full Text(PDF)]

15 **Szklarczyk D**, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019; **47**: D607-D613 [*RCA*] [PMID: 30476243 DOI: 10.1093/nar/gky1131] [FullText] [Full Text (PDF)]

16 **Tang Y**, Li M, Wang J, Pan Y, Wu FX. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems* 2015; **127**: 67-72 [*RCA*] [PMID: 25451770 DOI: 10.1016/j.biosystems.2014.11.005] [FullText]

17 **Wu T**, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)* 2021; **2**: 100141 [*RCA*] [PMID: 34557778 DOI: 10.1016/j.xinn.2021.100141]

[FullText] [Full Text(PDF)]
18 **Trott O**, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010; **31**: 455-461 [*RCA*] [PMID: 19499576 DOI: 10.1002/jcc.21334] [FullText] [Full Text(PDF)]
19 **Reagan-Shaw S**, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J* 2008; **22**: 659-661 [*RCA*] [PMID: 17942826 DOI: 10.1096/fj.07-9574LSF] [FullText]
20 **Brennan PN**, MacMillan M, Manship T, Moroni F, Glover A, Troland D, MacPherson I, Graham C, Aird R, Semple SIK, Morris DM, Fraser AR, Pass C, McGowan NWA, Turner ML, Manson L, Lachlan NJ, Dillon JF, Kilpatrick AM, Campbell JDM, Fallowfield JA, Forbes SJ. Autologous macrophage therapy for liver cirrhosis: a phase 2 open-label randomized controlled trial. *Nat Med* 2025; **31**: 979-987 [*RCA*] [PMID: 39794616 DOI: 10.1038/s41591-024-03406-8] [FullText]
21 **Yao Y**, Song L, Zuo Z, Chen Z, Wang Y, Cai H, Gu Y, Lv Z, Guan J, Chen R, Wang B, Yang L, Huang X, Wang L. Parthenolide attenuates hypoxia-induced pulmonary hypertension through inhibiting STAT3 signaling. *Phytomedicine* 2024; **134**: 155976 [*RCA*] [PMID: 39265445 DOI: 10.1016/j.phymed.2024.155976] [FullText]
22 **Poria DK**, Sheshadri N, Balamurugan K, Sharan S, Sterneck E. The STAT3 inhibitor Stattic acts independently of STAT3 to decrease histone acetylation and modulate gene expression. *J Biol Chem* 2021; **296**: 100220 [*RCA*] [PMID: 33839684 DOI: 10.1074/jbc.RA120.016645] [Full Text] [Full Text(PDF)]
23 **Amor C**, Fernández-Maestre I, Chowdhury S, Ho YJ, Nadella S, Graham C, Carrasco SE, Nnuji-John E, Feucht J, Hinterleitner C, Barthet VJA, Boyer JA, Mezzadra R, Wereski MG, Tuveson DA, Levine RL, Jones LW, Sadelain M, Lowe SW. Prophylactic and long-lasting efficacy of senolytic CAR T cells against age-related metabolic dysfunction. *Nat Aging* 2024; **4**: 336-349 [*RCA*] [PMID: 38267706 DOI: 10.1038/s43587-023-00560-5] [FullText] [Full Text(PDF)]
24 **Xu Y**, Zhang D, Yang H, Liu Y, Zhang L, Zhang C, Chen G, Hu Y, Chen J, Zhang H, Mu Y, Liu P, Liu W. Hepatoprotective effect of genistein against dimethylnitrosamine-induced liver fibrosis in rats by regulating macrophage functional properties and inhibiting the JAK2/STAT3/SOCS3 signaling pathway. *Front Biosci (Landmark Ed)* 2021; **26**: 1572-1584 [*RCA*] [PMID: 34994171 DOI: 10.52586/5050] [Full Text]
25 **Pang X**, Gao S, Liu T, Xu FX, Fan C, Zhang JF, Jiang H. Identification of STAT3 as a biomarker for cellular senescence in liver fibrosis: A bioinformatics and experimental validation study. *Genomics* 2024; **116**: 110800 [*RCA*] [PMID: 38286349 DOI: 10.1016/j.ygeno.2024.110800] [FullText]
26 **Park SJ**, Garcia Diaz J, Comlekoglu T, Hahn YS. Type I IFN receptor blockade alleviates liver fibrosis through macrophage-derived STAT3 signaling. *Front Immunol* 2025; **16**: 1528382 [*RCA*] [PMID: 40260261 DOI: 10.3389/fimmu.2025.1528382] [FullText] [Full Text(PDF)]

FOOTNOTES

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CORRESPONDENCE

Letter to the Editor: Natural product intervention in metabolic dysfunction-associated steatotic liver disease: From allyl isothiocyanate to signaling networks

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Abstract

The pursuit of effective therapeutic agents against metabolic dysfunction-associated steatotic liver disease continues, with natural products offering promise due to their multi-targeting capabilities. A study published in the *World Journal of Hepatology* by Gao *et al* demonstrates that allyl isothiocyanate (AITC) alleviates hepatic steatosis and insulin resistance by upregulating the hepatic vitamin D receptor (VDR), which activates the hepatocyte nuclear factor 4 alpha/microsomal triglyceride transfer protein/apolipoprotein B axis to promote lipid export and β-oxidation while suppressing lipogenesis. While compelling, this VDR-centric model exists in isolation, lacking integration with other known AITC targets like the sirtuin 1/AMP-activated protein kinase axis. Furthermore, although the experimental model provides insightful data, it relies exclusively on *in vitro* studies, awaiting validation *in vivo* and direct genetic evidence from VDR knockout models to solidify the causal relationship. This commentary aims to elucidate the multifaceted mechanisms of AITC against metabolic dysfunction-associated

steatotic liver disease, underscoring its systemic effects on lipid metabolism, insulin sensitivity, and hepatocyte transcriptional reprogramming, which collectively provide a foundation for developing precise, safe, and sustainable therapeutic strategies.

Key Words: Allyl isothiocyanate; Metabolically-dysfunction-associated steatotic liver disease; Vitamin D receptor; Multitarget therapy; Network pharmacology

Core Tip: This comment highlights that allyl isothiocyanate (AITC) exemplifies the principle of multi-target natural product therapy for metabolic dysfunction-associated steatotic liver disease. In addition to the novel vitamin D receptor/hepatocyte nuclear factor 4 alpha pathway identified by Gao *et al*, we integrate this axis with established AITC targets, such as sirtuin 1/AMP-activated protein kinase and nuclear factor erythroid 2-related factor 2, to construct a multi-signaling network. This network collectively reprograms lipid metabolism, insulin sensitivity, and oxidative stress. Future research must validate this integrative mechanism *in vivo* and leverage systems biology to advance AITC as a precise, network-based therapeutic strategy.

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TO THE EDITOR

Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as a prevalent chronic liver condition worldwide, affecting approximately one-quarter of adults and up to 75% of individuals with obesity[1,2]. The pathogenesis of MASLD involves multiple interacting factors, including excessive intrahepatic lipid accumulation, systemic insulin resistance, and low-grade chronic inflammation. These factors form a vicious cycle, collectively driving disease progression from simple steatosis to metabolic dysfunction-associated steatohepatitis, liver fibrosis, and potentially to cirrhosis or hepatocellular carcinoma[3]. Currently, no approved drugs can directly reverse hepatic steatosis or inflammatory fibrosis[4]. Although lifestyle interventions are the first-line therapy, their poor long-term adherence highlights the need for additional pharmacological strategies. Against this background, natural products represent a promising option for treating multifactorial complex diseases like MASLD, given their broad accessibility, favorable safety profile, and characteristic multi-component, multi-target synergistic actions[5]. In contrast to single-target chemical drugs that correct isolated aspects, natural active molecules can simultaneously regulate multiple key nodes, including lipid metabolism, insulin signaling, and oxidative stress/inflammatory responses, thus achieving a more comprehensive restoration of metabolic homeostasis.

Allyl isothiocyanate (AITC) is a natural organic sulfur compound characterized by its pungent, spicy odor. It primarily originates from cruciferous plants, where it is produced as a defensive substance through the enzymatic breakdown of glucosinolates upon tissue damage[6,7], which has garnered significant attention for its notable anti-inflammatory, antioxidant, and metabolism-modulating biological effects[8]. A recent study published in *World Journal of Hepatology* by Gao *et al*[9] investigated the role of AITC in MASLD and identified a mechanism involving vitamin D receptor (VDR) regulation. This study demonstrates that AITC increases VDR expression in hepatocytes, subsequently activating the hepatocyte nuclear factor 4 alpha (HNF-4α)/microsomal triglyceride transfer protein (MTTP)/apolipoprotein B (ApoB) signaling axis. This activation promotes hepatic lipid export, enhances fatty acid β-oxidation, and suppresses *de novo* lipogenesis, suggesting that the VDR pathway mediates the hepatic effects of AITC.

Specifically, the study's core finding shows that AITC treatment upregulates VDR expression, and the activated VDR subsequently interacts with HNF-4α to enhance its transcriptional activity, leading to upregulation of its downstream target genes MTTP and ApoB. This two proteins are critical for the assembly and secretion of very-low-density lipoprotein in the liver[10]. Their upregulation indicates that the liver reduces intrahepatic lipid accumulation, associated with enhanced lipid export. Furthermore, this pathway is implicated in improved fatty acid β-oxidation and insulin resistance. Given the multi-target characteristics of AITC, this VDR-involved pathway not only offers molecular evidence for its hepatoprotective effects but also positions VDR within the mechanistic framework underlying the action of natural products in MASLD.

AITC's known multi-target characteristics suggest that its biological effects are likely mediated through a synergistic network. The VDR pathway, as identified by Gao *et al*[9], is a crucial component of this network. However, this single mechanism alone cannot fully explain the anti-MASLD efficacy of AITC. Indeed, numerous prior studies have demonstrated that AITC is a molecule with complex regulatory functions[11-13]. Through specific binding and activation of the transient receptor potential ankyrin 1 (TRPA1) ion channel, the natural agonist AITC triggers a crucial influx of calcium ions (Ca^{2+})[14]. This initial Ca^{2+} signal acts as a central second messenger, initiating a multi-layered signalling cascade network that combats the complex pathology of MASLD from multiple angles. Based on this TRPA1/Ca^{2+}-initiated regulatory foundation, AITC targets multiple specific molecules and pathways to alleviate hepatic steatosis, mitigate oxidative stress and inflammation, improve insulin resistance and exert anti-fibrotic effects[15]. Within this

network, the TRPA1-mediated calcium influx serves as an essential prerequisite for activating the entire downstream cellular defense system. This highlights the unique mechanism by which natural products treat metabolic diseases through multi-target systematic intervention (Figure 1). The following sections will detail the specific molecular mechanisms by which it regulates lipid metabolism, enhances antioxidant defences, exerts anti-inflammatory and anti-fibrotic effects, and improves insulin sensitivity.

Regarding the regulation of lipid metabolism, the core action of AITC is the activation of the sirtuin 1/AMP-activated protein kinase (SIRT1/AMPK) pathway. This activation inhibits sterol regulatory element-binding protein 1, the key transcriptional factor for *de novo* lipogenesis, along with its downstream target genes (such as stearoyl-CoA desaturase 1 and fatty acid synthase), while simultaneously upregulating proteins involved in fatty acid β-oxidation (*e.g.*, peroxisome proliferator-activated receptor gamma coactivator 1 alpha, peroxisome proliferator-activated receptor alpha, carnitine palmitoyltransferase 1 alpha)[10]. This effectively reduces hepatic lipid synthesis and promotes its breakdown, alleviating hepatic steatosis. Loss-of-function experiments confirmed that knocking down SIRT1 or AMPK completely blocked the lipid-improving effects of AITC, establishing the central role of this pathway[10]. In studies on the anti-diabetic mechanisms of AITC, accumulating evidence further supports that AITC activates the TRPA1 receptor to induce Ca^{2+} influx, which in turn triggers the AMPK signaling pathway *via* calcium-sensitive upstream kinases such as Ca^{2+}/calmodulin-dependent protein kinase kinase 2, thereby playing a key role in the regulation of glucose and lipid metabolism[16].

In terms of enhancing antioxidant defense, AITC is a classic activator of nuclear factor erythroid 2-related factor 2 (Nrf2), effectively inducing the expression of a suite of antioxidant and detoxifying genes, thereby strengthening the hepatocyte's ability to resist oxidative damage[17]. It is noteworthy that research suggests activation of the VDR may also enhance the Nrf2 signaling pathway[18]. This implies that under AITC treatment, the VDR pathway may synergize with the Nrf2 pathway, jointly bolstering antioxidant defense and providing protection at more advanced disease stages (metabolic dysfunction-associated steatohepatitis).

Its anti-inflammatory and anti-fibrotic effects are primarily mediated through the modulation of relevant signaling pathways. AITC upregulates the inhibitor of nuclear factor kappa-B (NF-κB) alpha protein and inhibits the phosphorylation and activation of the IκB kinase/NF-κB pathway, effectively reducing the production of key pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β, thereby alleviating chronic liver inflammation[11]. This anti-inflammatory action synergizes with AITC's mechanism for improving lipid metabolism, jointly mitigating the pathological progression of MASLD. Furthermore, by activating TRPA1 and subsequently modulating the transforming growth factor-β1/small mother against decapentaplegic signaling, AITC can inhibit the differentiation of fibroblasts into myofibroblasts (manifested as reduced α-smooth muscle actin expression), demonstrating potential anti-fibrotic effects [19].

Regarding the improvement of insulin sensitivity, AITC acts through multi-target synergy. On one hand, it regulates energy metabolism and lipid flux by activating the AMPK/SIRT1 axis, directly alleviating lipotoxicity. On the other hand, it mitigates the damage caused by oxidative stress and inflammation to insulin signaling pathways by activating Nrf2 and inhibiting NF-κB. Simultaneously, AITC upregulates key insulin signaling molecules, including glucose transporter 2, peroxisome proliferator-activated receptor-γ, and phosphorylated insulin receptor substrate 1, and comprehensively improves blood glucose and lipid levels, thereby systematically restoring insulin sensitivity[11,20]. These pathways are interconnected and form a positive feedback network, systematically reshaping the metabolic homeostasis of the liver and the entire body, and ultimately restoring insulin sensitivity. This demonstrates its unique advantage as a natural product in the multi-targeted collaborative treatment of metabolic disorders.

The study by Gao *et al*[9] revealed a new mechanism by which AITC promotes liver lipid output by upregulating the VDR and activating the HNF-4α/MTTP/ApoB axis. This provides an important perspective for understanding its hepato-protective effect and officially incorporates VDR into the mechanism network of AITC's action[10]. However, it must be pointed out that this mechanism centered on VDR may not be able to fully explain all the effects exhibited by AITC, especially its extensive anti-inflammatory, antioxidant, and improvement of insulin resistance properties. The existing evidence suggests that the efficacy of AITC is more likely attributed to the aforementioned synergistic network triggered by TRPA1/Ca^{2+}, covering multiple pathways such as SIRT1/AMPK, Nrf2, and NF-κB. The VDR pathway may be an important component of this network and may interact with other pathways such as Nrf2[18].

CONCLUSION

In summary, AITC may act as an initial signal by activating the TRPA1 receptor and triggering Ca^{2+} influx. It then forms a multi-targeted network to exert its effects. This network simultaneously regulates several core pathways such as SIRT1/AMPK, Nrf2, VDR, and NF-κB, thereby systematically interfering with the complex pathological process of MASLD from multiple dimensions including reducing liver lipid accumulation, combating oxidative stress and inflammation, improving insulin resistance, and inhibiting fibrosis. This not only fully demonstrates the unique advantages and potential of natural products in treating complex metabolic diseases through multi-target systemic intervention, but also broadens our understanding of the mechanism of action of AITC, and provides a theoretical basis for formulating a synergistic treatment strategy targeting the multi-factor pathological characteristics of MASLD. Future research needs to rely on more in-depth *in vivo* experiments and genetic tools such as VDR knockout models to further verify the specific contribution of the VDR pathway to the overall effect of AITC and its interaction with other pathways, thereby more completely and systematically elucidating the multi-target synergistic mechanism of AITC in the treatment of MASLD.

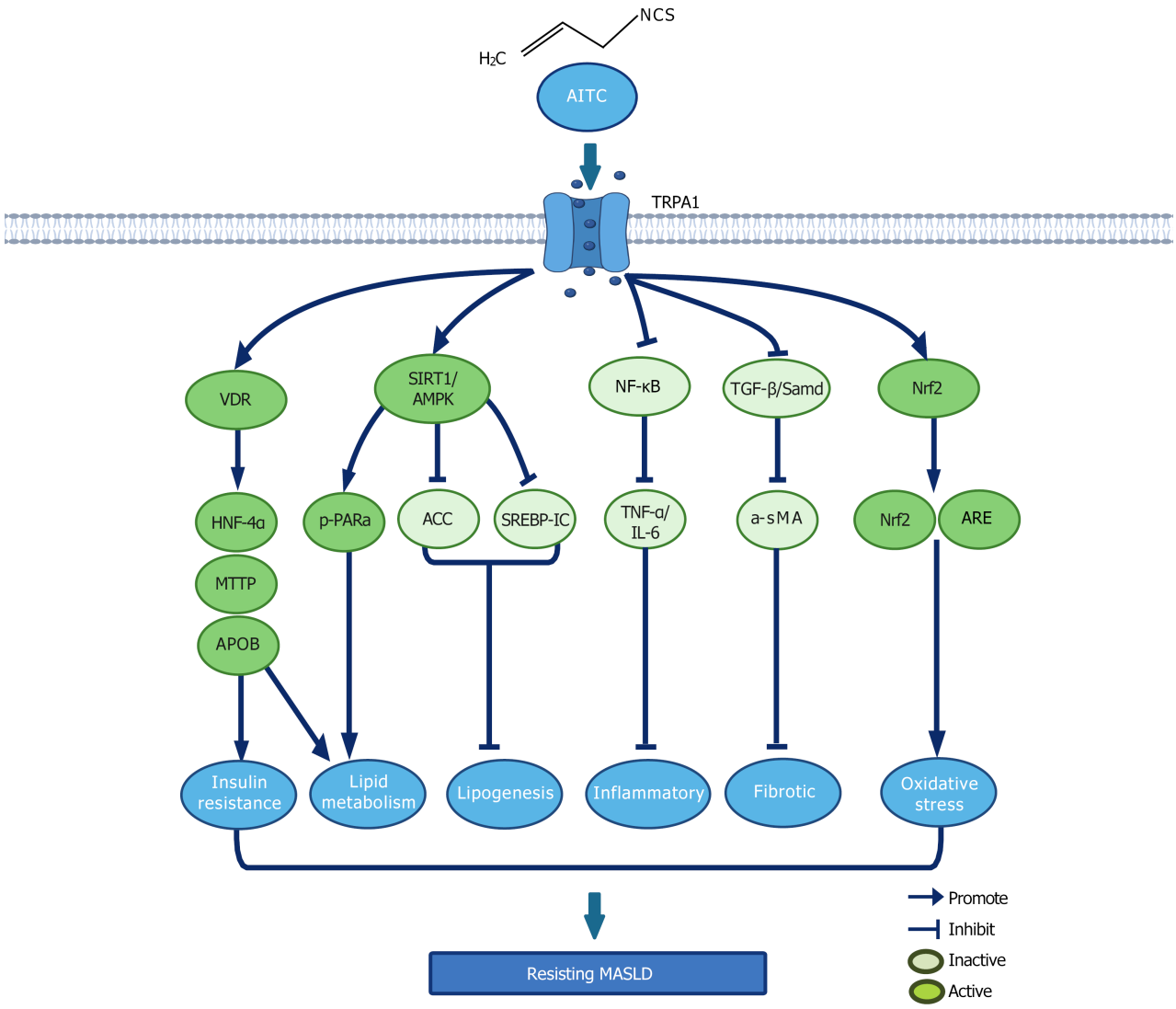


Figure 1 Schematic diagram of the molecular mechanism by which allyl isothiocyanate counteracts metabolic dysfunction-associated steatotic liver disease. MASLD: Metabolic dysfunction-associated steatotic liver disease; NCS: Isothiocyanate group; H_2C: Methylene group; AITC: Allyl isothiocyanate; TRPA1: Transient receptor potential ankyrin 1; VDR: Vitamin D receptor; SIRT1: Sirtuin 1; AMPK: AMP-activated protein kinase; NF-κB: Nuclear factor kappa B; TGF-β: Transforming growth factor-β; Samd: Smad family proteins; Nrf2: Nuclear factor erythroid 2-related factor 2; HNF-4α: Hepatocyte nuclear factor 4 alpha; MTTP: Microsomal triglyceride transfer protein; APOB: Apolipoprotein B; p-PARa: Phosphorylated peroxisome proliferator-activated receptor α; ACC: Acetyl-CoA carboxylase; SREBP-IC: Sterol regulatory element-binding protein-1c; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; a-sMA: A-smooth muscle actin; ARE: Antioxidant response element.

REFERENCES

1 **Younossi ZM**, Kalligeros M, Henry L. Epidemiology of metabolic dysfunction-associated steatotic liver disease. *Clin Mol Hepatol* 2025; **31**: S32-S50 [*RCA*] [PMID: 39159948 DOI: 10.3350/cmh.2024.0431] [FullText] [Full Text(PDF)]

2 **Huttasch M**, Roden M, Kahl S. Obesity and MASLD: Is weight loss the (only) key to treat metabolic liver disease? *Metabolism* 2024; **157**: 155937 [*RCA*] [PMID: 38782182 DOI: 10.1016/j.metabol.2024.155937] [FullText]

3 **Targher G**, Byrne CD, Tilg H. MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. *Gut* 2024; **73**: 691-702 [*RCA*] [PMID: 38228377 DOI: 10.1136/gutjnl-2023-330595] [FullText]

4 **Chan WK**, Chuah KH, Rajaram RB, Lim LL, Ratnasingam J, Vethakkan SR. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A State-of-the-Art Review. *J Obes Metab Syndr* 2023; **32**: 197-213 [*RCA*] [PMID: 37700494 DOI: 10.7570/jomes23052] [Full Text] [Full Text(PDF)]

5 **Ye Q**, Yuan S, Cai D. Synergistic potential of natural products and exercise: unveiling molecular mechanisms and innovative therapeutic approaches for liver diseases. *Front Nutr* 2025; **12**: 1656048 [*RCA*] [PMID: 41141263 DOI: 10.3389/fnut.2025.1656048] [FullText] [Full Text (PDF)]

6 **Tarar A**, Peng S, Cheema S, Peng CA. Anticancer Activity, Mechanism, and Delivery of Allyl Isothiocyanate. *Bioengineering (Basel)* 2022; **9**: 470 [*RCA*] [PMID: 36135016 DOI: 10.3390/bioengineering9090470] [FullText] [Full Text(PDF)]

7 **Li L**, Lin Y, Agyekumwaa Addo K, Yu Y, Liao C. Effect of allyl isothiocyanate on the growth and virulence of Clostridium perfringens and its application on cooked pork. *Food Res Int* 2023; **172**: 113110 [*RCA*] [PMID: 37689877 DOI: 10.1016/j.foodres.2023.113110] [FullText]

8 **Patil PB**, Patel JK. Chemopreventive aspects, investigational anticancer applications and current perspectives on allyl isothiocyanate (AITC): a

review. *Mol Cell Biochem* 2023; **478**: 2763-2777 [*RCA*] [PMID: 36929336 DOI: 10.1007/s11010-023-04697-0] [FullText]

9 **Gao T**, Zhong KP, Wang JZ, Chen Y, Li CX. Allyl isothiocyanate ameliorates metabolic dysfunction-associated steatotic liver disease *via* vitamin D receptors in hepatocytes. *World J Gastroenterol* 2026; **32**: 113647 [*RCA*] [PMID: 41640867 DOI: 10.3748/wjg.v32.i4.113647] [Full Text] [Full Text(PDF)]

10 **López-Pascual E**, Rienda I, Perez-Rojas J, Rapisarda A, Garcia-Llorens G, Jover R, Castell JV. Drug-Induced Fatty Liver Disease (DIFLD): A Comprehensive Analysis of Clinical, Biochemical, and Histopathological Data for Mechanisms Identification and Consistency with Current Adverse Outcome Pathways. *Int J Mol Sci* 2024; **25**: 5203 [*RCA*] [PMID: 38791241 DOI: 10.3390/ijms25105203] [FullText]

11 **Li CX**, Gao JG, Wan XY, Chen Y, Xu CF, Feng ZM, Zeng H, Lin YM, Ma H, Xu P, Yu CH, Li YM. Allyl isothiocyanate ameliorates lipid accumulation and inflammation in nonalcoholic fatty liver disease *via* the Sirt1/AMPK and NF-κB signaling pathways. *World J Gastroenterol* 2019; **25**: 5120-5133 [*RCA*] [PMID: 31558861 DOI: 10.3748/wjg.v25.i34.5120] [FullText] [Full Text(PDF)]

12 **Yap JMG**, Ueda T, Kanemitsu Y, Takeda N, Fukumitsu K, Fukuda S, Uemura T, Tajiri T, Ohkubo H, Maeno K, Ito Y, Oguri T, Ugawa S, Niimi A. AITC inhibits fibroblast-myofibroblast transition *via* TRPA1-independent MAPK and NRF2/HO-1 pathways and reverses corticosteroids insensitivity in human lung fibroblasts. *Respir Res* 2021; **22**: 51 [*RCA*] [PMID: 33579280 DOI: 10.1186/s12931-021-01636-9] [FullText] [Full Text(PDF)]

13 **Cevikbas F**, Wang X, Akiyama T, Kempkes C, Savinko T, Antal A, Kukova G, Buhl T, Ikoma A, Buddenkotte J, Soumelis V, Feld M, Alenius H, Dillon SR, Carstens E, Homey B, Basbaum A, Steinhoff M. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *J Allergy Clin Immunol* 2014; **133**: 448-460 [*RCA*] [PMID: 24373353 DOI: 10.1016/j.jaci.2013.10.048] [FullText] [Full Text(PDF)]

14 **Doihara H**, Nozawa K, Kawabata-Shoda E, Kojima R, Yokoyama T, Ito H. Molecular cloning and characterization of dog TRPA1 and AITC stimulate the gastrointestinal motility through TRPA1 in conscious dogs. *Eur J Pharmacol* 2009; **617**: 124-129 [*RCA*] [PMID: 19576208 DOI: 10.1016/j.ejphar.2009.06.038] [FullText]

15 **Talavera K**, Startek JB, Alvarez-Collazo J, Boonen B, Alpizar YA, Sanchez A, Naert R, Nilius B. Mammalian Transient Receptor Potential TRPA1 Channels: From Structure to Disease. *Physiol Rev* 2020; **100**: 725-803 [*RCA*] [PMID: 31670612 DOI: 10.1152/physrev.00005.2019] [FullText]

16 **Bhat R**, Saxena S, Khajuria M, Vyas D. Glucosinolates Hydrolysis Products: Promising Bioactives for the Prevention of Diabetes. *Mol Nutr Food Res* 2025; **69**: e70302 [*RCA*] [PMID: 41189420 DOI: 10.1002/mnfr.70302] [FullText]

17 **Hsu WH**, Lee BH, Li CH, Hsu YW, Pan TM. Monascin and AITC attenuate methylglyoxal-induced PPARγ phosphorylation and degradation through inhibition of the oxidative stress/PKC pathway depending on Nrf2 activation. *J Agric Food Chem* 2013; **61**: 5996-6006 [*RCA*] [PMID: 23731245 DOI: 10.1021/jf401197r] [FullText]

18 **Wang H**, Yu X, Liu D, Qiao Y, Huo J, Pan S, Zhou L, Wang R, Feng Q, Liu Z. VDR Activation Attenuates Renal Tubular Epithelial Cell Ferroptosis by Regulating Nrf2/HO-1 Signaling Pathway in Diabetic Nephropathy. *Adv Sci (Weinh)* 2024; **11**: e2305563 [*RCA*] [PMID: 38145959 DOI: 10.1002/advs.202305563] [FullText] [Full Text(PDF)]

19 **Matsushita H**, Mukudai S, Hashimoto K, Kaneko M, Sugiyama Y, Branski RC, Hirano S. Transient Receptor Potential Ankyrin 1 Channel Alters Transforming Growth Factor Beta 1/Smad Signaling in Rat Vocal Fold Fibroblasts. *Laryngoscope* 2024; **134**: 4593-4598 [*RCA*] [PMID: 38860441 DOI: 10.1002/lary.31570] [FullText]

20 **Gomoll AW**, Byrne JE, Mayol RF. Comparative antiarrhythmic actions of encainide and its major metabolites. *Arch Int Pharmacodyn Ther* 1986; **281**: 277-297 [*RCA*] [PMID: 3092755] [FullText]

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CORRESPONDENCE

Letter to the Editor: Filling the gaps in non-invasive assessment of liver fibrosis in metabolic dysfunction-associated steatotic liver disease

Roberta Forlano, Nicola Maurizio Castellaneta, Patrizia Suppressa

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Abstract

In this letter to the editor, we discuss the strengths and weaknesses of the paper from Joseph *et al*. We take to opportunity to review the clinical importance of screening metabolic dysfunction associated steatotic liver disease patients for significant fibrosis. The use of a first line blood test is crucial for cost and availability, although this may overestimate and underestimate in patients with normal liver enzymes. Liver stiffness as a second line test, decreases the number of false positives, although it may be influenced by operator experience, cost and patients' body habitus. Future studies are required to explore the application of further technologies in the screening. Nevertheless, for the time being, a 2 tier-system still represents the best option to screen a large number of patients and to optimise resources.

Key Words: Metabolic dysfunction associated steatotic liver disease; Liver fibrosis; Screening; Non-invasive assessment; Liver function tests

Core Tip: Non-invasive assessment of liver fibrosis remains challenging in patients suffering from metabolic dysfunction associated steatotic liver disease. A two-tier system including blood tests and elastography still remains the most sensible and practical approach to address this unmet need.

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TO THE EDITOR

We were delighted to read the article from Joseph *et al*[1] on the potential role of blood markers *vs* transient elastography for liver stiffness and steatosis in metabolic dysfunction-associated steatotic liver disease (MASLD). This article aims to fill an important gap in clinical practice regarding screening significant liver disease in patients suffering from MASLD [2].

In this paper, 300 patients with a clinical diagnosis of MASLD underwent transient elastography with liver stiffness measurements (LSM) and controlled attenuation parameter (CAP) score for further characterization[1]. A wide range of blood-based markers, such as aspartate transaminase to platelet ratio index (APRI), neutrophil-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and neutrophil percentage-to-albumin ratio (NPAR) were compared against LSM and CAP score. Despite an extensive literature on the application of fibrosis-4 index (FIB-4) in patients with MASLD, only little is known so far about the potential application of APRI, NLR and NPAR in this cohort of patients[3].

Identifying patients at high-risk of significant liver disease is of primary importance when managing MASLD patients [3,4]. Screening high-risk groups (*i.e.,* those with type-2 diabetes mellitus and or other features of metabolic syndrome) is strongly recommended by current international guidelines. The recent approval of a liver-targeted medication (resmetirom) has emphasized the importance of identifying those with moderate-advanced fibrosis (F2 or F3) from MASLD, who could benefit from treatment[5]. Moreover, several studies have shown that potentially any patient with MASLD could benefit from screening, as changes in lifestyle and weight loss with optimisation of metabolic risk factors, lead to better outcomes regardless of the severity of liver disease[6].

In this study, on a diagnostic performance analysis, FIB-4 exhibited the highest diagnostic performance for differentiating advanced fibrosis (F3 and F4) from lower stages (F0-F2), with an area under the curve of 0.806 ($P < 0.001$), sensitivity of 69.2%, and specificity of 80.4%. Most recent guidelines have suggested the implementation of a 2-step approach including a first line FIB-4, followed by LSM as this strategy has proved to maximise false negatives in high-risk groups of patients and to be cost-effective[6]. This study confirms that FIB-4, combined with LSM, is accurate at selecting those with significant liver disease. However, liver histology still represents the gold standard for diagnosing steatohepatitis as well as for staging fibrosis, therefore non-invasive markers performance should ideally be validated against liver biopsy rather than LSM directly[3]. Furthermore, a large-scale application of LSM may present some specific challenges, such as cost and availability. Moreover, despite a good reproducibility, experience may still have an impact on how exams are performed, especially in patients with challenging body habitus such as patients with visceral obesity. Other technologies based on elastography could potentially mitigate these limitations, such as ultrasound-based share wave elastography or magnetic resonance elastography, although these should better be applied in the a smaller setting of clinical trials. All these factors should be considered when setting up policies for large scale use of non-invasive markers including LSM.

Recent evidence has shown that many patients with advanced fibrosis secondary to MASLD may present with normal liver function tests, especially diabetics[6,7]. Conversely to what happened to other chronic liver diseases, liver function tests should not be used to assess the severity of fibrosis in MASLD. This poses a significant clinical challenge, as still many colleagues rely on transaminases when evaluating these patients. This is likely the reason why blood tests, such as APRI, which were developed from cohorts of biopsied patients with elevated liver function tests, perform worse in predicting severe liver disease in MASLD. Similarly, changes in white blood cells as well as in platelets may occur quite late in the natural history of the disease, making NLR and PLR not accurate for early diagnosis in this cohort. On the other hand, the application of blood-based, non-invasive markers which do not rely on liver function tests, such as enhanced liver fibrosis (ELF), could mitigate the effect of normal liver enzymes on screening detection rates. It would be interesting to see future studies analysing the performance of ELF in a specific cohort of patients with normal transaminases.

CONCLUSION

Identifying patients at high risk of significant liver disease from MASLD represents an important challenge in clinical practice. The use of non-invasive markers is recommended to increase the accessibility to screening outside of specialist care. FIB-4 proves to be a good predictor of fibrosis severity, while APRI, NLR and PLR may be impacted negatively by a long proportion of patients having completely normal blood tests. Algorithms including FIB-4 as first step for testing,

should be implemented in accordance with resources and local availability.

REFERENCES

1 **Joseph A**, Mathew S, Nair HR. Blood markers *vs* transient elastography for liver stiffness and steatosis in metabolic dysfunction-associated steatotic liver disease. *World J Hepatol* 2026; **18**: 113475 [*RCA*] [PMID: 41640950 DOI: 10.4254/wjh.v18.i1.113475] [FullText] [Full Text (PDF)]

2 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [*RCA*] [PMID: 28714183 DOI: 10.1002/hep.29367] [FullText]

3 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J Hepatol* 2024; **81**: 492-542 [*RCA*] [PMID: 38851997 DOI: 10.1016/j.jhep.2024.04.031] [FullText]

4 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [*RCA*] [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312] [Full Text]

5 **Harrison SA**, Bedossa P, Guy CD, Schattenberg JM, Loomba R, Taub R, Labriola D, Moussa SE, Neff GW, Rinella ME, Anstee QM, Abdelmalek MF, Younossi Z, Baum SJ, Francque S, Charlton MR, Newsome PN, Lanthier N, Schiefke I, Mangia A, Pericàs JM, Patil R, Sanyal AJ, Noureddin M, Bansal MB, Alkhouri N, Castera L, Rudraraju M, Ratziu V; MAESTRO-NASH Investigators. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. *N Engl J Med* 2024; **390**: 497-509 [*RCA*] [PMID: 38324483 DOI: 10.1056/NEJMoa2309000] [FullText]

6 **Forlano R**, Stanic T, Jayawardana S, Mullish BH, Yee M, Mossialos E, Goldin R, Petta S, Tsochatzis E, Thursz M, Manousou P. A prospective study on the prevalence of MASLD in people with type-2 diabetes in the community. Cost effectiveness of screening strategies. *Liver Int* 2024; **44**: 61-71 [*RCA*] [PMID: 37718933 DOI: 10.1111/liv.15730] [FullText]

7 **Uhanova J**, Minuk G, Premji K, Chandok N. Serial liver transaminases have no prognostic value in non-alcoholic fatty liver disease. *Can Liver J* 2019; **2**: 19-22 [*RCA*] [PMID: 35991834 DOI: 10.3138/canlivj.2018-0006] [FullText] [Full Text(PDF)]

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CORRESPONDENCE

Letter to the Editor: Efficacy and safety of the generic forms of sofosbuvir/ledipasvir in chronic hepatitis C virus adolescents in Upper Egypt

Nahed A Makhlouf

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Abstract

Mogahed *et al* recently published a retrospective study in the *World Journal of Hepatology*, which assessed the safety and efficacy of the generic sofosbuvir (SOF)/ledipasvir (LED) in Egyptian hepatitis C virus (HCV)-infected children in comparison with the brand form. They concluded that, the use of generic SOF/LED in HCV infected children is safe and effective. Previous study in 2021 on chronic HCV in adolescents used the generic SOF/LED at Assiut, Upper Egypt. Sustained virologic response (SVR12) was 100% both in treatment naïve with or without haematological disorders and in non-responder to pegylated interferon/ribavirin. The treatment was well tolerated, with minimal side effects. We also support the use of the generic forms of SOF/LED in HCV infected children and adolescents in Egypt and other resource limited countries.

Key Words: Adolescents; Hepatitis C virus; Generic; Ledipasvir; Sofosbuvir; Treatment naïve; Treatment experienced; Egypt

Core Tip: Using brand forms of direct acting antivirals (DAAs) against hepatitis C virus (HCV) is limited in low- and middle-income countries. Generic forms of DAAs were widely used in adults. Few studies addressed this issue in children and adolescents in Egypt. We support the use of the generic forms of sofosbuvir/ledipasvir in HCV infected children and adolescents.

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TO THE EDITOR

We are delighted to read the high-quality article by Mogahed *et al*[1], published in the *World Journal of Hepatology*. The main focus of this retrospective study is providing real-world evidence on the safety and efficacy of generic sofosbuvir (SOF)/Ledipasvir (LED) combination in children and adolescents infected with chronic hepatitis C virus (HCV) in comparison with their corresponding brand forms at Cairo (Lower Egypt). The study included 116 Egyptian children and adolescents with chronic HCV infection. Forty-three patients received the generic form of SOF/LED (Ledisbuvir) for 12 weeks, while 73 patients received the brand form (Harvoni®) for 12 weeks. End of treatment response and SVR12 were achieved in both groups (100% for each) with transient mild side effects. Headache and fatigue were most commonly reported side effects (37.2% for each). Irritability was reported in 30.2% and diarrhea in 11.6%[1]. Similarly, Makhlouf *et al* [2] prospectively included 65 chronic HCV adolescents (44 of them were treatment-naive without hematological disorders, 15 treatment-naive with hematological disorders, and 6 were previous nonresponders to pegylated interferon/ribavirin). All patients received generic SOF/LED combination in one tablet once daily for 12 weeks (generic drug, Sofolanork plus; Mash Premier, New Cairo City, Egypt). SVR12 was achieved in all patients (100%). All adverse events were mild, self-limited and observed 2 weeks of starting the drug regimen without treatment discontinuation. Headache was reported in 29.2%, diarrhea in 15.4%, while only 4.5% developed fatigue. In addition, at SVR12, significant improvement in the mean aspartate aminotransferase to platelet ratio index score and median liver stiffness were observed compared with baseline in all groups[2]. El-Khayat *et al*[3] evaluated SOF/LED treatment efficacy for 8 weeks *vs* 12 weeks in adolescent patients in Egypt. SVR12 was 97.6% in patients treated for 12 weeks, while it was 98.6% for those treated for 8 weeks. They concluded that eight weeks SOF/LED is equally effective and safe as 12 weeks with lower cost, higher treatment adherence and improved quality of life[3].

Adverse events were reported in 7% of 8 weeks group, where fatigue was 2.8%, while 1.4% was reported for each of headache, epigastric tenderness or nausea. In the other group, adverse events were reported in 8.3%, where 2.4% was reported for diarrhea and 2.4% for rash. However, each of nausea or epigastric tenderness or fatigue occurred in 1.2%[3]. However, El-Khayat *et al*[3] did not mention whether they used generic or brand SOF/LED combination.

Limitation

Small sample sizes of cited studies are considered as limitation.

Conclusion

We support the use of generic SOF/LED in children and adolescents with HCV infection. Generic SOF/LED regimen was safe and well tolerated with lower cost.

REFERENCES

1 **Mogahed E**, Ghita H, Enayet A, Yasin N, El-Karaksy H. Generic vs brand forms of direct acting antivirals for hepatitis C virus treatment in Egyptian children. *World J Hepatol* 2025; **17**: 110726 [*RCA*] [PMID: 41479503 DOI: 10.4254/wjh.v17.i12.110726] [FullText] [Full Text (PDF)]

2 **Makhlouf NA**, Abdelmalek MO, Ibrahim ME, Abu-Faddan NH, Kheila AE, Mahmoud AA. Ledipasvir/Sofosbuvir in Adolescents With Chronic Hepatitis C Genotype 4 With and Without Hematological Disorders: Virological Efficacy and Impact on Liver Stiffness. *J Pediatric Infect Dis Soc* 2021; **10**: 7-13 [*RCA*] [PMID: 32060510 DOI: 10.1093/jpids/piaa006] [FullText]

3 **El-Khayat H**, Kamal EM, Yakoot M, Gawad MA, Kamal N, El Shabrawi M, Sameh Y, Haseeb A, Fouad Y, Attia D. Effectiveness of 8-week sofosbuvir/ledipasvir in the adolescent chronic hepatitis C-infected patients. *Eur J Gastroenterol Hepatol* 2019; **31**: 1004-1009 [*RCA*] [PMID: 30676473 DOI: 10.1097/MEG.0000000000001360] [FullText]

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CORRESPONDENCE

Letter to the Editor: Induction immunotherapy in hyperimmunized liver transplantation: Rebalancing immunological risk and graft protection

Jin-Wei Zhang

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Abstract

Hyperimmunization-defined by the presence of pre-formed donor-specific antibodies or a positive donor–recipient crossmatch-remains a pivotal immunological challenge in liver transplantation (LT), linked to substantially elevated risks of acute rejection and graft dysfunction. In their recent work, EL-Domiaty *et al* published in the *World Journal of Hepatology* evaluate a targeted induction strategy combining rabbit anti-T-lymphocyte globulin and high-dose intravenous immunoglobulin in hyperimmunized liver transplant recipients. Using a rigorous matched case-control design, the team demonstrates that this induction approach yields rejection rates, graft survival, and patient outcomes on par with those seen in non-immunized recipients, with no excess of severe infectious complications. This editorial situates these valuable findings within the evolving body of literature on antibody-mediated injury in LT, offers a critical appraisal of the study's methodology and real-world clinical implications, and explores how such induction strategies can shape the future of personalized immunosuppressive care for immunologically high-risk liver transplant patients.

Key Words: Liver transplantation; Hyperimmunization; Donor-specific antibodies; Antibody-mediated rejection; Induction immunosuppression; Anti-T-lymphocyte globulin

Core Tip: For decades, hyperimmunized liver transplant recipients have faced poorer clinical outcomes due to their heightened immunological risk profile, leaving clinicians with limited evidence-based management options. This editorial delivers a comprehensive analysis of EL-Domiaty *et al*'s landmark study, which provides compelling clinical evidence that a short-course induction regimen of rabbit anti-T-lymphocyte globulin plus intravenous immunoglobulin can effectively mitigate early rejection risk and align outcomes with those of non-immunized recipients. This work highlights a viable, pragmatic strategy for managing this challenging patient population and paves the way for more personalized immunosuppression in liver transplantation.

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TO THE EDITOR

Liver transplantation (LT) stands as the curative gold standard for end-stage liver disease and select hepatic malignancies, yet the immunological complexity of the procedure continues to shape post-transplant outcomes in profound ways[1]. The liver has long been recognized as unique among solid organs for its relative resistance to antibody-mediated injury, but this resistance is far from absolute: It is context-dependent, and mounting clinical evidence confirms that preformed donor-specific antibodies (DSAs)-especially high-titer, complement-binding variants-and positive donor-recipient crossmatches drive increased risks of acute rejection, graft dysfunction, and mortality in transplant recipients[2-4]. Hyperimmunization affects a clinically significant subset of liver transplant candidates, typically stemming from prior sensitizing events such as blood transfusions, pregnancy, or previous solid organ transplantation[5-7].

The management of hyperimmunized liver transplant recipients remains one of the field's most contentious areas, with particular debate surrounding the role and optimal composition of induction immunosuppression. While induction therapy is a well-established cornerstone of kidney transplantation care, its use in LT has been inconsistent and heterogeneous across centers, rooted in valid concerns about infection, malignancy, and the risks of over-immunosuppression in a population already vulnerable to post-transplant complications[7,8]. EL-Domiaty *et al*[9] published in the *World Journal of Hepatology* address this long-unresolved clinical gap by evaluating a combined induction regimen of rabbit anti-T-lymphocyte globulin (rATLG) and intravenous immunoglobulin (IVIG) in hyperimmunized liver transplant recipients, offering critical, practice-changing insights into risk mitigation strategies for this high-risk group in contemporary LT.

Methods

EL-Domiaty *et al*'s study[9] was conducted at a high-volume liver transplant center using a retrospective 1:1 exact matched case-control design, pairing 46 hyperimmunized liver transplant recipients with 46 non-immunized controls. Matching was strictly based on age, sex, primary transplant indication, and model for end-stage liver disease score, with post-matching balance diagnostics confirming well-balanced baseline characteristics between the two cohorts-an essential step in minimizing confounding bias in retrospective analyses. Hyperimmunization was defined by the presence of DSAs with a mean fluorescence intensity ≥ 5000 (the center's established clinical threshold for high-titer DSAs) and/or a positive complement-dependent cytotoxicity crossmatch at the time of transplantation; no additional complement-binding assays (*e.g.*, C1q or C3d) were performed as part of the study's inclusion criteria.

High-risk hyperimmunized recipients received a standardized induction therapy regimen of rATLG (1.5 mg/kg/day for 3 consecutive days, initiated intraoperatively) combined with high-dose IVIG (2 g/kg total dose, administered in two divided doses in the first week post-transplant), followed by center-standard maintenance immunosuppression (tacrolimus-based triple therapy with mycophenolate mofetil and prednisone). In contrast, non-immunized control patients did not receive any induction immunosuppression and were managed with the identical maintenance regimen alone.

Clinical, immunological, and histopathological outcomes were systematically collected and adjudicated, with acute rejection classified strictly according to the 2016 Banff Working Group criteria for liver allograft pathology[10]. Graft and patient survival were analyzed using Kaplan-Meier curves, with standardized statistical comparisons (including log-rank tests and Cox proportional hazards models) employed to evaluate between-group differences. This rigorous analytical approach ensured methodological robustness, even within the constraints of the study's retrospective design.

Key findings

Despite their markedly higher baseline immunological risk, hyperimmunized recipients who received the rATLG/IVIG induction regimen exhibited rates of biopsy-proven acute T-cell–mediated rejection that were nearly identical to those of non-immunized controls. Most notably, no graft losses were attributed to acute rejection in either cohort over the study follow-up period. While acute antibody-mediated rejection occurred exclusively in the hyperimmunized group, these episodes were rare, clinically mild, and readily managed with targeted immunosuppressive interventions-with no subsequent impact on long-term graft survival.

Graft and patient survival at 1 and 3 years were comparable between the two groups, with a borderline statistical trend favoring non-immunized recipients (log-rank $P = 0.048$ for 3-year graft survival; $P = 0.051$ for 3-year patient survival). This subtle statistical finding is best interpreted as a reflection of the study's limited sample size (46 pairs) rather than a

clinically meaningful disparity in outcomes, as the absolute survival differences between groups were minimal (≤ 3% at all time points).

Notably, the rATLG/IVIG induction regimen was well-tolerated in this high-risk cohort: Severe infectious complications (defined as life-threatening infections requiring ICU admission, intravenous antimicrobial therapy for ≥ 14 days, or associated with organ dysfunction) occurred in 8.7% of hyperimmunized recipients *vs* 6.5% of controls (P = 0.68), with no cases of induction-related severe infection (*e.g.*, early opportunistic infection within the first month post-transplant). No new-onset malignancy was diagnosed in either group during the follow-up period, and induction-related adverse events (*e.g.*, infusion reactions, cytopenias) were mild, transient, and managed with supportive care alone-no recipients required discontinuation of induction therapy for adverse events. These findings strongly support the favorable safety profile of this induction strategy in hyperimmunized liver transplant recipients.

Critical appraisal

The findings of EL-Domiaty *et al*'s study[9] align closely with and extend the growing body of research demonstrating that antibody-mediated mechanisms are clinically impactful drivers of outcomes in LT-contrary to the historical view of the liver as uniformly resistant to AMR[2,6,11]. Earlier studies have consistently linked pre-formed DSAs, and particularly high-titer class II HLA antibodies, to elevated risks of acute rejection and early mortality in liver transplant recipients[3,4,12-14]. The low incidence of severe acute rejection (both T-cell-mediated and antibody-mediated) and the complete absence of rejection-related graft loss in the hyperimmunized cohort of this study stand in stark contrast to historical data, which report rejection rates ranging from 23% to 65% in sensitized liver transplant recipients without targeted induction therapy-underscoring the clinical efficacy of the rATLG/IVIG regimen[11,15-17].

The induction strategy evaluated here mirrors promising approaches previously explored in crossmatch-positive LT, including thymoglobulin-based regimens combined with B-cell-directed therapies[12], but offers a key advantage in its use of rATLG over thymoglobulin. While direct head-to-head comparative data of rATLG and thymoglobulin specifically in LT remain limited, multiple studies in solid organ transplantation (including kidney and heart transplantation) have demonstrated that rATLG confers a more favorable safety profile-with lower rates of severe cytopenia and opportunistic infection-while maintaining equivalent immunosuppressive efficacy[18-20]. This is a critical distinction for LT, where recipients are inherently immunocompromised due to underlying liver dysfunction and at high risk of post-transplant infection[21-23].

Equally important, EL-Domiaty *et al*'s study[9] demonstrates that standard maintenance immunosuppression levels are sufficient to maintain graft tolerance in hyperimmunized recipients who receive effective induction therapy. This finding challenges the historical practice of intensifying long-term immunosuppression in high-risk sensitized recipients-a strategy that often increases the risk of infection and malignancy without meaningful improvements in graft survival. Instead, the study supports a more nuanced approach: Front-loading immunosuppression with a short, targeted induction course to mitigate early immunological risk, followed by standard maintenance therapy to balance long-term graft protection and patient safety.

Of course, the study is not without limitations, which must be acknowledged to avoid overinterpretation of its findings. The retrospective, single-center design and relatively small sample size limit the generalizability of the results and preclude definitive conclusions about the regimen's efficacy in broader patient populations. Additionally, the study did not include systematic post-transplant DSA monitoring, which limits mechanistic insights into DSA kinetics, antibody decline, and the potential for subclinical AMR-an area increasingly recognized as a key driver of long-term graft fibrosis and dysfunction in LT[6,24,25]. Future studies addressing these gaps will be essential to refining this induction strategy for clinical practice.

Future perspectives

EL-Domiaty *et al*'s work[9] reinforces a central tenet of modern transplant medicine: The need for individualized immunosuppressive strategies in LT, and particularly for hyperimmunized recipients who have long been underserved by a one-size-fits-all approach. Prospective, multicenter randomized controlled trials are urgently warranted to validate the efficacy and safety of the rATLG/IVIG induction regimen in larger, more diverse cohorts of hyperimmunized liver transplant recipients. These trials should incorporate pre-transplant stratification of patients based on DSA specificity, class, and titer (including complement-binding status *via* C1q/C3d assays) to define optimal patient selection criteria-ensuring that the regimen is targeted to those who will derive the greatest clinical benefit.

To further refine risk stratification and improve long-term outcomes, future clinical practice should integrate routine post-transplant DSA surveillance (at 1, 3, 6, and 12 months post-transplant, and annually thereafter) with protocol liver biopsies in hyperimmunized recipients. This integrated approach will enable early detection of subclinical AMR and antibody-mediated graft injury, allowing for timely, targeted interventions (*e.g.*, IVIG, plasma exchange, or B-cell depletion) before irreversible graft damage occurs-a critical step in preserving long-term graft function.

Advances in immunological profiling-including high-dimensional flow cytometry to characterize T-cell/B-cell subsets, immune cell activation status, and cytokine profiles-also open the door to tailoring induction regimens to the distinct immunological phenotypes of individual hyperimmunized recipients. For example, recipients with high levels of circulating memory B cells and plasma cells may benefit from the addition of B-cell depletion therapy (*e.g.*, rituximab) to the rATLG/IVIG backbone, while those with predominant T-cell sensitization may thrive with the standard regimen alone. This precision immunology approach will allow clinicians to balance rejection prevention against the inherent risks of immunosuppression (infection, malignancy, cardiovascular disease)-a delicate rebalancing act that is the core of optimal transplant care[7,8].

Notably, future research should prioritize the generation of direct head-to-head comparative data between rATLG and thymoglobulin in LT, to formally validate the safety and efficacy advantages of rATLG suggested by solid organ transplantation data. This research will fill a key evidence gap and provide clinicians with clear, evidence-based guidance for induction agent selection in hyperimmunized recipients.

CONCLUSION

EL-Domiaty *et al*'s study[9] delivers a landmark contribution to the field of LT, providing robust clinical evidence that a short-course induction regimen combining rATLG and high-dose IVIG can effectively mitigate immunological risk in hyperimmunized liver transplant recipients. By achieving rejection rates and graft/patient survival outcomes comparable to those of non-immunized patients-without excessive toxicity or an increased risk of severe infection or malignancy-this strategy represents a meaningful step forward in the shift from universal to personalized immunosuppression in LT.

This work addresses a long-standing clinical unmet need, offering clinicians a pragmatic, evidence-based approach to managing one of the most challenging subsets of liver transplant candidates. Continued investigation through larger, prospective, multicenter studies-incorporating immunological phenotyping, routine DSA surveillance, and protocol biopsies-will be essential to confirm these findings, refine the regimen, and optimize long-term care for hyperimmunized liver transplant recipients. Ultimately, this work paves the way for a more precise, patient-centered approach to immunosuppression in LT, where immunological risk is balanced with graft protection to achieve the best possible outcomes for every recipient.

REFERENCES

1 **Kim WR**, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2015 Annual Data Report: Liver. *Am J Transplant* 2017; **17** Suppl 1: 174-251 [*RCA*] [PMID: 28052604 DOI: 10.1111/ajt.14126] [Full Text]

2 **Lee BT**, Fiel MI, Schiano TD. Antibody-mediated rejection of the liver allograft: An update and a clinico-pathological perspective. *J Hepatol* 2021; **75**: 1203-1216 [*RCA*] [PMID: 34343613 DOI: 10.1016/j.jhep.2021.07.027] [FullText]

3 **Levitsky J**, Goldberg D, Smith AR, Mansfield SA, Gillespie BW, Merion RM, Lok AS, Levy G, Kulik L, Abecassis M, Shaked A. Acute Rejection Increases Risk of Graft Failure and Death in Recent Liver Transplant Recipients. *Clin Gastroenterol Hepatol* 2017; **15**: 584-593.e2 [*RCA*] [PMID: 27567694 DOI: 10.1016/j.cgh.2016.07.035] [FullText] [Full Text(PDF)]

4 **McCaughan JA**, Robertson V, Falconer SJ, Cryer C, Turner DM, Oniscu GC. Preformed donor-specific HLA antibodies are associated with increased risk of early mortality after liver transplantation. *Clin Transplant* 2016; **30**: 1538-1544 [*RCA*] [PMID: 27653177 DOI: 10.1111/ctr.12851] [FullText]

5 **Ruiz R**, Tomiyama K, Campsen J, Goldstein RM, Levy MF, McKenna GJ, Onaca N, Susskind B, Tillery GW, Klintmalm GB. Implications of a positive crossmatch in liver transplantation: a 20-year review. *Liver Transpl* 2012; **18**: 455-460 [*RCA*] [PMID: 22139972 DOI: 10.1002/lt.22474] [FullText]

6 **Del Bello A**, Congy-Jolivet N, Muscari F, Lavayssière L, Esposito L, Cardeau-Desangles I, Guitard J, Dörr G, Suc B, Duffas JP, Alric L, Bureau C, Danjoux M, Guilbeau-Frugier C, Blancher A, Rostaing L, Kamar N. Prevalence, incidence and risk factors for donor-specific anti-HLA antibodies in maintenance liver transplant patients. *Am J Transplant* 2014; **14**: 867-875 [*RCA*] [PMID: 24580771 DOI: 10.1111/ajt.12651] [FullText]

7 **Cillo U**, Bechstein WO, Berlakovich G, Dutkowski P, Lehner F, Nadalin S, Saliba F, Schlitt HJ, Pratschke J. Identifying risk profiles in liver transplant candidates and implications for induction immunosuppression. *Transplant Rev (Orlando)* 2018; **32**: 142-150 [*RCA*] [PMID: 29709248 DOI: 10.1016/j.trre.2018.04.001] [FullText]

8 **Charlton M**, Levitsky J, Aqel B, O'Grady J, Hemibach J, Rinella M, Fung J, Ghabril M, Thomason R, Burra P, Little EC, Berenguer M, Shaked A, Trotter J, Roberts J, Rodriguez-Davalos M, Rela M, Pomfret E, Heyrend C, Gallegos-Orozco J, Saliba F. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. *Transplantation* 2018; **102**: 727-743 [*RCA*] [PMID: 29485508 DOI: 10.1097/TP.0000000000002147] [FullText]

9 **El-Domiaty N**, Coilly A, Sebagh M, Taupin J, Ibrahim W, Sacleux S, Ichai P, Duhaut L, Pittau G, Ciacio O, Salloum C, Sa-cunha A, Azoulay D, Feray C, Cherqui D, Shiha G, Samuel D, Saliba F. Induction with anti-T-lymphocyte globulins and human immunoglobulins: A strategy for hyperimmunized liver transplant patients. *World J Hepatol* 2026; **18**: 115221 [*RCA*] [DOI: 10.4254/wjh.v18.i3.115221] [FullText] [Full Text (PDF)]

10 **Demetris AJ**, Bellamy C, Hübscher SG, O'Leary J, Randhawa PS, Feng S, Neil D, Colvin RB, McCaughan G, Fung JJ, Del Bello A, Reinholt FP, Haga H, Adeyi O, Czaja AJ, Schiano T, Fiel MI, Smith ML, Sebagh M, Tanigawa RY, Yilmaz F, Alexander G, Baiocchi L, Balasubramanian M, Batal I, Bhan AK, Bucuvalas J, Cerski CTS, Charlotte F, de Vera ME, ElMonayeri M, Fontes P, Furth EE, Gouw ASH, Hafezi-Bakhtiari S, Hart J, Honsova E, Ismail W, Itoh T, Jhala NC, Khettry U, Klintmalm GB, Knechtle S, Koshiba T, Kozlowski T, Lassman CR, Lerut J, Levitsky J, Licini L, Liotta R, Mazariegos G, Minervini MI, Misdraji J, Mohanakumar T, Mölne J, Nasser I, Neuberger J, O'Neil M, Pappo O, Petrovic L, Ruiz P, Sağol Ö, Sanchez Fueyo A, Sasatomi E, Shaked A, Shiller M, Shimizu T, Sis B, Sonzogni A, Stevenson HL, Thung SN, Tisone G, Tsamandas AC, Wernerson A, Wu T, Zeevi A, Zen Y. 2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection. *Am J Transplant* 2016; **16**: 2816-2835 [*RCA*] [PMID: 27273869 DOI: 10.1111/ajt.13909] [FullText]

11 **Taner T**, Gandhi MJ, Sanderson SO, Poterucha CR, De Goey SR, Stegall MD, Heimbach JK. Prevalence, course and impact of HLA donor-specific antibodies in liver transplantation in the first year. *Am J Transplant* 2012; **12**: 1504-1510 [*RCA*] [PMID: 22420671 DOI: 10.1111/j.1600-6143.2012.03995.x] [FullText]

12 **Kubal CA**, Mangus RS, Saxena R, Lobashevsky A, Higgins N, Agarwal A, Fridell JA, Tector AJ. Crossmatch-positive liver transplantation in

patients receiving thymoglobulin-rituximab induction. *Transplantation* 2014; **97**: 56-63 [*RCA*] [PMID: 24030603 DOI: 10.1097/TP.0b013e3182a688c0] [FullText]

13 **Del Bello A**, Neau-Cransac M, Lavayssiere L, Dubois V, Congy-Jolivet N, Visentin J, Danjoux M, Le Bail B, Hervieu V, Boillot O, Antonini T, Kamar N, Dumortier J. Outcome of Liver Transplant Patients With Preformed Donor-Specific Anti-Human Leukocyte Antigen Antibodies. *Liver Transpl* 2020; **26**: 256-267 [*RCA*] [PMID: 31612580 DOI: 10.1002/lt.25663] [FullText]

14 **O'Leary JG**, Kaneku H, Jennings LW, Bañuelos N, Susskind BM, Terasaki PI, Klintmalm GB. Preformed class II donor-specific antibodies are associated with an increased risk of early rejection after liver transplantation. *Liver Transpl* 2013; **19**: 973-980 [*RCA*] [PMID: 23780820 DOI: 10.1002/lt.23687] [FullText]

15 **Dumortier J**, Conti F, Hiriart JB, Dharancy S, Duvoux C, Besch C, Houssel-Debry P, Latournerie M, Chermak F, Meszaros M, Pageaux GP, Radenne S, Boillot O, Hardwigsen J, Kounis I, Kamar N, Saliba F, Erard D, Del Bello A. Treatment of donor-specific anti-HLA antibodies-mediated rejection after liver transplantation: A French nationwide retrospective study. *Liver Transpl* 2023; **29**: 1313-1322 [*RCA*] [PMID: 37367954 DOI: 10.1097/LVT.0000000000000200] [FullText]

16 **Wiesner RH**, Demetris AJ, Belle SH, Seaberg EC, Lake JR, Zetterman RK, Everhart J, Detre KM. Acute hepatic allograft rejection: incidence, risk factors, and impact on outcome. *Hepatology* 1998; **28**: 638-645 [*RCA*] [PMID: 9731552 DOI: 10.1002/hep.510280306] [FullText]

17 **Lunz J**, Ruppert KM, Cajaiba MM, Isse K, Bentlejewski CA, Minervini M, Nalesnik MA, Randhawa P, Rubin E, Sasatomi E, de Vera ME, Fontes P, Humar A, Zeevi A, Demetris AJ. Re-examination of the lymphocytotoxic crossmatch in liver transplantation: can C4d stains help in monitoring? *Am J Transplant* 2012; **12**: 171-182 [*RCA*] [PMID: 21992553 DOI: 10.1111/j.1600-6143.2011.03786.x] [FullText]

18 **Gharekhani A**, Entezari-Maleki T, Dashti-Khavidaki S, Khalili H. A review on comparing two commonly used rabbit anti-thymocyte globulins as induction therapy in solid organ transplantation. *Expert Opin Biol Ther* 2013; **13**: 1299-1313 [*RCA*] [PMID: 23875884 DOI: 10.1517/14712598.2013.822064] [FullText]

19 **Ducloux D**, Kazory A, Challier B, Coutet J, Bresson-Vautrin C, Motte G, Thalamy B, Rebibou JM, Chalopin JM. Long-term toxicity of antithymocyte globulin induction may vary with choice of agent: a single-center retrospective study. *Transplantation* 2004; **77**: 1029-1033 [*RCA*] [PMID: 15087766 DOI: 10.1097/01.tp.0000116442.81259.60] [FullText]

20 **Bamoulid J**, Crepin T, Gaiffe E, Laheurte C, Moulin B, Frimat L, Rieu P, Mousson C, Durrbach A, Heng AE, Rebibou JM, Saas P, Courivaud C, Ducloux D. Immune reconstitution with two different rabbit polyclonal anti-thymocytes globulins. *Transpl Immunol* 2017; **45**: 48-52 [*RCA*] [PMID: 28941751 DOI: 10.1016/j.trim.2017.09.002] [FullText]

21 **Ray DS**, Gang S, Khullar D, Bhalla AK, Sharma A, Abraham A, Raju SB, Siddhi V, Dutta AR, Abraham G, Shekar MS, Shah BV, Kothari J, Bahadur M, Ahmed I, Narula AS, Margabandhu S, Shetty S, Chodankar D, Mandrekar A, Salvi VS. Rabbit Anti-thymocyte Globulin (rATG) as Induction Immunosuppression Therapy in Patients Undergoing Renal Transplantation: Clinical Experience from RISE Registry. *Indian J Transplant* 2023; **17**: 294-300 [*RCA*] [DOI: 10.4103/ijot.ijot_81_22] [FullText]

22 **Brennan DC**, Daller JA, Lake KD, Cibrik D, Del Castillo D; Thymoglobulin Induction Study Group. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. *N Engl J Med* 2006; **355**: 1967-1977 [*RCA*] [PMID: 17093248 DOI: 10.1056/NEJMoa060068] [FullText]

23 **Mourad G**, Rostaing L, Legendre C, Garrigue V, Thervet E, Durand D. Sequential protocols using basiliximab versus antithymocyte globulins in renal-transplant patients receiving mycophenolate mofetil and steroids. *Transplantation* 2004; **78**: 584-590 [*RCA*] [PMID: 15446319 DOI: 10.1097/01.tp.0000129812.68794.cc] [FullText]

24 **Höfer A**, Jonigk D, Hartleben B, Verboom M, Hallensleben M, Manns MP, Jaeckel E, Taubert R. Non-invasive screening for subclinical liver graft injury in adults *via* donor-specific anti-HLA antibodies. *Sci Rep* 2020; **10**: 14242 [*RCA*] [PMID: 32859929 DOI: 10.1038/s41598-020-70938-7] [FullText] [Full Text(PDF)]

25 **Vandevoorde K**, Ducreux S, Bosch A, Guillaud O, Hervieu V, Chambon-Augoyard C, Poinsot D, André P, Scoazec JY, Robinson P, Boillot O, Dubois V, Dumortier J. Prevalence, Risk Factors, and Impact of Donor-Specific Alloantibodies After Adult Liver Transplantation. *Liver Transpl* 2018; **24**: 1091-1100 [*RCA*] [PMID: 29665189 DOI: 10.1002/lt.25177] [FullText]

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