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EDITORIAL

Editorial: Metabolomics in chronic hepatitis C: Decoding fibrosis grading and underlying pathways

Jorge Quarleri, M Victoria Delpino

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Abstract

In the management of the growing population of hepatitis C virus-infected patients, a significant clinical challenge exists in determining the most effective methods for assessing liver impairment. The prognosis and treatment of chronic hepatitis C depend, in part, on the evaluation of histological activity, specifically cell necrosis and inflammation, and the extent of liver fibrosis. These parameters are traditionally obtained through a liver biopsy. However, liver biopsy presents both invasiveness and potential sampling errors, primarily due to inadequate biopsy size. To circumvent these issues, several non-invasive markers have been proposed as alternatives for diagnosing liver damage. Different imaging techniques and blood parameters as single markers or combined with clinical information are included. This Editorial discusses the identification of a set of six distinctive lipid metabolites in every fibrosis grade that appear to show a pronounced propensity to create clusters among patients who share the same fibrosis grade, thereby demonstrating enhanced efficacy in distinguishing between the different grades.

Key Words: Hepatitis C virus; Chronic hepatitis C; Liver fibrosis; Biomarker; Liquid biopsy

Core Tip: Accurate diagnosis of liver damage in chronic hepatitis C is pivotal for decision-making. Liver biopsy, the traditional "gold standard" for assessing tissue damage, offers valuable insights but is invasive, with potential complications and sampling errors. Non-invasive methods have made progress in the last decade, but challenges remain. Various non-invasive techniques are in development, including serum biomarker assays and advanced imaging. They often struggle to distinguish intermediate fibrosis stages and are affected by hepatic and extrahepatic factors. This Editorial discusses which identified potential biomarkers in plasma samples linked to each fibrosis grade and hepatitis C virus-induced pathogenesis.

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INTRODUCTION

The natural evolution of chronic hepatitis C (CHC) involves a continuous inflammatory response triggered by recurring liver injuries. This is subsequently accompanied by the activation of hepatic stellate cells, the accumulation of fibrillar collagen within the extracellular matrix (ECM), and the gradual development of fibrosis. These sequential events can potentially lead to ECM degradation, which in turn may result in vascular and architectural modifications, ultimately culminating in the occurrence of cirrhosis or hepatocellular carcinoma (HCC)[1].

Timely diagnosis and intervention are pivotal in preventing the progression to liver cirrhosis and HCC, particularly in light of the advent of direct-acting antiviral therapy, which has revolutionized the treatment of CHC. Nevertheless, effectively reducing the morbidity and mortality associated with this condition necessitates a more comprehensive understanding of liver involvement, improved prognostication, and rigorous monitoring[2]. In this context, accurate determination of the degree of liver fibrosis assumes paramount significance in the clinical management of HCC, as it not only informs treatment decisions but also aids in predicting patient outcomes. However, this endeavor is fraught with challenges, as the methods employed for fibrosis staging encompass both histological assessment through liver biopsies and various imaging modalities. The Metavir classification system, which employs a 0-4 scale, is commonly utilized for staging the various grades of fibrosis in biopsied liver tissue[3]. While liver biopsy remains the acknowledged "gold standard" for diagnosing and staging liver fibrosis, its invasiveness and associated discomfort, coupled with the risk of complications, subject to sampling errors and subjectivity between observers make it a less-than-ideal option[4-6].

Conventional imaging modalities (ultrasonography, computed tomography, and magnetic resonance imaging) are valuable but their sensitivity is limited when it comes to detecting moderate or advanced fibrosis[7]. Besides, advanced acoustic technologies (hepatic elastography) enhance the precision of imaging approaches but the cost of the equipment is a limitation, among others[8].

In the present issue of the JWH, the Ferrasi *et al*[9] study aims to investigate the plasma metabolome using mass spectrometry on samples obtained from individuals with CHC and varying degrees of fibrosis with the goal of identifying prospective biomarkers for categorizing these fibrotic conditions.

The potential clinical utility of these markers presents a compelling avenue for not only staging liver fibrosis but also evaluating the rate and progression of liver fibrogenesis. This assessment, in turn, translates into valuable prognostic insights and serves as a tool for assessing treatment response and monitoring the effectiveness of antifibrotic medications. Nevertheless, the available data regarding their performance in defining the stage of liver fibrosis is variable, and their routine availability may be limited in certain hospital settings[10]. These markers encompass various glycoproteins (such as hyaluronan and laminin), members of the collagen family (including procollagen III, type IV collagen, and type IV collagen 7s domain), collagenases and their inhibitors (metalloproteinases and tissue inhibitors of metalloproteinases), along with numerous cytokines implicated in the fibrogenic process, notably transforming growth factor-β1. These markers have been individually and collectively assessed to gauge the severity and progression of hepatic fibrosis and to monitor changes associated with viral treatment[2] or, even HCC[11-18].

The metabolome comprises the entirety of metabolites that are internally generated within a particular physiological state and can be considered as the ultimate outcome of gene expression. This approach enhances the biomarker identification in human plasma as an invaluable tool in clinical practice and research. They facilitate early detection, accurate diagnosis, personalized treatment, and improved patient outcomes, ultimately contributing to more effective healthcare and better public health.

For every stage of fibrosis, the researchers identified a distinct metabolite profile, and the significance of each molecule varies based on the fibrosis stage, potentially intensifying or diminishing over the course of the disease. Hence, the employment of metabolomics techniques in liquid biopsies exhibits potential as diagnostic, prognostic, and therapeutic monitoring tools.

The pro-viral implications of lipid metabolic reprogramming during virus infection encompass four distinct functions. Firstly, lipids play crucial roles in virus entry and trafficking, serving as attachment factors, internalization receptors, or transportation shuttles during the initial stages of viral entry. Secondly, lipids contribute to virus replication and assembly by providing subcellular spaces essential for key events in the viral life cycle. Thirdly, lipids are indispensable for the generation of energy and essential nutrients required for viral replication. Lastly, lipids serve as pivotal

components in viral envelopment and fulfill diverse functions in the process of virus egress[19]. The study from Ferrasi *et al*[9] analyzes the link between hepatitis C virus (HCV)-induced lipid metabolism abnormalities with the fibrosis grade score, at first with an emphasis on those involved in cholesterol biosynthesis[9].

In the case of grade F1, certain biomarkers that appeared to be more associated with HCV infection rather than fibrosis progression were noticed when compared to individuals with more advanced fibrosis stages. Consequently, the initial molecule detected in grade F1 belonged to the sterol category, featuring distinct characteristics related to cholesterol ester, already recognized as a critical component of HCV lipoviral particles[20]. Furthermore, a diacylglycerol was also identified in grade F1, and its elevated levels were associated with a less advanced state of fibrosis, specifically.

When considering lipid metabolism and the accrual of lipids, it became feasible to pinpoint the presence of the sphingolipid class in the intermediate-grade F2, specifically represented by ceramide. Their accumulation potentially leads to steatosis, which, in turn, may contribute to the progression of liver fibrosis[21]. Furthermore, the authors identified in F2 grade a molecule from the eicosanoid class. This particular molecule is a bioactive lipid that serves as a potent mediator of inflammation in infectious diseases and HCC.

In the case of intermediate-grade F3 and advanced-grade F4, another lipid class (glycerophospholipids) was identified, with the specific biomarkers recognized as phosphoethanolamines. Besides, in F3 grade samples the authors identified the farnesylcysteine, a prenol lipid, as a plausible biomarker for assessing the risk of tumor development, that was previously linked to liver carcinogenesis.

As mentioned above, several studies indicate the potential disruption of fatty acid lipid metabolic pathways during HCV infection. This disruption leads to the accumulation of acyl-coenzyme A (CoA) and intermediary products in fatty acid metabolism belonging to the CoA class. Among them, the authors identified the cis, cis-3,6-dodecadienoyl-CoA among those patients exhibiting F1 grade, while in those with F3 the S-2-octenoyl CoA was found.

Finally, in the advanced grade (F4), a metabolite associated with CoA was detected, along with malonyl carnitine. The presence of malonyl carnitine is noteworthy as it is intricately tied to disease progression and the development of HCC, primarily due to the dysregulation of energy-supplying metabolic pathways.

In addition to the lipid-based biomarkers, the authors identified other plausible markers such as polypeptide angiotensin III [Ang III, also called Ang-(2-8), is generated from Ang II by aminopeptidase A] in grade F1 which may augment collagen production, methyladenosine in F2 and (S)-2,3,4,5-tetrahydropiperidine-2-carboxylate in F3 grade.

CONCLUSION

In conclusion, despite a limited number of samples, Ferrasi *et al*[9] analysis found potential biomarkers specific to each grade of liver fibrosis. These biomarkers showed a propensity to group patients with similar fibrosis grades, although there were instances of overlap such as those proposed for grades F2 and F3. The score plot analysis showed greater efficiency in discriminating between the extreme grades (F1 and F4). This study represents an advancement in the quest for non-invasive serum markers that reflect the progression of liver damage.

FOOTNOTES

Author contributions: Quarleri J and Delpino MV contributed equally to this work. Both authors have read and approved the final manuscript.

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REVIEW

Budd-Chiari syndrome in children: Challenges and outcome

Arghya Samanta, Moinak Sen Sarma, Rajanikant Yadav

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Abstract

Budd-Chiari syndrome (BCS) is an uncommon disease of the liver, characterised by obstruction of the hepatic venous outflow tract. The etiological spectrum of BCS as well as venous obstruction pattern show wide geographical and demographic variations across the globe. Compared to adults with BCS, children have primary BCS as the predominant etiology, earlier clinical presentation, and hence better treatment outcome. Underlying prothrombotic conditions play a key role in the etiopathogenesis of BCS, though work-up for the same is often unyielding in children. Use of next-generation sequencing in addition to conventional tests for thrombophilia leads to better diagnostic yield. In recent years, advances in radiological endovascular intervention techniques have revolutionized the treatment and outcome of BCS. Various non-invasive markers of fibrosis like liver and splenic stiffness measurement are being increasingly used to assess treatment response. Elastography techniques provide a novel non-invasive tool for measuring liver and splenic stiffness. This article reviews the diagnostic and therapeutic advances and challenges in children with BCS.

Key Words: Budd-Chiari syndrome; Radiological endovascular intervention; Transjugular intrahepatic porto-systemic shunt; Direct intrahepatic porto-systemic shunt; Liver stiffness; Splenic stiffness; Shear-wave elastography

Core Tip: Budd-Chiari syndrome (BCS) is a rare disease of the liver, characterised by obstruction of hepatic venous outflow tract. The effectiveness of radiological endovascular interventions in alleviating clinical symptoms as well as hepatic congestion has been shown both in adults and children. However, unlike in adults, established treatment guidelines have not been developed in children with BCS. Long-term follow-up studies including the prevalence of hepatopulmonary syndrome and hepatocellular carcinoma in this patient population are lacking in children. The role of liver and splenic stiffness measurement by elastography techniques is poorly studied in patients with BCS.

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INTRODUCTION

Budd-Chiari syndrome (BCS) is a rare vascular disease of the liver with a promising outcome if treated optimally on time. It occurs due to obstruction in the hepatic venous outflow anywhere from the hepatic vein (HV) to the entry of the inferior vena cava (IVC) to the heart[1]. Involvement of at least two HV leads to an increase in hepatic sinusoidal pressure and congestion resulting in symptoms of BCS[2]. Global epidemiological data on BCS is scarce[3]. The incidence of BCS reported in the published literature ranges from 0.2 to 4.1 cases per million population per year, with an estimated prevalence of 2.4–7.7 per million population in Asian countries[4,5] and of 1.4–4.0 per million population in Western countries[6-9]. Population-based study on the epidemiology of BCS in children is lacking. Large case series on pediatric BCS are available[10-15]. Chronic BCS constitutes 3%-7% of cases of portal hypertension (PHTN) in the pediatric population[10,11]. So far, interventional and long-term outcome studies in children are limited[12-15]. Over the years there has been remarkable progress in understanding the evolution of this disease. This review aims to discuss the various aspects of BCS, including recent updates on diagnostic and treatment modalities for BCS in children as well as the challenges in them. The review will particularly focus on chronic BCS which accounts for the majority of cases in children.

VARIATIONS IN THE ETIOLOGICAL SPECTRUM OF BCS

Primary BCS is an obstruction of the hepatic venous outflow tract that occurs due to an endoluminal venous lesion (thrombosis or web) resulting from an unidentifiable cause or an inherent prothrombotic condition. Secondary BCS results from obstruction from an invasive lesion (malignant tumor or a parasitic mass) or extrinsic compression by space-occupying lesions (abscesses, cysts, and benign or malignant solid tumors)[1]. The above inflammatory or neoplastic conditions can also result in a secondary prothrombotic state which further adds to thrombosis in the HV. Secondary causes of BCS are common in adults as compared to children[16]. Global variations in the type of BCS may reflect different predisposing factors in different countries. Studies from Western countries have shown HV to be the most common site of obstruction in BCS[17,18]. There seems to be a shift in the pattern of obstruction in studies from Asian countries. Earlier, isolated IVC obstruction was the most type of BCS in Asian patients. Recent studies from China[19,20] as well as India[16,21,22] have noted that a combined IVC and HV obstruction (40%-75%) is the most common type of BCS. A similar pattern has also been documented in Indian children with BCS[12-15]. Over the years, the changes in the spectrum have been better documented due to the advancements in radiology such as high-resolution Doppler ultrasonography (DUS) and non-invasive venography by computed tomography (CT), and magnetic resonance imaging.

BCS AND THROMBOPHILIA

Primary BCS is often regarded as a result of a unique constellation of prothrombotic conditions. The inherited prothrombotic conditions associated with BCS are protein C deficiency, protein S deficiency, antithrombin III deficiency, factor V Leiden mutation, prothrombin gene mutation, and hyper-homocysteinemia with methyltetrahydrofolate reductase mutation[23]. Acquired prothrombotic conditions like antiphospholipid syndrome (APLS), paroxysmal nocturnal hemoglobinuria (PNH), sickle cell disease, and myeloproliferative disorders (MPD) also predispose to BCS[24,25]. Systemic disorders like inflammatory bowel disease, Behcet's disease, and other inflammatory intraabdominal lesions can cause BCS in adults but rarely in children[26]. Recent studies reported an identifiable etiology in 80-84% of cases in adults[27-30]. Underlying myeloproliferative disorders (45%–51%) and exposure to oral contraceptives (50%) are the commonest etiologies[27,28]. In over 25% of cases, more than one thrombophilic state may be present[29,30]. From the available pediatric literature, we understand that the prothrombotic workup in children is often unyielding, inconclusive, or ambiguous. Table 1 summarizes the findings of prothrombotic workup of prior pediatric studies on BCS[11-14,31-35]. As shown, most of the pediatric publications are from India including authors' own experience. One of the major

Table 1 Summary of various pediatric studies (national) of thrombophilia profile in children with Budd-Chiari syndrome

Ref.	Country	No. of cases	Age group	Disease under evaluation	Proportion of cases with prothrombotic work-up	Yield of prothrombotic work-up, *n* (%)	Conditions detected
Nagral *et al*[12]	India	16	4 yr (2-11 yr)	Primary BCS	15/16	4/15 (27)	Protein C deficiency 2; APLA syndrome 1; Antithrombin III deficiency 1
Kathuria *et al*[13]	India	45	10 yr (2-16 yr)	Primary BCS	12/45 children	8/12 (67)	PNH 1; APLA 4; Protein C deficiency 5; Protein S deficiency 3; Hyperhomocystinemia 2 (60% multiple prothrombotic conditions)
Sharma *et al*[14]	India	32	9 yr (5-15.5 yr)	Primary BCS	Not available		
Alam *et al* [11]	India	13	9 yr (5-13.5 yr)	Primary BCS	13/13	10/13 (77)	MTHFR mutation 5; Factor V mutation 1; Celiac disease 1
Malik *et al* [31]	India	11	10 yr (3-16 yr)	Primary BCS	11/11	4/11 (37)	APLA 2; PNH 1; JAK2 1
Shukla *et al*[32]	India	36	1-10 yr	Primary BCS	36/36	15/36 (41.7)	JAK 2V617F 0; Protein C deficiency 2; Protein S deficiency 2; Antithrombin III deficiency 3; APLA 4; Factor V Leiden 4; High homocysteine level 0; Multiple conditions 0
Shukla *et al*[32]	India	43	10-19 yr	Primary BCS	43/43	16/43 (37)	JAK 2V617F 5; Protein C deficiency 1; Protein S deficiency 1; Antithrombin III deficiency 3; APLA 4; Factor V Leiden 1; High homocysteine level 4; Multiple conditions 4
Zhou *et al* [33]	China	35	22 yr (10-25 yr)	Primary BCS	35/35	22/35 (63)	Hyperhomocyteinemia14; APLA 5; JAK2 V617F 1; IBD 1; Behcet disease 1
Dobre *et al*[34]	England	7	7 yr (4-14 yr)	Primary BCS	7/7	7/7 (100)	JAK2 V617F 2; Protein C deficiency 3; Antithrombin III deficiency 2; PNH 1; Factor V Leiden 1; APLA syndrome 2
Revil-Vilk *et al*[35]	Canada	171 BCS -31 cases	3.5 yr (2-13 yr)	Venous thrombosis of different sites	171/171	23/171 (13.4)	Factor V Leiden 8; Prothrombin gene mutation 4; Protein S deficiency 2; Protein S deficiency 1; High lipoprotein a 8

PNH: Paroxysmal nocturnal haemoglobinuria; APLA: Anti phospholipid antibody; IBD: Inflammatory bowel disease; JAK: Janus kinase; MTHFR: Methyl tetrahydro folate reductase.

drawbacks is the lack of in-depth testing for prothrombotic conditions. Shukla *et al*[32] found thrombophilia in 42% of children and 37% of adolescents. The commonest etiologies were Factor V Leiden in children and JAK 2V617F mutation in adolescents. Although thrombophilia may be present in 68%-75% of children, the cause-and-effect relationship is not established[12,32]. Relative and multiple deficiencies of proteins C and S, antithrombin III deficiency, and hyperhomocysteinemia (9%-15%) may be a result of an advanced liver disease resulting in poor synthetic function of the liver rather than a true thrombophilia state[29]. In a systematic review of adults with BCS, MPD was found to be more associated with HV block (16%-62%) than IVC block (4%-5%)[36].

Oral anticoagulants may alter proteins C and S, activated protein C levels, and lupus anticoagulant levels. Hence these tests are best performed before starting thromboprophylaxis. Further, the presence of hypersplenism and hemodilution in cirrhosis with portal hypertension masks the peripheral blood findings of a concomitant MPD[29]. Genetic testing is the gold standard for documentation of thrombophilia. In a study of 80 adults with non-cirrhotic splanchnic vein thrombosis, next-generation sequencing was able to identify JAK2 mutations, previously undetected by conventional techniques in one-third of cases[37].

It is also suggested that the prevalence of various thrombophilic disorders is different between younger children and adolescents. In a study from India, the JAK2V617F was found to be common in adolescents but not in children[32]. Recently it has been shown that adolescent venous thromboembolism is multifactorial in the majority with more than two risk factors at diagnosis[38]. Warfarin is the most commonly used oral anticoagulant in BCS patients, especially after radiological intervention. As it has a narrow therapeutic window, the dose needs to be titrated with regular monitoring of international normalized ratio (INR) (target: 2-3). Vitamin K epoxide reductase complex subunit C1 (VKORC1) and cytochrome P450 2C9 (CYP2C9) are the two major genes involved in the metabolism of warfarin and they determine the dose requirement of warfarin[39]. CYP2C9 metabolizes the more potent S-enantiomer of warfarin while VKORC1 is the target protein for warfarin. Multiple single nucleotide polymorphisms have been described in these genes, of which the most important ones are CYP2C9*2, CYP2C9*3, and VKORC1 heterozygous haplotype GA and homozygous AA[40]. Thus, the patient's genotype is a major determinant of not only the dose requirement but also the risk of anticoagulation-

related complications. In a study from India in adults with BCS, patients with the presence of mutations in VKORC1 or CYP2C9 were associated with an increased risk of bleeding[41]. More intensive monitoring while on warfarin is recommended for these patients. Some guidelines have now started recommending incorporating the results of genetic testing for clinical use while on warfarin therapy[42].

Identification of thrombophilia in a child has implications in terms of the need for lifelong oral anticoagulation, increased risk of other venous thromboembolic events, lifestyle modifications like avoiding oral contraceptive pills, risk of complications like leukemia in myeloproliferative disorders, and implications for other family members. In addition, there is a need to educate asymptomatic family members regarding the risk factors and lifestyle modifications.

CLINICAL FEATURES

Clinical manifestations can be diverse, ranging from acute liver failure to completely asymptomatic patients, making BCS a possible differential diagnosis in many acute and chronic liver diseases. Most patients with BCS have a chronic presentation, whereas only a small number of patients present with a fulminant type of BCS[12-14]. The clinical and radiological features of children with BCS in various pediatric studies are summarized in Table 2. The usual age at presentation in children is 10 (range 1.5–17) years but BCS has been reported in children as young as 4.5 mo[11,12,32]. The commonest symptom is rapidly reaccumulating ascites (83%–90%) with dilated tortuous abdominal and back veins (60%-70%)[11-14,31]. Up to 15%-20% of patients can be completely asymptomatic, hence a high index of suspicion should be kept, especially when there is no clear detectable etiology for chronic liver disease and/or a prothrombotic condition exists[43]. In such scenarios, proper imaging by an experienced radiologist is warranted[44]. The presentation of BCS depends on the extent and rapidity of hepatic venous outflow obstruction and the development of decompressing venous collaterals. With this concept, BCS can be classified as fulminant, acute, subacute, or chronic[45,46]. However, a pathological examination of the liver illustrates a dissociation between the rapidity of the clinical presentation and the acuteness of the histological damage. Up to 50% of patients clinically classified as acute have histological features of chronicity (*e.g.*, fibrosis or cirrhosis)[46]. The prognostic value of this clinical classification in predicting mortality has not been prospectively evaluated[47]. Over time, several prognostic indices have been designed to predict mortality and response to therapeutic interventions[15,18,46,47]. These scoring systems incorporate clinical and laboratory features to stratify patients, although their use for the management of an individual patient is debatable[48].

DIFFERENCE BETWEEN ADULTS AND CHILDREN WITH BCS

BCS can occur at any age group but it most commonly affects young adults. In adults, secondary causes of BCS are much more frequently seen than children in whom primary causes are predominant. Regarding the underlying etiology, thrombophilic conditions are reported in more than 80% of adults with BCS. In Asian children, the etiology is largely idiopathic. Thrombophilia work-up is often under-reported and the results are variable. Acquired prothrombotic conditions like MPD, APLS, PNH, sickle cell disease, and oral contraceptive pill use are predominantly seen in adults. On the contrary, inherited thrombophilias like protein C deficiency and protein S deficiency are more commonly found in children. The clinical presentation is also different for adults and children. Shukla *et al*[32] compared 43 children and 129 adult patients with BCS. They found hepatomegaly without ascites as the most common presentation in children as compared to ascites being the most common presentation in adults. The authors hypothesized that children have better angiogenesis and collateral formation, and shorter disease duration, leading to milder clinical presentation and better treatment outcome.

DIAGNOSTIC CHALLENGES

The diagnosis of BCS depends on the demonstration of HV and/or IVC obstruction. Invasive venography remains the gold standard; however, it is performed during the time of endovascular intervention procedure. DUS has therefore emerged as the primary imaging modality with a diagnostic accuracy of > 90%[12,13,15]. DUS evaluates hepatic, portal, and IVC patency, site, and length of block and liver parenchymal changes (including caudate lobe hypertrophy and intrahepatic comma-shaped collaterals). HVs may be engorged, irregular, or filled with thrombus. The "health" and residual stump of the HV are the most critical to plan an endovascular intervention. Triphasic flow may be dampened or reversed. The IVC may be narrowed or displaced by caudate hypertrophy or contain an intraluminal thrombus. Intrahepatic comma-shaped collaterals and caudate lobe hypertrophy are almost universally seen in patients with BCS. The presence of dense intrahepatic collaterals suggests chronicity. Subcapsular collaterals may bleed during a percutaneous intervention and need to be carefully documented. Non-invasive venography (CT or MRI) is required when there is a diagnostic ambiguity. Hypoenhancement of the peripheral hepatic parenchyma and relatively normal enhancement of the caudate lobe result in a mottled appearance on CT. These changes are more obvious on MRI[49]. However, if they are unyielding, invasive venography and liver biopsy should be considered.

Another challenging diagnostic dilemma in the context of BCS is hepatocellular carcinoma (HCC). Long-standing congestion and fibrosis of hepatic parenchyma in patients with chronic BCS are known to give rise to hepatic nodules. The etiology of hepatic nodules detected on radio imaging in BCS patients can be benign regenerative nodules and HCC.

Table 2 Clinical presentation, site of block, radiological intervention, and outcomes of major studies in children with Budd-Chiari syndrome

	Nagral *et al*[12], %	Kathuria *et al*[13], %	Sharma *et al*[14], %	Singh *et al*[15], %	Shukla *et al*[32], %
No. of patients	16	45	32	113	43
Age at presentation, median (range)	22 (4-132) mo	10.5 (2-16) yr	9 (4.5-214) mo	10 (1.5-17) yr	16.7 (10-19) yr
Symptom duration, median (range)	3 (0.5-48) mo	8.9 mo (5 d–8 yr)	-	-	12.7 (0.5-150) mo
Diagnosis by USG	63	95.6	60	-	-
Symptoms					
Ascites	81	82	96	84	91
GI bleed	25	35	8	22	23
Jaundice	12.5	20	24	12	14
Hepatomegaly	N/A	85	96	N/A	67
Splenomegaly	N/A	70	N/A	N/A	37
Abdominal vein dilation	N/A	70	70	N/A	N/A
Type of block					
Only HV	94	71	76	74	67
Only IVC	6	4	0	2	9
Both HV + IVC	0	25	24	24	24
RI, *n*	13	25	24	53	24
Angioplasty alone	4	2	7	7	-
Angioplasty + stenting	3	20	3	40	10
DIPS	-	-	-	5	-
TIPSS	6	3	14	1	14
Follow-up duration, median (IQR)	31 (12-54) mo	6.5 (0.5-86) mo	44 (5-132) mo	13.5 (1-155) mo	41 (12-168) mo
Successful RI					
Angioplasty	25	100	43	100	-
Angioplasty + stenting	100	87	67	90	90
DIPS	-	-	-	80%	-
TIPSS	80	67	72	100	80
Procedure related complications	TIPSS encephalopathy = 1; Neck hematoma = 1	Anaesthesia related death = 1; Neck Hematoma-1; Hemoperitoneum = 1	No major	TIPSS; Enceph-alopathy = 1	N/A
Stent patency rate	-	Overall 75%	-	87% at 1 yr, 82% at 5 yr, 62% at 10 yr	Overall 75%
Mortality	2: GI bleeding 1, liver failure = 1	3: Intracranial bleeding = 1, anaesthesia related = 1, liver failure = 1	5: Intracranial bleeding = 2, GI bleeding = 1, HCC = 1, liver failure = 1	3: Procedure related = 2, head trauma = 1	4: Intracranial bleeding = 1, HCC = 1, liver failure = 2
HPS	N/A	N/A	4 (12, 5%)		-
HCC	N/A	N/A	1 (3%)		1 (2.3%)

N/A: Not available; HV: Hepatic vein; IVC: Inferior vena cava; USG: Ultrasonography; GI: Gastro-intestinal; HV: Hepatic vein; IVC: Inferior vena cava; RI: Radiological intervention; DIPS: Direct intrahepatic porto-systemic shunt; TIPSS: Transjugular intrahepatic porto-systemic shunt; HPS: Hepato-pulmonary syndrome; HCC: Hepatocellular carcinoma.

It is important to distinguish between these two conditions as treatment differs significantly. HCC lesions are usually larger in size (> 2 cm) and hypervascular on DUS and CT/MR[50]. Also, for HCC, contrast enhancement in T1-weighted MR imaging can show different enhancement patterns between HCC with and without BCS[51]. Yang *et al*[51], in a comparative study between 10 adult HCC patients with associated primary BCS and 32 other HCC patients without BCS, found that significantly more lesions with BCS were hyperintense during the arterial phase and slightly hyperintense or isointense during the venous phase than lesions without BCS ($P < 0.05$ for both). Therefore, histological confirmation is required in the workup of HCC in patients with BCS.

TREATMENT OPTIONS

Therapeutic options for BCS include medical management with anticoagulation therapy, radiological interventions such as angioplasty and stenting, surgical shunting and transjugular intrahepatic portosystemic shunt (TIPSS), and lastly, liver transplantation (LT).

Medical therapy

Anticoagulation alone with oral anticoagulants is sufficient in only 10% of adult patients, especially those with mild disease, and the vast majority of them progress, requiring intervention strategies in follow-up[52-54]. Pediatric experience is limited. Sharma *et al*[14] reported a 33% response in a small cohort of Indian BCS children who were treated with warfarin alone. Data on the comparative efficacy of directly acting anticoagulants *vs* vitamin K antagonists in BCS patients is scarce[55]. A recent Austrian multicentre study on the efficacy and safety of DOAC in 22 adult BCS patients (DOAC first-line anticoagulation in 6, switched over from warfarin and LMWH in 16) showed that DOAC showed clinical response in 63% of cases while bleeding occurred in 11 (4 major bleeding, 7 minor bleeding)[55]. Confirmation of efficacy and safety by larger prospective studies is needed.

Radiological endovascular intervention

The progressive improvement in radiological endovascular intervention (REI) techniques in the past two decades has provided better procedural and clinical success for BCS treatment compared to other treatment modalities in both adults and children[14,15,56,57].

Angioplasty vs stenting

The rationale of angioplasty is to decompress the liver while restoring hepatic blood flow. Angioplasty can be done with or without stenting in BCS patients with short-segment stenosis (< 5 cm). Wang *et al*[58], in a landmark study, compared the treatment efficacy and long-term patency of angioplasty with and without stenting in 88 adult Chinese patients with BCS. They had shown that the superiority of angioplasty with stenting over angioplasty alone in the rate of re-stenosis (2% *vs* 40% over a median follow-up period of 27 mo, $P < 0.0001$)[58]. Balloon angioplasty without stenting of the obstructed veins is preferred in infants and younger children as appropriate sizes of stents are not available, thus making stenting technically difficult in this population. Also due to the relatively shorter duration of disease, the veins are likely to be much more pliant. Hence angioplasty alone may reduce and normalize the venous pressure significantly after the procedure. In older children, angioplasty alone has a technical success rate and clinical response rate of 90% but is fraught with a high risk of blockage[11-15]. Restenosis is almost always inevitable. Nagral *et al*[12], Kathuria *et al*[13], and Sharma *et al*[14] have reported higher re-occlusion rates with angioplasty alone (75%, 33%, and 57%, respectively) compared to angioplasty + stenting (0%, 13%, and 33%, respectively) and TIPSS (20%, 33%, and 28%, respectively). This highlights that angioplasty with stenting rather than angioplasty alone, should be the preferred modality of radiological intervention (RI) in children. The smaller size of the liver and caliber of the veins in children pose a challenge when choosing an appropriate size and length of the stent. An uncovered self-expandable metallic stent is preferred over fully covered stents because of the lower risk of post-procedural thrombosis. Longer stents (20–30 mm) are required for long-segment IVC blocks than HV blocks. Overall, the technical success and clinical response rate of stenting in children is excellent (> 90%) with a much less re-stenosis rate than angioplasty[11-15]. In the authors' experience, the length of the stent may appear longer as the liver decongests after the intervention. However, with the growth of the child, the liver also grows in size and the final stent size is usually appropriate for the child. Customized stents are available. It is also important to realize that once the liver is recovering and the physiology is restored, there is an expected sudden growth spurt in children with chronic liver disease, especially during puberty. With growth, the blood flow and turbulence through the recovering liver increase. Hence anticoagulation is most important in this phase till the stent undergoes complete endothelialization. Oral contraceptives in adolescence are to be prescribed with caution.

TIPSS

TIPSS is a shunt created between the portal and the systemic circulation, leading to a reduction in hepatic congestion and symptom resolution. Currently, TIPSS is performed in failed angioplasty-stenting with an available HV stump and as a bridge to LT[27]. TIPSS is technically more demanding than cirrhotic patients due to the blocked HVs. Nonetheless, various studies have established TIPSS as a safe and effective treatment for BCS in adults[22,48,59,60]. Amarapurkar *et al*[22] and Shalimar *et al*[59] have shown good technical success rates (87.5%-100%) and clinical success rates (80%-93%). Post-procedure encephalopathy occurs less frequently (3% to 5% cases) than in other cirrhotic patients, reflecting the relatively preserved hepatic function in most patients with BCS[59,60]. The main concern about TIPSS is the higher occurrence of stent dysfunction. Compared to other cirrhotic patients undergoing TIPSS, BCS patients show higher stent

dysfunction (70% *vs* 50% within 1 year), probably due to underlying prothrombotic conditions in BCS[61]. The advent of polytetrafluoroethylene covered stents has dramatically reduced the incidence of stent dysfunction (30%-70% in bare stents *vs* 10-20% in covered stents)[61,62]. The available TIPSS stents are usually expensive and are inappropriate in terms of sizes for children, thus limiting their use in the pediatric population, although reports of successful treatment of children with BCS with TIPSS are available[12,13]. TIPSS in children with BCS risks placement of the bare end of the stent beyond the portal vein and into the superior mesenteric vein if the size is too long. Hence, a "stent within a stent" is a minor modification of this technique that allows customization for the size of the liver. In this technique, a fully covered graft stent (10 mm × 3–7 cm) is placed inside the bare uncovered stent (10 mm × 8–10 cm).

Modified TIPSS/DIPS (direct intrahepatic portosystemic shunt)

Long-segment (> 5 cm) HV block is usually not amenable to angioplasty, stenting, or TIPSS. A modified TIPSS or DIPS is a procedure where a shunt is made between IVC and the right branch of the portal vein. Though technically challenging, DIPS has been shown to effectively decompress hepatic congestion and clinical resolution in patients with BCS. In a large pediatric study from the authors' center, children with chronic BCS undergoing DIPS had a procedural success and clinical response rate of 80% and 90%, respectively[15].

Surgical shunts

The principle of a surgical portosystemic shunt is to relieve the obstruction causing PHTN using a venous conduit, thereby decompressing the hepatic sinusoids. Surgical portosystemic shunts have now been almost completely abandoned because of high perioperative mortality (25%) and poor shunt patency (70%)[63-65]. Surgical portosystemic shunting can also be technically difficult when there is caudate lobe hypertrophy[66]. Most studies on surgical portosystemic shunts in adult patients with BCS failed to show any survival benefit[65,67]. In a case series of 25 Indian children with BCS, only one out of the four patients who underwent surgical shunts survived[68].

LT

LT is considered a salvage therapy in the setting of fulminant presentation, progression to end-stage liver disease, or development of HCC[27,53]. BCS accounts for approximately 1% of all pediatric LT cases[69]. Involvement of retrohepatic IVC, the proximity of thrombus near the right atrium, and an underlying prothrombotic condition causing vascular complications make LT challenging. The challenge is even greater when considering living donor liver transplantation (LDLT) since the graft does not contain the retrohepatic IVC, as in deceased-donor liver transplantation (DDLT). Therefore, HV reconstruction is more complex, especially if the IVC is also obliterated[69]. The smaller size of vessels in children complicates the situation further. Several large retrospective analyses have evaluated the benefit of DDLT in adult BCS patients with 5-year survival rates varying between 71% and 89.4%, similar to those undergoing LT for other diseases[70,71]. Due to the scarcity of deceased-donor liver grafts, LDLT has been the mainstay for BCS patients undergoing LT in most Asian countries with 5-year survival rates ranging from 75% to 81%[72-75]. Data on LT in pediatric BCS is only in the form of case reports and small case series and long-term prognosis has been reported to be good so far[76,77].

STEPWISE APPROACH OR UPFRONT REI IN CHILDREN WITH BCS: THE WAY FORWARD?

The rarity of BCS in general makes it difficult to perform randomized controlled trials in patients with BCS. Hence, most recommendations regarding treatment are based on case reports, retrospective studies, and expert opinions. Concerning the timing of the interventions, the European Association for the Study of the Liver[78] and the Asia Pacific Association for the Study of the Liver (APASL)[27] recommended a stepwise therapeutic algorithm for BCS in adults. The algorithm depends on treatment response, medical therapy with anticoagulant drugs, angioplasty, stent placement, TIPS, and LT. In contrast to the strict step-up principle, an AASLD practical guideline suggested checking for a venous obstruction amenable for angioplasty in all symptomatic patients right at the beginning and treating accordingly[79]. The step-up algorithm has also been criticized because it pays little attention to hemodynamics and its possible improvement or even relief by interventional treatment. The step-up algorithm also does not restore physiology at the onset and possibly delays the definitive procedure, leading to advanced liver disease. Guidelines are silent regarding what should be the optimal treatment approach in children with BCS. Longitudinal studies on pediatric BCS have shown that children with chronic BCS tend to decompensate early with ascites and variceal bleeding[13,14]. In those receiving medical therapy alone, it has been seen that 26% of adults die over 2 years[53,55]. Response to medical therapy has been variable in children (33-43%) and two-thirds of these children ultimately require an intervention in the long run[14]. There is also concern about the safety profile of long-term use of oral anticoagulants in children[80]. Hence, we suggest that all children with BCS (whether symptomatic or not) should undergo angioplasty and stenting as a primary treatment modality.

It is debatable whether asymptomatic or incidentally detected BCS should be subjected to REI or not, especially in children. On one hand, decompensation may rapidly set in as children do not tolerate portal hypertension for longer periods as compared to adults. On the other hand, there are no sound ethical justifications as to whether these children should be subjected to invasive procedures and maintained on lifelong anticoagulation. In a personal opinion, the authors would prefer the former in asymptomatic children given their longer life expectancy, milder liver disease, and possibly better health and compliance with the HV. Hence there is a small window of opportunity to restore normal physiology in optimal conditions.

POST-INTERVENTION COMPLICATIONS AND NEED FOR MONITORING AFTER REI

Immediate complications of REI include subcapsular hematoma, hemoperitoneum, congestive heart failure, transient hepatic encephalopathy, and pulmonary thromboembolism and are encountered in 1%–3% of procedures in experienced centers[12-16,27]. Long-term complications of bleeding secondary to anticoagulation have also been reported[12,13,22]. However, the most commonly encountered post-procedure complication is re-stenosis after REI. Singh *et al*[15], in a large cohort of Indian children with BCS, reported follow-up vascular patency rates of 87%, 82%, and 62% at 1, 5, and 10 years after intervention, respectively. In the above study, 29% of the cohort with REI (27% HV/IVC stenting; 60% modified TIPSS) had restenosis[15]. Adult studies report 17% to 41% restenosis after REI[21,22,24]. To prevent re-stenosis, heparin infusion should be started during the intervention and continued thereafter. Warfarin must be initiated within 24 h of completing the procedure. The physician should consider stopping heparin and continuation of long-term warfarin if the target INR of 2–3 is achieved. Periodic clinical examination, liver function test, and shunt patency by DUS (post-stenting: 24 h, 1 wk, 1 mo, 3 mo, and subsequently every 6 mo; post TIPS/DIPS: at 7–14 d, 3 mo, 6 mo, 9 mo, and 12 mo) is performed. Post-intervention surveillance aims to re-intervene before critical stenosis or occlusions recur.

Assessment of response to therapy

Response to therapy implies restoration of blood flow across previously blocked HV/IVC and consequent improvement in organomegaly and liver function test (LFT), no recurrence of ascites without need of diuretics, and resolution of signs of PHTN. Kathuria *et al*[13], in a study among 20 children with BCS undergoing REI, demonstrated clinical and biochemical resolution in all; however, the median follow-up duration was 6.5 mo only. Sharma *et al*[14], in another study of 20 Indian children with BCS who underwent REI, showed that HV stenting or TIPS is efficacious in improving clinical features, LFT, PHTN features, and growth parameters in 66% and 72% of cases, respectively, over a median follow-up duration of 44 (range 5-132) mo. Further long-term studies need to holistically address the natural history and timelines of resolution of organomegaly, liver stiffness, varices, liver functions, growth, pubertal maturity, and quality of life in children with BCS.

LIVER AND SPLENIC STIFFNESS MEASUREMENT IN MONITORING

Recently, liver stiffness measurement (LSM) and splenic stiffness measurement (SSM) have been extensively studied as potential non-invasive markers of hepatic and splenic fibrosis and congestion and hepatic venous pressure gradient in patients with chronic liver disease[81-84]. LSM and SSM can be measured by various imaging techniques [transient elastography (TE), shear-wave elastography (SWE), and MR elastography]. Fraquelli *et al*[82], in a study of 132 patients with chronic hepatitis B and C, showed that LSM and SSM measured by TE, were reliable in predicting significant fibrosis [odds ratio (OR) = 5.2 and 4.6, respectively] and cirrhosis (OR = 7.8 and 9.1, respectively). SSM of < 48 kPa by TE was useful in ruling out esophageal varices. In another study by the same authors in 186 chronic liver disease patients, LSM and SSM measured by SWE were equally effective and reliable in predicting significant liver fibrosis as compared to TE, with SWE having the advantage of applicability in patients with obesity or ascites[83]. The latest Baveno VII guidelines recommended that all cases of clinically advanced chronic liver disease should undergo LSM testing[85].

However, these tests are yet not standardized in children. Pediatric literature regarding LSM and SSM by elastography techniques is emerging. Chongsrisawa *et al*[86] reported significantly higher LSM in biliary atresia patients with esophageal varices than those without (37.72 ± 21.55 *vs* 10.97 ± 8.71 kPa, $P < 0.001$). Yoon *et al*[87] showed that an LSM value of > 18.4 kPa predicted clinically significant PH (CSPH) in children with CLD with a high sensitivity (87.5%) and specificity (84.0%). LSM has been used to monitor and assess treatment response in adult patients with BCS[88]. In a study to assess short- and long-term outcomes in 32 Chinese adults with BCS undergoing REI, Wang *et al*[88] measured LSM using SWE at 2 d, 3 mo, and 6 mo post-procedure. Mean LSM value before the procedure was 35.17 ± 10.60 kPa, which decreased to 15.36 ± 4.34 kPa and 15.68 ± 5.58 kPa at 3 mo and 6 mo post-procedure, respectively ($P < 0.001$). Published literature on the role of LSM in monitoring children with BCS is scarce[89]. Dohare *et al*[89] evaluated the role of LSM in 32 children undergoing REI and showed that LSM values decreased significantly after REI. A maximal decrease is seen 24 h after REI (43.7 kPa at baseline *vs* 22.5 kPa 24 h post-procedure, $P = 0.001$)[75]. Among the nine children developing re-stenosis after REI, re-stenosis was typically associated with an increase in LSM compared with the patient's prior measurement (median absolute increase 11.0 kPa; interquartile range [IQR] 6.1-24.4).

SSM reflects congestion as well as structural changes in the spleen as a direct consequence of the increased PHT[90,91]. SSM is considered a direct and more suitable surrogate marker of PHTN and performs better for the prediction of CSPH [91,92] and esophageal varices[93,94]. Sutton *et al*[94] evaluated SSM in 67 children with chronic liver disease and showed that SSM is a reliable predictor of CSPH at a value > 38.0 kPa [area under receiver operating curve (AUROC) = 0.92, sensitivity = 89%, specificity = 82%, $P < 0.01$]. Sintusek *et al*[95] studied 51 BA children and showed a higher SSM of 46.85 (IQR 25.95-54.55) kPa in patients with varices as compared to the no-varices group [median SSM-16.54 (IQR 11.75-21.75) kPa; $P < 0.001$]. SSM has been performed in adults with BCS ($n = 7$) who underwent TIPSS[96]. In this case series, SSM in combination with LSM may reflect the severity of the disease at presentation and the need for invasive treatment. SSM values also showed a significant decline after TIPSS over a median follow-up period of 1 year. Further studies are required to elucidate the role of LSM and SSM in monitoring BCS patients after REI.

PROGNOSTIC INDICES AND THEIR IMPLICATIONS

To date, many prognostic scores have been developed in patients with BCS to quantify the disease severity and prognosis (Table 3). The authors evaluated the prognostic accuracy of these indices in BCS children and found that pre-intervention PELD score with a cut–off of 4 (AUROC = 0.809, 86% sensitivity, and 75% specificity) significantly determined poor outcomes following REI. Zeitoun score independently predicted poor outcome [OR = 15.4, 95% confidence interval (CI): 1.17-203.56, P = 0.04] with a cut-off of 4.3 (AUROC = 0.923, 83% sensitivity, and 77% specificity) in the unintervened chronic BCS[15]. Hence BCS children with a Zeitoun index > 4.3 should undergo REI without any delay.

LONG-TERM COMPLICATIONS OF BCS

Hepatopulmonary syndrome

Hepatopulmonary syndrome (HPS) occurs in a substantial portion (28%) of adult patients with BCS and balloon angioplasty can reverse HPS in patients with BCS[97]. The mechanism is unknown but may be related to portal decompression. This may also explain the favorable outcomes of TIPSS creation for HPS in patients with cirrhosis and idiopathic PHTN[98]. Sharma *et al*[14] in a previous pediatric study reported the detection of HPS among five children in long-term follow-up, one in an un-intervened child and four after RI with patent stent (3 TIPSS, 1 HV angioplasty). It is not clear as to which patients will have resolution or progress to HPS as contradicting outcomes have been noted. The possible reason for developing HPS even after reduction of portal hypertension post-RI could be increased exposure of pulmonary vasculature to vasodilator mediators like increased levels of nitric oxide, endothelin-1, tumor necrosis factor-α , and endotoxemia[98].

HCC is an uncommon but dreaded long-term complication of BCS[99,100]. In a recent systematic review of adults, the prevalence of HCC in BCS is geographically varied[99]. It is documented as 2.0%–46.2% in 12 Asian studies, 40.0%–51.6% in two African studies, 11.3% in one European study, and 11.1% in one American study[99]. Irrespective of hepatitis as the underlying risk factor of HCC, the pooled prevalence of HCC was 17.6% in BCS patients (95%CI: 10.1%–26.7%), 26.5% in IVC obstruction (95%CI: 14.4%–40.7%), and 4.2% in HV obstruction (95%CI: 1.6%–7.8%). When patients with HCC and concomitant hepatitis were excluded, the pooled prevalence of HCC was 15.4% (95%CI: 6.8%–26.7%). Only 3 out of the 16 included studies evaluated the risk factors for the development of HCC in BCS patients. However, there was significant heterogeneity among these studies and the results were contradicting[99]. Further long-term prospective studies are necessary to evaluate risk factors for HCC in BCS patients. Data regarding the prevalence of HCC in children with BCS is limited. So far, only three cases of HCC in children with BCS have been reported[14,32,101]. All of them had HCC in the 2nd decade of life, liver nodules > 3 cm, and elevated alpha-fetoprotein. One patient was on anticoagulation only[101] while the other two had blocked stents[14,32]. In the authors' own unpublished experience from the authors' center, an 18-year-old boy developed HCC 6 years after blockage of DIPS. Routine surveillance for HCC is thus warranted in BCS patients, even after undergoing REI.

FUTURE DIRECTIONS

Future studies need to elucidate the underlying thrombophilic conditions and their role in the etiopathogenesis of BCS. The use of next-generation sequencing in addition to the conventional thrombophilia work-up is warranted for better understanding and higher diagnostic yield. Further studies are needed to determine the precise role of MRA in differentiating benign liver nodules with HCC in BCS patients. Liver and splenic stiffness measurement by elastography techniques (transient elastography, shear-wave elastography, and MR elastography) may serve as a useful non-invasive marker for assessing treatment response. Despite its rarity, pediatric BCS provides a unique opportunity to study the natural history, long-term complications, and treatment outcome.

CONCLUSION

BCS is a rare but potentially treatable cause of portal hypertension with an excellent prognosis after definitive treatment. Due to recent advances in interventional radiology, radiological endovascular intervention is currently the preferred primary treatment modality. Better patient and procedure selection, choice of appropriate size and type of stent, and mandatory follow-up assessment are of utmost importance for better long-term outcomes. In future, prospective and larger studies should be undertaken to study the epidemiology and establish standardized diagnostic and therapeutic management protocols for pediatric BCS.

Table 3 Budd-Chiari syndrome-specific prognostic indices

Score	Equation	Cut-off	Predicted survival rate
CTP	Serum bilirubin, serum albumin, INR, ascites, HE	-	-
MELD	9.57 × log (creatinine) + 3.78 × log (total bilirubin) + 11.2 × log (INR) + 6.43	-	-
Zeitoun index	Ascites score[a] × 0.75 + Child-Pugh score × 0.28 + age × 0.037 + creatinine × 0.0036	5.4 (range from 3.4 to 9.1)	At 5 yr; ≤ 5.4: 95%; > 5.4: 65%
New Clichy score	0.95 × ascites score + 0.35 × CTP score + 0.047 × age + 0.0045 × serum creatinine + 2.2 × type IIIb[b] −2.6	5.1 (range from 2.0 to 9.7)	At 5 yr; < 5.1: 100%; ≥ 5.1: 65%
Rotterdam BCS index	1.27 × encephalopathy + 1.04 × ascites + 0.72 × prothrombin time + 0.004 × bilirubin	Bilirubin; class I: 0–1.1; class II: 1.1–1.5; class III: ≥ 1.5 (range from 0.02 to 4.03)	At 5 yr; class I: 89%; class II: 74%; class III: 42%
TIPS-BCS index	Age (yr) × 0.08 + bilirubin (mg/dL) × 0.16 + INR × 0.63	7	1-yr OLT-free survival, ≤ 7: 95%; > 7: 12%
BCS-intervention-free survival prognostic score	Ascites (yes = 1, no = 0) × 1.675 + ln creatinine (μmol/L) × 0.613 + ln bilirubin (μmol/L) × 0.440	Interval 1: ≤ 5; interval 2: 5-6; interval 3: ≥ 6	Intervention-free survival interval 1: 78.3%, interval 2: 27.8%; interval 3: 6.8%
AIIMS-HVOTO score	1.2 × response to therapy + 0.8 × child class)	Score: < 3; 3-4; > 4	% yr survival score ≤ 3: 92%; score 3-4: 79%; score > 4: 39%

[a]Ascites score: 1, absent with free sodium intake and no diuretic agents; 2, easy to control with sodium restriction or diuretic agents; and 3, resistant to this treatment because of hyponatremia or functional renal failure.
[b]Type III' is a binary variable coded as 1 for patients with clinicopathological findings of acute injury superimposed on chronic lesions, and 0 for the other patients.
CTP: Child-Turcotte-Pugh, MELD: Model for end stage liver disease; BCS: Budd-Chiari syndrome; AIIMS: All India Institute of Medical Sciences; HVOTO: Hepatic vein outflow tract obstruction; OLT: Orthotopic liver transplantation.

FOOTNOTES

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MINIREVIEWS

Risk of hepatitis B reactivation in patients with myeloproliferative neoplasms treated with ruxolitinib

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Abstract

Classical Philadelphia-negative myeloproliferative neoplasms (MPNs), *i.e.*, polycythemia vera, essential thrombocythemia, and primary/secondary myelofibrosis, are clonal disorders of the hematopoietic stem cell in which an uncontrolled proliferation of terminally differentiated myeloid cells occurs. MPNs are characterized by mutations in driver genes, the JAK2V617F point mutation being the most commonly detected genetic alteration in these hematological malignancies. Thus, JAK inhibition has emerged as a potential therapeutic strategy in MPNs, with ruxolitinib being the first JAK inhibitor developed, approved, and prescribed in the management of these blood cancers. However, the use of ruxolitinib has been associated with a potential risk of infection, including opportunistic infections and reactivation of hepatitis B. Here, we briefly describe the association between ruxolitinib treatment in MPNs and hepatitis B reactivation.

Key Words: Ruxolitinib; Myeloproliferative neoplasms; Hepatitis B; Polycythemia vera; Myelofibrosis; JAK inhibitor

Core Tip: The JAK inhibitor ruxolitinib has been approved for the treatment of classical Philadelphia-negative myeloproliferative neoplasms (MPNs), *i.e.*, polycythemia vera, essential thrombocythemia and primary/secondary myelofibrosis. However, its use has been associated with a potential risk of opportunistic infections, including hepatitis B reactivation. Herein, we briefly overview the association between ruxolitinib treatment in MPNs and hepatitis B reactivation.

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INTRODUCTION

Introduction to hepatitis B virus reactivation

Hepatitis B virus (HBV) infection is the most common chronic viral infection in the world. It affects more than 350 million people worldwide as chronic carriers, and more than 2 billion (30% of the world's population) people show evidence of past exposure. Additionally, HBV infection has accounted for roughly half of total liver cancer mortality in 2010[1,2]. Once contacted, the virus cannot be eliminated, even with proper and rapid antiviral treatment, but the infection is self-limiting in more than 95% of immunocompetent adults. These patients are now known as carriers 'anti-HBc positive'. They do not require specific management or monitoring unless immunosuppression is suspected[3].

If HBV persists for more than 6 mo in the body, the affected individual is considered to have chronic hepatitis B. Its incidence depends on the time of exposure: 95% of newborns, 20%-30% of children aged 1 to 5 years, and less than 5% of adults[3]. The reason for this dormant state of HBV is the presence of covalently closed circular viral DNA (cccDNA) that penetrates and persists indefinitely in hepatocyte DNA[2-4]. This cccDNA acts as a template for future viral components in the case of HBV reactivation (HBVr). Viral transmission has been greatly slowed recently by the advent of a safe and effective vaccine, available since 1981 and introduced in 2011 in routine vaccination schedules in more than 180 countries [1,5].

DEFINITION, EPIDEMIOLOGY AND MANIFESTATIONS OF HBVR

The number of cases of HBVr after treatment with immunosuppressive agents is increasing worldwide, mostly attributed to an increase in the prevalence of positive HBV serology and, at the same time, an increase in the number of clinical indications for potent immunosuppression, including solid malignancies, inflammatory bowel disease, autoimmune disorders, blood cancers, *e.g.* myeloproliferative neoplasms (MPNs), and rheumatic diseases[3].

There are, although very similar, several definitions of HBVr, proposed by several medical associations from around the globe. All of them take into account both virological and serological criteria and describe HBVr as either an exacerbation of chronic hepatitis B or a reactivation of past hepatitis B infection. The most used definition is the one proposed by the American Association for the Study of Liver Diseases, last updated in 2020, which defines HVBr according to the virological status of the patient[4,6-8].

For HBsAg-positive patients with or without detectable HBV DNA: (1) At least 2 Log (or 100-fold) increase in HBV DNA compared to the baseline level; (2) HBV DNA at least 3 Log (or 1000) IU/mL in patients with previously undetectable HBV DNA; or (3) HBV DNA at least 4 Log (or 10000) IU/mL if the baseline level is unavailable[4,6-8].

For patients with HBsAg negative and HBV DNA negative: (1) HBV DNA becomes detectable; or (2) reverse HBsAg seroconversion (reappearance of HBsAg)[4,6-8].

The natural history of HBVr depends, among others, on the underlying disease requiring immunosuppressives, host immunity and the immunosuppressive agents used. Evolution can be classified into multiple stages[4,6-8].

After the initiation of immunosuppressive therapy, viral replication resumes, leading to a gradual increase in serum HBV DNA levels. The patient is still asymptomatic and, in general, HBVr-related hepatitis, described as an increase in alanine transaminase (ALT) or aspartate transaminase (AST) to 3 times upper limit of normal (ULN), does not develop[4,6-8].

HBVr-related hepatitis

ALT or AST increases to ≥ 3 times ULN (in some cases between 5-10 times ULN). Although most patients may remain asymptomatic, a small number might experience constitutional symptoms, such as pain in the right upper quadrant and jaundice. In rare cases, hepatic injury could further progress and cause liver failure, fulminant hepatitis or even death[4,6-

8].

Spontaneous or antiviral-induced resolution

Normalization of serum ALT and AST levels, due to completion of immunosuppressive therapy, due to antiviral therapy, or due to host immunological mechanisms[4,6-8].

Acute liver failure/persistent liver injury

Found in a small number of individuals who continue to have a progressive decline in liver function, it is characterized by increased levels of bilirubin, prolonged prothrombin time, and, in very rare cases, even signs and symptoms of acute liver failure and hepatic decompensation (ascites and encephalopathy)[4,6-8].

MECHANISMS OF HBVR

As previously mentioned, after entering the hepatocytes, the viral genome is converted into plasmid-like cccDNA which can persist in liver cells in a latent state, serving as a reservoir for HBVr, in spite of active anti-HBV immune response. Compared to the hepatitis C virus (HCV) infection, complete eradication of both HBV cccDNA and integrated DNA is impossible with current antiviral treatment with nucleos(t)ide analogs. Thus, these cells constitute a reservoir of persistent HBV. Although HBVr can occur in a variety of settings, immunosuppressive therapies are the most commonly reported. A detailed description of the HBVr induction mechanisms of immunosuppressive therapies is provided in Table 1[3,4,6-12].

RISK FACTORS FOR HBVR

Host-related risk factors for HBVr include male sex, younger age and older age (the elderly are more likely to have HBsAg seroclearance but persistent levels of total HBV DNA and cccDNA in the liver) and have been associated with increased risk of HBVr. Preexisting conditions, for example, cirrhosis or MPNs, also play a role in HBVr. HBVr has been reported in patients with MPNs, lymphomas, myeloma, and acute myeloid leukemia. However, it is not yet clear whether this association is attributed to the underlying disease or to the potent immunosuppressants used in the management of these blood cancers[7-9].

Virological factors include HBsAg and HBeAg positivity (adding a 5- to 8-fold risk for HBVr), non-A HBV genotypes, elevated HBV DNA levels before starting immunosuppressive therapy, and co-infection of HBV with other viruses such as HIV and HCV[4,7,8].

Type of immunosuppression: the greatest risk of HBVr is represented by the use of B-cell depleting therapies, used in the therapeutic armamentarium of blood and solid cancers and in the setting of bone marrow or solid organ transplantation[3,4,6-12]. More details are presented in Table 1.

PREVENTION OF HBVR

Identifying infected individuals is the first and most important step for HBVr prophylaxis. According to the latest specialty guidelines, HBV infection screening must be performed in all patients who are receiving immunosuppressive treatment. Furthermore, all patients who are HBcAg positive, regardless of the status of HBsAg or the HBV DNA values, must receive prophylactic antiviral treatment. In numerous studies, prophylactic antiviral treatment has been shown to reduce the rate of HBVr, liver failure, and death in these categories of patients. Even if lamivudine was the first and for many years the most used oral antiviral agent for HBVr prophylaxis, *YMDD* gene mutations cause a high incidence of viral resistance if used for > 6 mo. This is why entecavir or tenofovir are recommended as therapies for HBVr prevention if intended for longer periods of time[4,6-8].

Duration of antiviral prophylaxis

In general, the duration of antiviral therapy varies depending on the type of immunosuppressives used. General recommendations include the use of antiviral therapy for at least 6 mo after the last dose of immunosuppressive agents is administered. However, in the case of B cell-depleting therapies (such as rituximab or obinutuzumab), it is recommended that antiviral prophylaxis be continued up to 12 mo after the last dose. Another important step is routine testing for HBV DNA and serum ALT and AST 3-6 mo after discontinuation of immunosuppressives[3,7].

Moreover, particular attention should be given to preventive measures, such as instructing patients to withdraw from alcohol consumption, as well as close monitorization of liver function tests in subjects who are prescribed pharmacological agents with a potentially hepatotoxic effect[13,14]. According to the findings of the Dionysos Study, individuals diagnosed with HBV who consume alcohol experience elevated rates of hepatic fibrosis and death[13].

Table 1 Immunosuppressive agents associated with HBVr

Immunosuppressive therapies with high risk of HBVr							
B-cell depleting therapies (rituximab and ofatumumab)		Anthracycline derivatives (doxorubicin and epirubicin)	Corticosteroids	TNF-α inhibitors (infliximab, adalimumab, certolizumab)			Anti-CD52 monoclonal antibody (alemtuzumab)
Increased HBVr risk in positive HBsAg and negative HBsAg and anti-HBc subjects by acting against the B-lymphocyte antigen CD20; The Food and Drug Administration has placed a black box warning for rituximab regarding HBVr in rituximab-treated individuals; used to treat CD20+ blood cancers (lymphomas, CLL) and IRD; B cells play a previously underestimated role in HBV immune control by producing neutralizing antibodies; rituximab associated with > 5× increase in HBVr risk (incidence 3%–55%, overall mortality rate 30%–38%)		High-risk for patients with hepatocellular carcinoma and hepatitis B undergoing TACE; used to treat lymphomas and acute leukemias, breast and ovarian cancer, and sarcoma; HBVr rate = 41% in patients with HBsAg positive	Prednisone use > 20 mg p.o. daily > 4 wk	TNF-α can activate the APOBEC antiviral pathway which causes the degradation of cccDNA in HBV-infected cells. HBVr pooled incidence in patients with resolved HBV infection = 3.0% *vs* 15.4% in HBsAg positive patients			Used for refractory CLL; causes reverse HBsAg seroconversion and reactivation-related hepatitis
Immunosuppressive agents with moderate risk of HBVr							
Less potent TNF-α inhibitors (etanercept)	Cytokine or integrin inhibitors (abatacept, ustekinumab, natalizumab, vedolizumab)	Tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib)	Proteasome inhibitors: (Bortezomib)	Histone deacetylase inhibitors (HDIs) (romidepsin)		Prednisone 10-20 mg p.o. daily > 4 wk	Calcineurin inhibitors (cyclosporine or tacrolimus)
Moderate risk of HBVr in patients with HBsAg positive (1%-5%) and even lower in patients with HBsAg negative	Commonly utilized in the treatment of IBD, IRD and dermatologic conditions; inhibit local inflammatory response associated with immune-mediated diseases by blocking the localization and traffic of activated lymphocytes	Standard of treatment for all phases of CML; also used in the treatment of GIST; inhibit various kinase signaling pathways, essential for immune activation and proliferation of lymphocytes, with an important role in immune control of HBV replication; prophylactic antiviral therapy and regular monitoring of HBV DNA and liver enzymes are essential; reported HBVr rates of 26%–34.8%	Used for the treatment of MM and induction therapy for transplant- eligible patients prior to stem cell harvest; target cellular pathways that interfere with the functions of healthy B cells, which are important in HBV immune control	Used in the treatment of T-cell lymphomas; inhibit histone deacetylase, a histone-modifying enzyme that is important for epigenetic regulation of gene expression with possible deacetylation status of silent cccDNA, resulting in active HBV transcription and then HBVr		The mechanism is two-fold: The HBV genome contains a transcription regulatory element responsive to glucocorticoid that is up-regulated by corticosteroids, resulting in increased viral replication; a directly suppressive effect on cytotoxic T cells that are involved in HBV control; risk of HBVr of 10%-15.8% in HBsAg positive individuals	Suppress T cell function by inhibiting calcineurin required for signal transduction of T cell activation and inhibiting transcription of interleukin required for T cell proliferation
Immunosuppressive agents with low risk of HBVr							
Methotrexate, azathioprine or 6-mercaptopurine				Intra-articular steroid injections or prednisone < 10 mg p.o. daily			
Documented cases of HBVr are rather rare							
Novel therapies							
Immune checkpoint inhibitors such as anti-PD-L1 (nivolumab) and anti-CTLA4 (ipilimumab)	BTK inhibitor ibrutinib and PI3K delta inhibitor idelalisib	Ruxolitinib	Mogamulizumab	Brentuximab	Obinutuzumab	Hypomethylating agents: Decitabine, azacitidine	Daratumumab

HBVr rarely reporter; anti-HBV prophylaxis is recommended	B-cell receptor signaling modulators; approved by the FDA for the treatment of CLL and certain low-grade NHL; HBVr has been rarely been reported; anti-HBV prophylaxis is recommended	A novel inhibitor of JAK1 and JAK2 that has been approved for the treatment of patients with MPNs; There are reported cases of HBVr	Humanized monoclonal antibody targeting the C-C chemokine receptor 4; Used for ATLL; HBVr cases have been reported	Anti-CD30 drug conjugated antibody; used in the treatment of relapsed or refractory HL and CD30 positive T-cell lymphoma; There are reported cases of HBVr	Newer generation anti-CD20 monoclonal antibody, similar to rituximab but with greater efficacy; FDA has mandated a warning of the risk of HBVr with obinutuzumab and HBVr has been reported	Used in the treatment of AML; anti-HBV prophylaxis is recommended	Monoclonal antibody against CD38; used in the treatment of hematologic malignancies of B cells; HBVr cases have been reported

CML: Chronic myeloid leukemia; GIST: Gastrointestinal stromal tumors; ATLL: Adult T-cell leukemia/lymphoma; AML: Acute myeloid leukemia; CLL: Chronic lymphocytic leukemia; IRD: Inflammatory rheumatic diseases; TACE: transarterial chemoembolization, APOBEC: Catalytic polypeptide-like apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; MM: Multiple myeloma; NHL: Non-Hodgkin's lymphomas; HL: Hodgkin's lymphoma; HBVr: Hepatitis B virus reactivation.

HBVR RISK IN MPNS TREATED WITH RUXOLITINIB

Ruxolitinib is a commonly used medication to treat MPNs, a group of blood disorders characterized by excessive blood cell production in the bone marrow. One of the common manifestations of MPNs is splenomegaly. Ruxolitinib acts by inhibiting Janus kinases (JAK1 and JAK2), which are enzymes involved in signaling pathways associated with cytokine receptors. By inhibiting these enzymes, ruxolitinib effectively helps control MPNs, particularly intermediate and high-risk myelofibrosis (MF) and high-risk polycythemia vera (PV). Importantly, its effect is not specific to any particular mutation. Ruxolitinib shows good oral bioavailability and reaches its maximum plasma concentration within 1-2 h after administration. Plasma half-life of this drug is approximately 3 h when administered at a maximum tolerated dose of 100 mg once a day. It is mainly metabolized through the CYP3A4 pathway, an important liver enzyme system involved in drug metabolism. Consequently, ruxolitinib has the potential for interactions with medications that induce or inhibit the CYP3A4 pathway. Ruxolitinib is primarily eliminated from the body through metabolism in urine and feces. Therefore, dosage adjustments are necessary for patients with renal or liver impairments, as these conditions can affect the clearance of the drug from the body[15].

It is important to note that this pharmacological agent exhibits immunomodulatory effects, meaning that it can modify the functioning of the immune system. As a result, ruxolitinib treatment may increase susceptibility to opportunistic infections in patients prescribed this drug. Thus, regular monitoring for signs of infection is important when subjects diagnosed with MPNs start taking this medicine[16]. In particular, this pharmacological agent exhibits immunomodulatory and anti-inflammatory actions and can interfere with or impair the innate/adaptive immune response due to its interplay with dendritic cells, regulatory/T-helper lymphocytes or natural killer cells[17,18].

In a case report by Sjoblom *et al*[19], a patient with a history of PV received initial treatment with hydroxyurea. However, due to progressive splenomegaly and fatigue, his treatment was changed to pegylated interferon. Furthermore, to more effectively manage his symptoms, ruxolitinib was introduced. The patient experienced HBVr while on ruxolitinib, which was confirmed by abnormal liver function test results, positive viremia, and newly positive surface antigen for hepatitis B (HbsAg). With the initiation of tenofovir disoproxil, the patient's liver function gradually normalized, indicating successful management of HBVr[19]. In another report by Shen *et al*[20], a patient with MF and a history of HBV infection experienced HBVr during ruxolitinib treatment. The initial elevation in transaminase levels was mistakenly attributed to drug toxicity. Subsequent detection of high plasma levels of HBV DNA confirmed the reactivation. Ruxolitinib was discontinued and antiviral therapy was started, resulting in a gradual decrease in transaminase levels[19,20]. Additionally, in another report by Passucci *et al*[21], a patient with PMF and previous HBV infection achieved resolution of splenomegaly with ruxolitinib therapy. However, HBVr occurred after the patient discontinued prophylactic lamivudine. De-escalation of ruxolitinib and the initiation of anti-HBV therapy led to a gradual decline in HBV DNA levels without signs of active hepatitis[21]. Kirito *et al*[22] highlight the importance of considering prophylactic antiviral therapy in patients with chronic HBV infection before starting treatment with ruxolitinib, as such a proactive measure can help prevent HBVr, as observed in their patient[22].

Ruxolitinib has an immunosuppressive effect, leading to an increased risk of serious infections. The immunosuppressive effect of ruxolitinib is due to its interaction with multiple pathways of the immune system, affecting both adaptive and innate immune responses. This can result in the reactivation of silent infections such as tuberculosis, HBV, and varicella-zoster virus. Therefore, proactive infection surveillance, baseline screening for latent infections, and considering prophylactic or preventive interventions for specific infections such as varicella-zoster virus and HBV virus are crucial[23]. A pilot study conducted by Crodel *et al*[24] investigated the frequency of infections in patients with MPNs. The study included multiple centers and relied on patient-reported data. The findings revealed that over 50% of MPN patients experienced one or more episodes of infection within a 12-mo period. The most frequently reported infections were upper respiratory tract infections, herpes virus infections, and gastrointestinal infections. Among the different subtypes of MPNs, subjects with MF had the highest percentage of infectious events, followed by PV and essential thrombocythemia[24]. Furthermore, Lussana *et al*[25] conducted a systematic review and meta-analysis

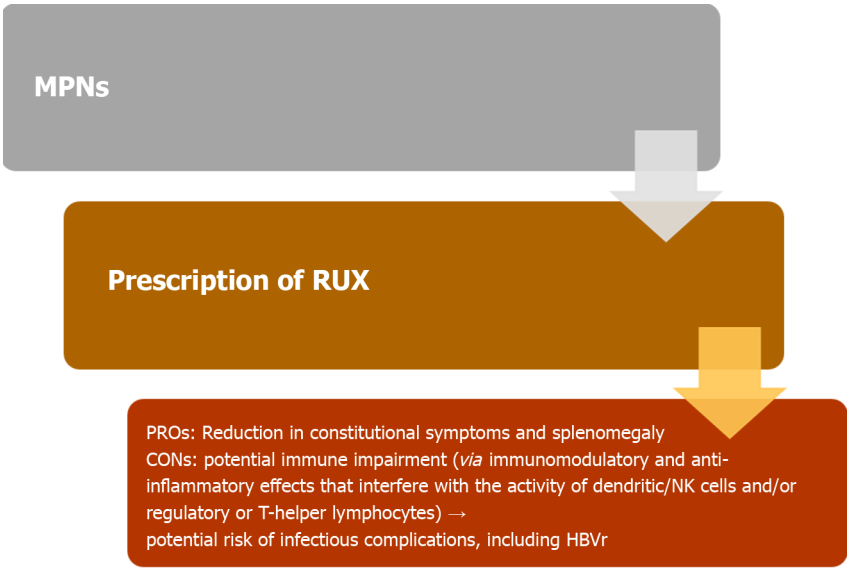

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Figure 1 Benefits and risks of ruxolitinib use in terms of opportunistic infections in myeloproliferative neoplasms. MPNs: Myeloproliferative neoplasms; RUX: Ruxolitinib; HBVr: Hepatitis B virus reactivation; NK cells: Natural killer cells.

examining the safety and efficacy of ruxolitinib in the treatment of MF and PV. The study specifically focused on the incidence of infections in patients receiving ruxolitinib. It was found that ruxolitinib, with its immunosuppressive effects, can affect immune functions and increase the risk of infections. Herpes zoster, pneumonia, bronchitis, and urinary tract infections were among the most frequently reported infectious complications. The aforementioned quantitative assessment emphasized the importance of carefully evaluating infection risk before initiating ruxolitinib therapy and highlighted the need to monitor and address infections in patients receiving ruxolitinib for MF and PV[25].

In a paper by Perricone *et al*[26], two case reports of HBVr in MF patients treated with ruxolitinib are discussed. The immunosuppressive effects of ruxolitinib, particularly in dendritic cells and T cells, may contribute to an increased risk of infections, including HBVr. The article emphasizes the need for vigilance among physicians when considering infectious causes when using immunosuppressive agents such as ruxolitinib[26]. In a prospective study by Gill *et al*[27], 40 patients with MPNs were included. Among the 37 subjects who were negative for HBsAg, 15 tested positive for anti-HBc antibodies, indicating occult HBV infection. Prophylactic treatment for HBV was administered to the three HBsAg positive patients. During a median follow-up of 19.2 mo, four patients (26.7%) experienced HBVr, occurring at a median of 10.5 mo after starting ruxolitinib therapy. The estimated cumulative incidence rates of HBVr at 6 and 12 mo were 7.7% and 30.8%, respectively. This investigation emphasizes the need to monitor HBVr in patients with occult HBV infection who receive ruxolitinib therapy[27]. Garcia-Horton *et al*[28] conducted a retrospective cohort study involving 1171 individuals with MPNs to evaluate the risk of HBVr in subjects treated with ruxolitinib. Among the 58 patients with prior HBV infection, 20 received ruxolitinib. Only one patient experienced HBVr during ruxolitinib therapy, and their HBV DNA levels peaked, but subsequently returned to undetectable levels without interrupting or reducing the ruxolitinib dose[28]. Duan *et al*[29] conducted a retrospective analysis to evaluate the incidence of HBVr in MPN patients treated with ruxolitinib. The study included 62 patients with a history of HBV infection, 56 with resolved infection and 6 with chronic HBV infection. Among patients with chronic HBV infection, two experienced HBVr and hepatitis flare-up after ruxolitinib therapy. None of the patients with resolved HBV infection experienced reactivation. In particular, the two patients with chronic HBV infection did not receive antiviral prophylaxis[29]. Caocci *et al*[30] presented a case report of a patient with MF who experienced HBVr during treatment with ruxolitinib. The patient had a history of HBV infection and initially received ruxolitinib for symptoms related to MF. Although there was improvement in MF symptoms, HBVr was observed through increased levels of HBV-DNA. Adjusting the dose of ruxolitinib resulted in an improvement in symptoms, but HBV-DNA levels remained fluctuating. This case report raises concerns about the management of MF patients with HBV infection receiving ruxolitinib and emphasizes the importance of careful monitoring and potential prophylactic treatment[30].

A schematic representation between the benefits and risks of ruxolitinib use in terms of opportunistic infections in MPNs is depicted in Figure 1.

CONCLUSION

In conclusion, ruxolitinib is an effective medication to manage MPNs such as MF and PV, particularly in intermediate and high-risk cases. By inhibiting JAK1 and JAK2, ruxolitinib helps control excessive blood cell production and reduce splenomegaly. However, its use carries certain risks and considerations. The interaction of ruxolitinib with the immune system can increase the susceptibility to opportunistic infections, highlighting the need for vigilant monitoring and timely

intervention. Furthermore, there is a potential for HBVr, especially in patients with a history of HBV infection. Close monitoring of liver function and proactive measures, such as prophylactic antiviral therapy, are crucial to managing these risks. In general, ruxolitinib offers therapeutic benefits for MPNs, but careful evaluation of infection risk, regular monitoring, and appropriate interventions are essential to ensure patient safety.

FOOTNOTES

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MINIREVIEWS

Function of macrophage-derived exosomes in chronic liver disease: From pathogenesis to treatment

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Abstract

Chronic liver disease (CLD) imposes a heavy burden on millions of people worldwide. Despite substantial research on the pathogenesis of CLD disorders, no optimal treatment is currently available for some diseases, such as liver cancer. Exosomes, which are extracellular vesicles, are composed of various cellular components. Exosomes have unique functions in maintaining cellular homeostasis and regulating cell communication, which are associated with the occurrence of disease. Furthermore, they have application potential in diagnosis and treatment by carrying diverse curative payloads. Hepatic macrophages, which are key innate immune cells, show extraordinary heterogeneity and polarization. Hence, macrophage-derived exosomes may play a pivotal role in the initiation and progression of various liver diseases. This review focuses on the effects of macrophage-derived exosomes on liver disease etiology and their therapeutic potential, which will provide new insights into alleviating the global pressure of CLD.

Key Words: Chronic liver disease; Macrophage; Exosomes; Function; Etiology; Treatment

Core Tip: Chronic liver disease (CLD) affects hundreds of millions of individuals worldwide, and identifying the causes and researching viable therapies could lessen the global burden. As nanovesicles produced by cells, exosomes are able to facilitate intercellular communication and play a crucial role in a variety of systemic disorders. Immune cells such as macrophages are intimately associated with liver diseases. In this review, the importance of macrophage-derived exosomes in CLD, from pathophysiology to therapeutic potential, is highlighted.

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INTRODUCTION

Chronic liver disease (CLD) imposes a heavy burden on millions of people worldwide. A total of 1.5 billion people worldwide had suffered from CLD by 2017[1]. Approximately 2 million individuals worldwide die each year from liver disease[2]. Chronic hepatitis, cirrhosis and liver cancer are the three main CLDs. Among the ranked global causes of death, cirrhosis is 11th, while hepatocellular carcinoma (HCC) is 16th[3]. We reviewed numerous studies and found that the pathogenesis of CLD is multifactorial. Most liver diseases are associated with steatosis, oxidative stress, alcoholism, inflammation[4], the environment, the microbiome, metabolism, and genetic factors[5]. However, in addition to liver transplantation, current therapy consists only of eliminating the etiology and treating the complications of cirrhosis[6]. Therefore, the exploration of the pathogenic mechanism and identification of optimal treatment strategies are urgently needed.

Exosomes can be released into biological fluids by all cells under physiological or pathological conditions. Exosomes are produced by budding and have apparent molecular heterogeneity because of various membrane-related protein complexes. Exosomes contain components including RNAs, DNAs, lipids, proteins, amino acids, and metabolites[7]. These soluble and extracellular components can enter cells through endocytosis and plasma membrane invagination, which involve the surface proteins on exosomes[8]. Substances within exosomes stimulate recipient cells, thereby altering signal transduction pathways[9]. Therefore, by carrying these payloads, exosomes can mediate intercellular communication[10]. When exosomes are taken up by other cells, the components alter the phenotype of recipient cells and disrupt the dynamic equilibrium of cellular transformation, demonstrating their unique functions in maintaining cellular homeostasis[11,12]. Furthermore, exosomes may serve as biomarkers or therapeutic targets in the diagnosis and treatment of various diseases[9,11,13]. At present, exosomes have been widely studied in liver diseases. In alcoholic liver disease (ALD)[14], alcohol increases the generation of exosomes, which is due to autophagic damage and the destruction of autophagosomes or lysosomes. During the progression of nonalcoholic fatty liver disease (NAFLD)[15], hepatocyte-derived exosomal microRNA (miR)-192-5p can activate proinflammatory macrophages. In liver fibrosis[16], activated hepatic stellate cells (HSCs) release fibrogenic vesicles. Furthermore, engineered exosomes can be used for drug delivery. For instance, Tang *et al*[17] showed that exosomes from mesenchymal stem cells (MSCs) modified to carry small interfering RNA or an antisense oligonucleotide targeting signal transducer and activator of transcription 3 (STAT3) could directly inhibit STAT3 to treat fibrosis. Lou *et al*[18] also modified exosomes from MSCs to carry miR-199a-3p, which improved the chemotherapy sensitivity of liver cancer, proving that exosomes can be used as novel therapeutic agents by acting as nanocarriers to deliver drugs or molecules. In conclusion, exosomes can drive or inhibit disease progression and have potential utility in liver disease therapy.

Macrophages are the core cellular component of the liver and are crucial in maintaining organ homeostasis and coping with liver injury[19]. As key immune cells, they contribute to the development of hepatic disease by polarizing into diverse phenotypes in response to microenvironmental stimulation[20] or expressing their heterogeneity through the production of cytokines, cell surface markers, and transcriptomes[21]. Due to the specific physiological roles of macrophages, the involvement of macrophage-derived exosomes in the development and progression of liver disease has been extensively studied. For instance, relaxin is an antifibrotic peptide hormone that affects vasodilation, thereby alleviating fibrosis and protecting organs through its cognate G protein-coupled receptor relaxin family peptide receptor 1. A study showed that after binding to receptors expressed by hepatic macrophages, relaxin can change the phenotype of macrophages, and macrophages can release exosomes to promote relaxin-mediated HSC dormancy and alleviate hepatic fibrosis (HF)[22]. *In vitro*, interleukin-6 (IL-6) treatment upregulated exosome generation-related genes, stimulating the release of miR-223-rich exosomes from macrophages, which can transfer and reduce the expression of fibrogenic TAZ in liver cells to alleviate liver fibrosis[23]. However, macrophage-derived exosomes can also accelerate disease progression; for example, exosomes derived from macrophages treated with lipopolysaccharide (LPS) promote liver fibrosis[24], and exosomes derived from M2 macrophages can mediate HCC metastasis[25]. At present, determining how to use macrophage-derived exosomes to treat disease has become a research hotspot. Studies have shown that macrophage-derived exosomes can be used as natural nanocarriers to deliver proteins[26] or drugs[27], induce immune activation and participate in immunotherapy[28]. Overall, investigating the various physiological functions of macrophage-derived exosomes can lead to a better understanding of the pathogenesis and treatment of CLD.

This review discusses the biology and physiological functions of exosomes, focusing on exosomes derived from macrophages in the etiology of liver disease, as well as the possible use of exosomes in diagnosis, prognosis, and treatment, which will help in the search for the best therapeutic strategies for liver disease and contribute to reducing the global burden of liver disease (Figure 1).

OVERVIEW OF MACROPHAGE-DERIVED EXOSOMES

Exosomes are a subtype of extracellular vesicles secreted by all cells and are widely distributed in various body fluids. These factors mediate cell-to-cell communication and play specific roles in normal physiological functions and the occurrence of diseases. Macrophages are important immune cells in the human body, and their unique heterogeneity and phenotype result in different physiological effects. In addition, macrophage-derived exosomes participate in the occurrence of diseases in various systems.

Biology of exosomes

Exosomes are small nanoscale vesicles with diameters of 30-150 nm[29]. They originate in endosomes and form mature exosomes during interactions with other vesicles or organelles[8]. First, the cell invaginates the cell membrane through budding to generate clathrin bodies, which enter the cytoplasm and form early endosomes[30,31]. Late endosomes mature from early endosomes by interacting with the Golgi complex, which can form intraluminal vesicles (ILVs) by invagination of the restrictive membrane[10]. ILVs are then further endocytosed to generate multivesicular bodies (MVBs), which are known as multivesicular endosomes[8]. Ultimately, MVBs have two outcomes: Some MVBs enter the lysosomal pathway and are degraded by the lysosome, while others fuse with the cell membrane and release multiple vesicular structures into the extracellular matrix as exosomes[31]. Therefore, the biogenesis of exosomes can be divided into the following processes: Budding, envelope invagination, MVB production, and MVB release. All cells, whether normal or abnormal, can release exosomes, and cancer cells release more exosomes than other cells[9]. Exosomes can exhibit unique characteristics based on their cell origin and material composition. As a double lipid-encapsulated vesicle, exosomes contain a variety of substances, such as DNAs, RNAs, lipids, and proteins, and the composition or contents of these vesicles vary depending on the cell source[32]. Moreover, exosomes can be isolated from various types of body fluids, such as plasma[33], serum[34], and urine[35]. In summary, exosomes are tiny vesicles secreted by various cells, and their secretion mechanism is related to membrane fusion. In addition, secreted exosomes can be widely distributed throughout the body, enabling them to play a role in a variety of systems.

Function of exosomes

Because structure determines function, the function of exosomes is dependent on their complex and specific characteristics, which are determined by the cell type from which they are derived. Exosomes can be involved in the immune response, antigen presentation, cell migration, cell differentiation, tumor invasion, and other processes. The most important function of exosomes is to mediate intercellular communication[9]. Exosomes transfer cargo to recipient cells by binding to cell surface receptors, undergoing plasma membrane fusion, or through the endocytic system[36], which can activate signaling pathways, alter gene expression, or regulate the overall function of the recipient cell. For example, the decrease in hsa_circ_0074854 carried by exosomes secreted by HCC cells can inhibit M2 macrophage polarization, thus delaying the migration and invasion of HCC cells[37]. Cancer-associated fibroblasts can transfer exosomal miRNAs directly to tumor cells and enhance their cell-related functions, such as epithelial-mesenchymal transition and chemoresistance[38]. Therefore, exosomes can mediate material exchange between cells, are involved in cellular communication, and reflect the different physiological functions of cells; thus, they are often used as biomarkers of diseases or targeted delivery vectors of substances. The expression levels of some miRNAs in serum exosomes are significantly upregulated in patients with pancreatic cancer; thus, some miRNAs are considered useful markers for the early diagnosis and progression of pancreatic cancer[39]. The delivery of engineered exosomes loaded with an iron shedding inducer [erastin (ER)] and photosensitizer (rose bengal) into tumor tissue can specifically induce ferroptosis in HCC cells, which can be used as a new treatment strategy for malignant tumors[40]. In conclusion, exosomes have wide-ranging functions. Whether as carriers of substances or as signaling factors, exosomes have powerful cellular communication functions, which is an area for future basic research to determine clinical applications.

Exosomes derived from macrophages

Macrophages are immune cells that can be found in most tissues and serve numerous roles[41]. For instance, macrophages are derived from monocytes and have phagocytic functions; these cells can engulf and kill intracellular parasites, bacteria, and tumor cells, as well as aging and abnormal cells, which is critical for immune defense, immune stability, and immune surveillance. In addition, macrophages are uniquely heterogeneous. In a dynamically changing microenvironment, macrophages can exhibit two phenotypes that perform different functions: Classically activated macrophages (M1) and alternatively activated macrophages (M2)[42]. M1 macrophages can be activated by LPS alone or in combination with T-helper 1 (Th1) cytokines [such as interferon (IFN)-γ and granulocyte-macrophage colony-stimulating factor] and can produce proinflammatory cytokines, such as IL-1β, IL-6, IL-12, IL-23, tumor necrosis factor (TNF)-α, chemokine (C-X-C motif) ligand (CXCL) 1-3, CXCL8-10, chemokine (C-C motif) ligand (CCL)2-5, and CCL11. Therefore, M1 macrophages are able to mediate functions such as antigen presentation, Th1 immune reactions, proinflammatory effects, pathogen elimination, and antitumor activity[43-46]. However, M2 macrophages can be further divided into M2a, M2b, M2c, and M2d subtypes and can release complex cytokines. Specifically, when injury causes an acute

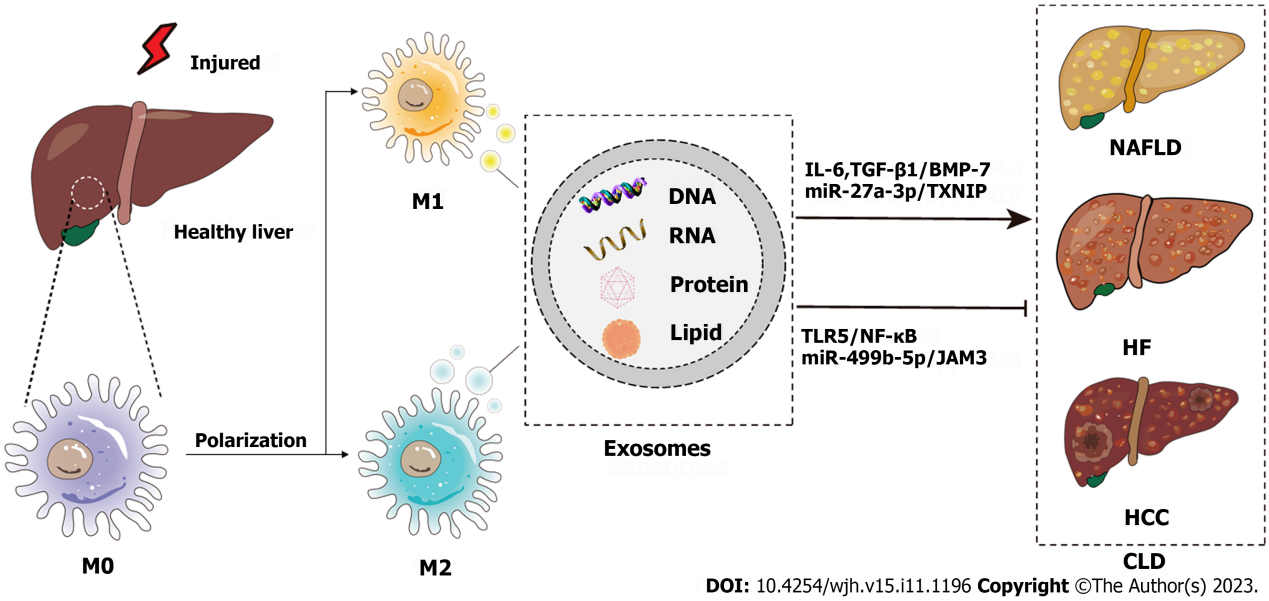

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Figure 1 Schematic diagram of the pathogenesis of chronic liver disease from the perspective of macrophage-derived exosomes. Injured livers activate macrophages to secrete exosomes that encapsulate RNAs, DNAs, lipids, proteins, *etc.*, which influence the development of chronic liver disease through various signaling pathways. CLD: Chronic liver disease; NAFLD: Nonalcoholic fatty liver disease; HF: Hepatic fibrosis; HCC: Hepatocellular carcinoma; IL-6: Interleukin-6; TGF: Transforming growth factor; BMP: Bone morphogenetic protein; TXNIP: Thioredoxin-interacting protein; TLR: Toll-like receptor.

inflammatory response or organ fibrosis, inflammatory factors such as IL-4 and IL-13 activate M2 macrophages to transform into M2a macrophages to inhibit inflammation, mediate damage repair and promote fibrosis. In response to LPS and immune complexes, M2 macrophages can be transformed into M2b macrophages, which can regulate immunity and induce the occurrence of infection and cancer. Glucocorticoids, IL-10, and transforming growth factor-beta (TGF-β) can induce the polarization of M2 macrophages to M2c macrophages, which perform functions such as phagocytosis, immunosuppression, and tissue remodeling. Moreover, M2d macrophages are induced by toll-like receptor (TLR) agonists and adenosine A2A receptor ligands to cause angiogenesis and promote cancer. M2 macrophages can produce various cytokines, including Arg1, CCL17, CCL22, IL-10, IL-1β, IL-6, TNF-α, IL-12, IL-10, TGF-β, CXCL13, and IL-10, which can mediate numerous functions, including inhibiting inflammation, wound healing, the Th2 immune response, anaphylaxis, fibrosis, immune regulation, supporting tumors, promoting infection, and angiogenesis[47-51]. Therefore, when homeostasis is disrupted, the polarization of different macrophage phenotypes occurs, which means that macrophages are remarkably plastic cells (Figure 2).

Macrophages are capable of secreting exosomes, and macrophage-derived exosomes are present in multiple systems. In addition, macrophage-derived exosomes are involved in various diseases and act as therapeutic targets and drug carriers. In recent years, research has mainly focused on the involvement of macrophage-derived exosomes in systemic diseases. For instance, in multimicrobial sepsis, lactate promotes macrophages to release exosomes containing lactated/acetylated high mobility box-1 (HMGB1), which increases endothelial cell permeability and accelerates sepsis. Therefore, reducing circulating levels of exosomal HMGB1 be a therapeutic strategy for the treatment of sepsis[52]. In head and neck squamous cell carcinoma (HNSCC), the long non-coding RNA (lncRNA) HOTTIP in exosomes secreted by M1 macrophages upregulates the TLR5/NF-κB signaling pathway *via* the competing sponges miR-19a-3p and miR-19b-3p to inhibit the progression of HNSCC[53]. In the respiratory system, Wei *et al*[54] found that the exosomes derived from M2 macrophages in patients with lung adenocarcinoma could encapsulate miR-942, and these exosomes enhanced the invasion and migration of lung adenocarcinoma cells and promoted angiogenesis by regulating FOXO1 protein to alleviate β-catenin inhibition. In addition, macrophage-derived exosomes are also valuable in the treatment of chronic pulmonary fibrosis[55] and asthma[56]. In the circulatory system, macrophage-derived exosomes accelerate atherosclerosis in patients with diabetes[57], but they can also be used for myocardial tissue repair in acute myocardial infarction[58]. Moreover, macrophage-derived exosomes can serve as carriers for drug delivery. In a mouse model of Alzheimer's disease (AD), macrophage-derived exosomes could carry silybin, allowing it to cross the blood-brain barrier and reduce astrocyte-mediated neuroinflammation and improve cognitive deficits in AD mice[59]. In conclusion, macrophage-derived exosomes are widely distributed in the body and are associated with a variety of systemic diseases, and exosomes can play an important role in the treatment of diseases by acting as therapeutic targets or drug carriers. Given the importance of macrophages in the physiology of the liver, much research has been devoted to the link between macrophage-derived exosomes and the onset of CLD. Therefore, this review focuses on the impact of macrophage-derived exosomes on the etiology and treatment of CLD.

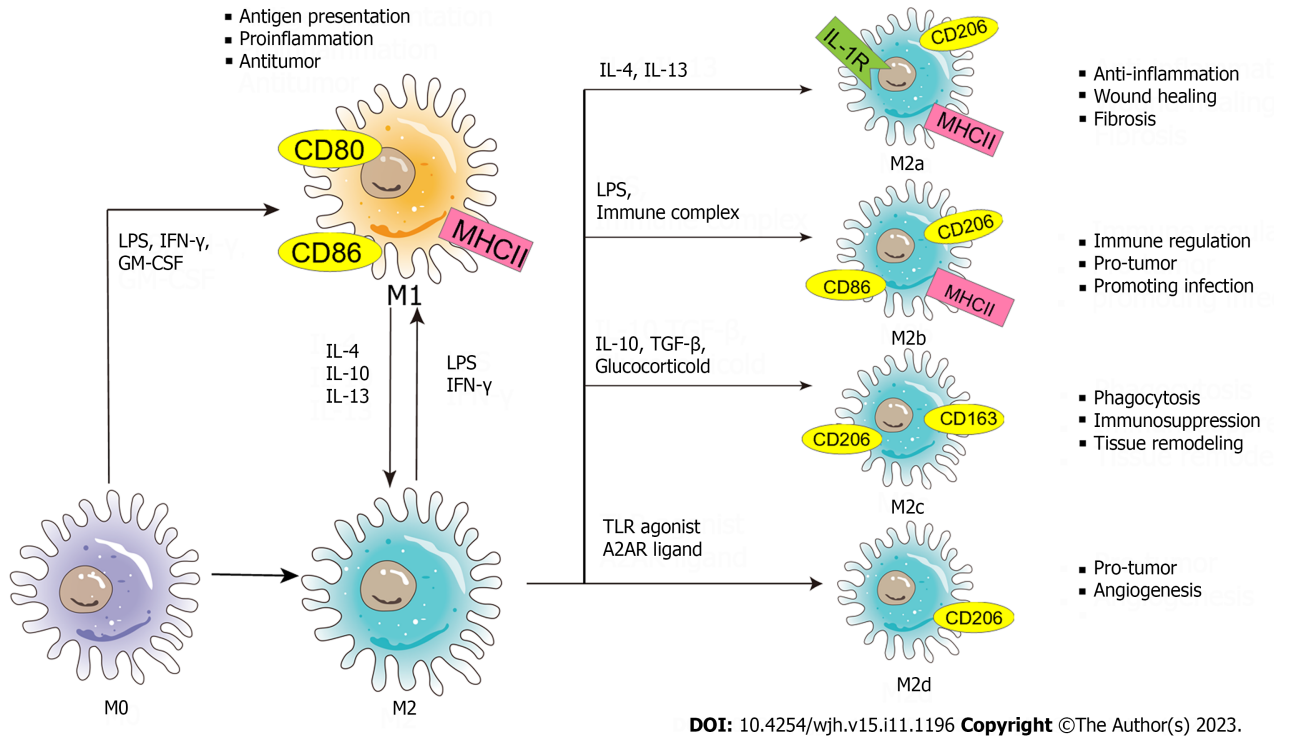

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Figure 2 Schematic diagram of the phenotypes and functions of macrophage polarization. The nature macrophage can be activated by a variety of influencing factors (such as lipopolysaccharide, interferon-γ, granulocyte-macrophage colony-stimulating factor, *etc.*) and polarized into two phenotypes - classically activated macrophages and alternatively activated macrophages. Exosomes carry stimulatory factors to activate macrophages. Macrophages themselves secrete exosomes to form a signal transmission network between macrophages and other cells. (M0: M0 macrophage; M1: M1 macrophage; M2: M2 macrophage; M2a: M2a macrophage; M2b: M2b macrophage; M2c: M2c macrophage; M2d: M2d macrophage; LPS: Lipopolysaccharide; IFN-γ: Interferon-γ; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; TLR: Toll-like receptor; A2AR: A2A receptor; MHC: Major histocompatibility complex; TGF: Transforming growth factor.

MACROPHAGE-DERIVED EXOSOMES IN CLD

CLD has impacted tens of thousands of patients worldwide, its pathogenesis has been explored, and its therapeutic regimen has been optimized. In recent years, there has been intensive study of the function of macrophage-derived exosomes in some diseases. In this review, the use of macrophage-derived exosomes to treat NAFLD, HF, HCC, and other liver diseases is discussed in the context of pathogenesis and therapeutic potential.

NAFLD

NAFLD is the most common CLD and is recognized as a global public health problem. The pathogenesis of NAFLD is complex[5,60]. During the pathogenesis of NAFLD, hepatic damage caused by inflammation, oxidative stress, and lipotoxicity eventually cause collagen deposition and fiber regeneration, which lead to liver fibrosis[61]. During this process, obesity, type 2 diabetes, resistance to insulin, HSC activation, the environment, genetics, and other factors accelerate the progression of liver injury. However, due to the complexity of the pathophysiology and the heterogeneity of disease phenotypes, there is currently no specific drug to treat NAFLD. Healthy lifestyle interventions and weight loss are mostly used to prevent this disease in high-risk groups, and individualized combination therapy is often used to treat patients[62]. Only a better understanding of the pathogenesis and progression of this disease can provide a more accurate treatment plan. Therefore, we focused on the role of exosomes in NAFLD and their potential use in treatments.

The study of macrophage-derived exosomes in the pathogenesis and treatment of NAFLD has made considerable and remarkable progress and has potential applications in NAFLD therapy. For example, in sepsis associated with NAFLD, exosomes released by Trem2-deficient macrophages carry a large amount of miR-106b-5p and cause abnormal mitochondrial structure and energy metabolism in hepatocytes by blocking mitofusin 2 (Mfn2), which accelerates the progression of NAFLD and increases the susceptibility of NAFLD patients to sepsis[63]. In addition, miR-155-rich exosomes secreted by adipose tissue macrophages improved insulin sensitivity and maintained glucose homeostasis in obese mice[64]. Similarly, miR-69 released from M2 macrophages had the same effect[65]. In obese individuals, macrophage-derived exosomes are increased and delivered to hepatocytes, thereby regulating obesity-related insulin resistance[66] (Figure 3). In conclusion, exosomes derived from macrophages typically carry different cargos and transfer them between liver cells, and these exosomes can not only initiate but also delay the progression of NAFLD.

HF

HF is a dynamic and highly integrated molecular, cellular, and tissue process involving most types of CLD that undergo

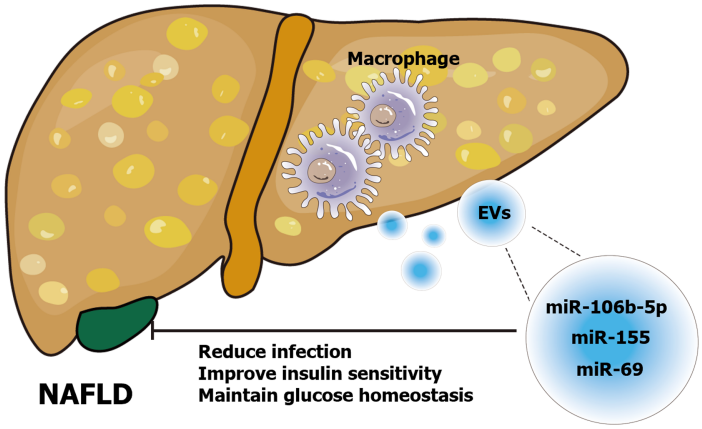

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Figure 3 Schematic diagram of the role of macrophage-derived exosomes in nonalcoholic fatty liver disease. Exosomes derived from macrophages carry different microRNAs (miR-106b-5p[63], miR-155[64], miR-69[65]) to act on nonalcoholic fatty liver disease cells and alleviate the disease progression by regulating liver homeostasis. NAFLD: Nonalcoholic fatty liver disease; EVs: Extracellular vesicles; miRNA: MicroRNA.

repeated substantial liver damage and continuous activation of inflammatory responses[67]. During these processes, the activation of hepatic myofibroblasts results in the secretion of extracellular matrix proteins, including collagen, which can lead to fibrous scars and, eventually, the development of HF[68]. If not treated in time, HF will progress to cirrhosis, HCC, and eventually liver failure, but unfortunately, the only treatment that can achieve a complete cure is liver transplantation[69]. Previously, hepatic macrophages, which are specific immune cells, were shown to be important in the progression of HF. Macrophages mediate various functions in fibrotic liver homeostasis, disease progression, and injury recovery and are considered potential targets to protect against fibrosis[21]. Notably, intercellular crosstalk between hepatic macrophages and HSCs is vital for stimulating HSC activation[24]. Therefore, exosomes derived from macrophages are important for intercellular crosstalk during the pathogenesis of HF.

Recent studies have shown that exosomes derived from different sources are involved in the pathogenesis, diagnosis, and potential treatment of HF. This review will focus on research on macrophage-derived exosomes in HF. On the one hand, exosomes derived from macrophages accelerate HF. For instance, when THP-1 macrophages were treated with LPS, the exosomes secreted by these cells were changed, and the alteration in miRNA correlated with HF progression, increasing fibrotic gene expression and promoting HSC replication and activation[24]. Similarly, another study showed that exosomes secreted by LPS-treated macrophages could overexpress miR-500, which promoted the proliferation and activation of HSCs to accelerate the progression of fibrosis by inhibiting MFN2[70]. Deng *et al*[71] found that exosomes derived from LPS-treated macrophages could increase the expression levels of collagen-1 and alpha-smooth muscle actin in JS1 cells. According to recent research, LPS stimulation enhances the expression of miR-155-5p in macrophage-derived exosomes, which facilitates the activation of HSCs, resulting in oxidative stress and collagen production[72]. Autophagy contributes to the progression of liver damage in the early stages of liver fibrosis, and CCL4 can exacerbate autophagy, which causes M1 macrophages to polarize and secrete exosomes rich in miR-423a-5p to encourage HSC activation and control HF[73]. The miRNAs carried by macrophage-derived exosomes in these examples could accelerate HF, but they were also shown to be expressed at high levels in serum, which suggested that these miRNAs can be used as biomarkers for the diagnosis of fibrosis. On the other hand, exosomes derived from macrophages can be used to delay HF. Hepatic macrophages are important mediators of relaxin-mediated amelioration of HF. When the relaxin receptor on macrophages binds to relaxin, their phenotype can be changed from the profibrogenic phenotype to the pro-resolution phenotype, and the pro-resolution phenotype can secrete exosomal miR-30a-5p to inhibit the growth of HSCs. Therefore, nanoparticle-mediated delivery of miR-30a-5p can alleviate liver fibrosis[22]. In another recent study, phillygenin, an active ingredient in the Chinese medicine Forsythiae Fructus, was shown to inhibit StarD13-targeted M1 macrophage exosomal miR-125b-5p to reduce HSC activation, thereby alleviating the progression of liver fibrosis[74]. Macrophage-derived exosomes can also delay or treat the occurrence of NAFLD-HF. For instance, miR-411-5p is present in exosomes secreted by M2 macrophages and can suppress HSC activation in NAFLD[75]. In contrast, miR-223-enriched exosomes suppressed the expression of profibrotic TAZ to inhibit the development of NAFLD[23]. Kupffer cells (KCs) are a special type of macrophage in the liver that can also produce exosomes. In nonalcoholic steatohepatitis (NASH), KCs deliver endogenous miR-690 to HSCs *via* exosomes, which can help treat HF in NASH by suppressing the expression of profibrotic genes[65]. These results demonstrate that macrophage-derived exosomes can alleviate the progression of HF and interact with HSCs (Figure 4). Therefore, research on macrophage-derived exosomes in HF mainly focuses on the crosstalk between macrophages and HSCs, and the mechanisms are diverse and should be analyzed from multiple perspectives. Since the search for a better treatment strategy for HF is ongoing, future studies should focus on how to use macrophage-derived exosomes to diagnose or treat HF.

HCC

Worldwide, HCC is one of the most common causes of cancer death, and HCC is a major type of liver cancer, accounting for more than 90% of cases[76]. HCC is caused by many pathogenic factors, and its prognosis is poor. Most patients are

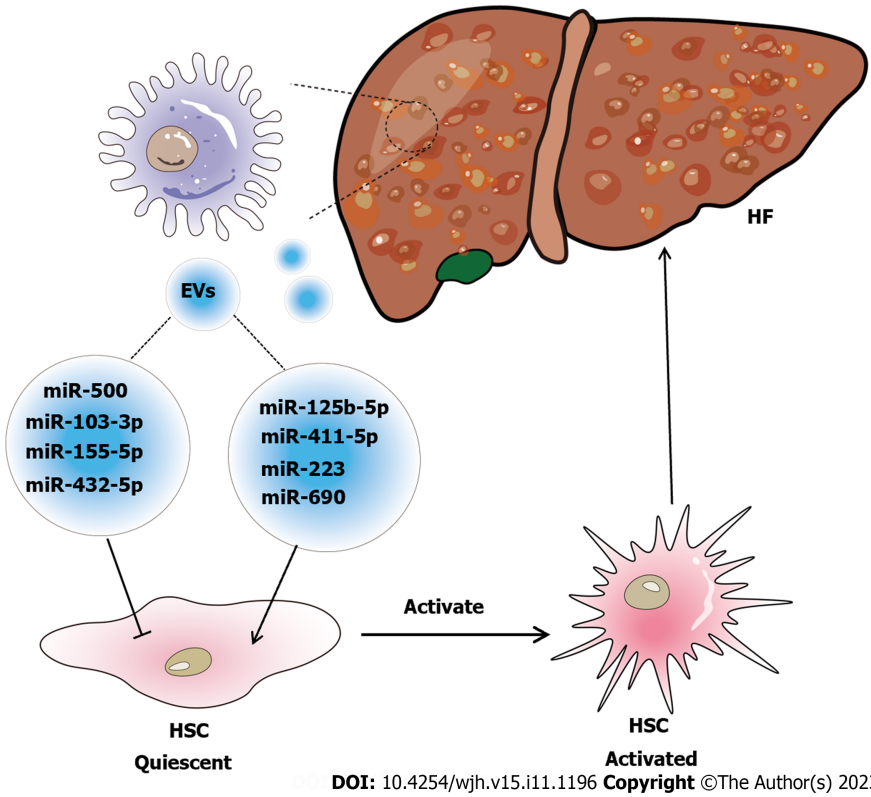

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Figure 4 Schematic diagram of the relationship between macrophage-derived exosomes and hepatic fibrosis. Exosome-mediated communication between macrophages and hepatic stellate cell (HSC) influences the disease progression of hepatic fibrosis (HF). Special substances carried in exosomes, such as microRNAs (miR-103-3p[24]; miR-500[70]; miR-155-5p[72]; miR-432-5p[73]; miR-125b-5p[74]; miR-411-5p[75]; miR-690[65]; miR-223[23], are signaling factors that induce the activation of HSC to form cirrhosis or inhibit the activation of HSC to alleviate HF. EVs: Extracellular vesicles; HSC: Hepatic stellate cell; HF: Hepatic fibrosis.

diagnosed with advanced HC, for which chemotherapy and immunotherapy are by far the best treatment options[77]. Therefore, the burden of HCC in the world is still severe, and it is important to continue to find new methods for early diagnosis and treatment to improve HCC prognosis. Because exosomes mediate cell communication and carry substances that can be exchanged between cells, macrophages can take up exosomes released by tumor cells in the tumor microenvironment, which affects tumor growth or metastasis by altering macrophage phenotypes. For example, Li *et al*[78] found that exosomes produced by HCC contain abundant levels of lncRNA TUC339, which alters macrophage phenotype, ultimately accelerating the rapid growth of tumors by promoting tumor immune evasion. In addition, another study showed that ER-stressed HCC cells released exosomal miR-23a-3p, which upregulated programmed cell death ligand 1 expression in macrophages and inhibited T-cell functions, thereby ameliorating tumor progression[79]. Similarly, ER-stressed HepG2 cell-derived exosomes promoted the expression of cytokines through the activation of the JAK2/STAT3 pathway, and these exosomes ultimately led to the immunosuppression of macrophages and promoted tumor growth [80]. These results indicate the close connection between exosomes secreted by hepatic carcinoma cells and macrophages, and the impact on macrophages has a robust effect on the growth of HCC. Therefore, we discuss the role of macrophage-derived exosomes in HCC, including the mechanism and application value in diagnosing or treating HCC (Figure 5 and Table 1).

Studies have shown that exosomes derived from macrophages mainly promote the growth and invasiveness of HCC. According to recent research, tumor-associated macrophage (TAM)-derived exosomal lncRNAs increase aerobic glycolysis and cell growth in HCC by controlling the miR-548s/ALDH1A3 pathway, thereby contributing to disease malignancy[81]. Liu *et al*[82] discovered that the miRNAs in exosomes secreted by macrophages were altered, and these miRNAs could reduce androgen receptor (AR) expression and translation to enhance the invasion of HCC. In addition, miR-15b was increased in the exosomes of arsenite-treated macrophages, and this factor could be delivered to HCC cells to promote HCC[83]. Exosomes produced by M2-polarized macrophages could induce TGF-β1/bone morphogenetic protein 7 pathway imbalances and promote the invasiveness of liver cancer[84]. Similarly, Li *et al*[85] reported that M2 macrophage-derived exosomes were rich in miR-27a-3p, and these exosomes could promote cancer cell stemness *via* the miR-27a-3p/thioredoxin-interacting protein pathway. Moreover, miR-660-5p-rich M2 macrophage-derived exosomes could promote liver cancer development by downregulating KLF3[86]. These results indicate that some substances loaded in exosomes can promote tumor growth, invasion, and metastasis, which can provide new insights into the pathogenesis of HCC.

In addition, these results increase enthusiasm for using exosomes in cancer treatment. For example, exosomal miR-628-5p generated by M1 macrophages prevented the m6A alteration of circFUT8, which prevented the proliferation of HCC [87]. Additionally, M1 macrophage-derived exosomal miR-326 inhibited HCC cells from proliferating, forming colonies,

Table 1 Association of the macrophage-derived exosomes with hepatocellular carcinoma

Ref.	Macrophage cell type/phenotype	HCC cell lines/model/tissue	Exosome contents	Findings
Liu *et al* [82], 2020	THP-1/M2	Human HCC cell lines: SK-HEP-1 and HepG2 cell; mouse HCC cell lines: Hepa 1-6	MiR-92a-2-5p	Promote HCC invasion *via* altering the AR/PHLPP/p-AKT/β-catenin signaling
Li *et al* [83], 2021	THP-1/M2	Human HCC cell lines: SMMC-7721	MiR-15b	Promotes the progression of HCC by blocking the LATS1-mediated Hippo pathway
Li *et al* [85], 2021	THP-1/M2	Human HCC cell lines: Huh7, 97H, HepG2, LM3 and SMMC-7721	MiR-27a-3p	Promote cancer stemness of HCC *via* the miR-27a-3p/TXNIP pathways
Tian *et al* [86], 2021	THP-1/M2	Human HCC cell lines: HepG2 and Bel-7402; human HCC tumor tissues	MiR-660-5p	Promote the development of HCC by regulating KLF3
Bai *et al* [88], 2020	THP-1/M1	Human HCC cell line BEL-7404, HepG2, SMMC-7721 and QGY-7703; Xenograft nude mouse model	MiR-326	Suppresses HCC progression *via* NF-κB signaling pathway
Pu J *et al* [89], 2021	C57BL/6 mouse bone marrow-derived macrophages/M2	Murine model of primary HCC (C57BL/6)	MiR-21-5p	Facilitate CD8+T cell exhaustion in HCC *via* the miR-21-5p/YOD1/YAP/β-catenin pathway
Wang *et al* [90], 2019	TAMs/M1	Human HCC cell lines: Huh7, HepG2 and BEL-7404; human HCC tumor tissues	MiR-125a/b	Suppressed HCC cell proliferation and stem cell properties by targeting CD90
Zhang *et al* [92], 2022	THP-1/M2	Human HCC cell lines: SMMC-7721and HepG2; Xenograft nude mouse model (BALB/C)	hsa_circ_0004658	Inhibits HCC progression *via* miR-499b-5p/JAM3

HCC: Hepatocellular carcinoma; M1: M1 macrophage; M2: M2 macrophage; TAMs: Tumour-associated macrophages; miRNA: MicroRNA; AR: Androgen receptor; LATS1: Suppressor kinase 1; TXNIP: Thioredoxin-interacting protein.

migrating, invading, and promoting apoptosis by decreasing the expression of NF-κB[88]. Exosomes released by M2 macrophages could also prevent HCC. M2 macrophage-derived exosomal miR-92a-2-5p enhanced liver cancer invasion, and preclinical research showed that inhibiting miR-92a-2-5p in macrophages could reverse the effect of coculture on AR and weaken the invasion of HCC[82]. MiR-21-5p in exosomes derived from M2 macrophages could enter HCC tissue and deplete CD8+ T cells, providing new insights into tumor immunotherapy[89]. Another team showed that the levels of miR-125a and miR-125b in exosomes secreted by TAMs could inhibit HCC stem cells[90]. Chen *et al*[91] recently showed that IL-2 was an important factor that further regulated TAM-derived exosomal miRNAs to enhance the inhibition of cancer progression. Likewise, RBPJ+/+ macrophage-derived exosomes could also suppress neoplasms[92]. Further study of the effects of these exosomes in the treatment of liver cancer would provide important value in the search for early diagnostic screening markers for patients, which will help in the development of novel schemes for clinical treatment and is critical for reducing the burden of HCC patients worldwide.

Other CLD

The range of CLDs is varied; in addition to NAFLD, HF, and HCC, CLDs also include alcoholic fatty liver disease, viral liver disease, and immune liver disease. Current research into the role of macrophage-derived exosomes in other liver diseases also deserves attention.

Viral hepatitis is a liver disease caused by infection by various hepatitis viruses. The prognosis is generally good, but due to inappropriate lifestyles and untimely treatment, some cases progress to more serious liver diseases, such as liver failure. Therefore, some studies have explored the relationship between exosomes and viral hepatitis. A study showed that macrophage-derived exosomes could spread to hepatocytes and promote IFN-α-induced hepatitis B virus (HBV) resistance, and these factors relied on the main pathways of viral invasion[93]. Antiviral molecules can also enter hepatocytes through internalized INF-α-treated macrophage-derived exosomes, thereby reducing the replication of HBV [94]. Similarly, exosomes released from Tlr3-activated macrophages are enriched in many hepatitis C virus (HCV)-resistant miRNAs, and when these exosomes are taken up by HCV hepatocytes, they can mediate anti-HCV activity by inhibiting HCV replication in cells, which suggests a potential treatment for HCV[95]. These studies show that in viral liver diseases, exosomes carrying antiviral substances are transmitted from macrophages and absorbed by diseased liver cells; thus, these exosomes are therapeutic carriers, indicating a potential novel treatment method for viral hepatitis. In ALD, the main causative factor is alcohol intake. Alcohol stimulation increases the expression of miR-155 and increases the release of macrophage-derived exosomes by reducing lysosome-associated proteins in the liver, leading to the dysregulation of lysosomal autophagy[14]. Alcohol exposure can increase the number of miR-27a-rich exosomes produced by monocytes, which can polarize primitive monocytes into M2 macrophages[96]. In another study, macrophage-derived exosomes were shown to participate in immune regulation in concanavalin A-induced hepatitis[97]. A recent study showed that Concanavalin A could promote the release of exosomes from type I macrophages and that these exosomes contained the lncRNA H19, which induced apoptosis in autoimmune liver disease cells, suggesting a new avenue for developing treatments for autoimmune liver diseases[98]. In summary, research on exosomes in liver diseases has

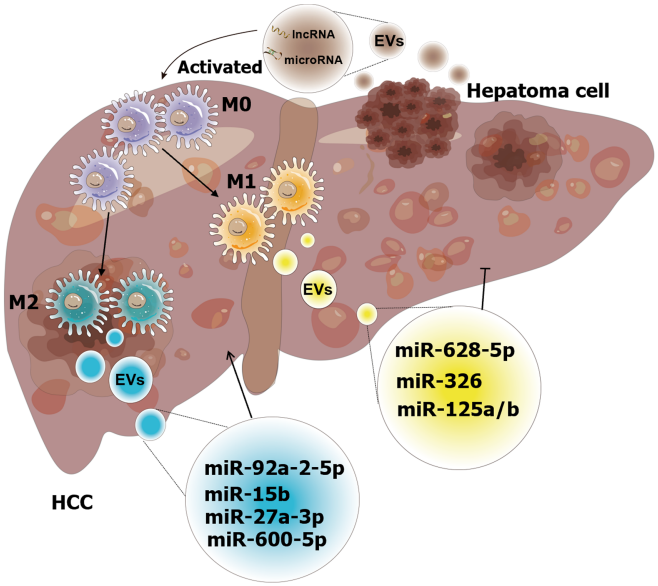

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Figure 5 Schematic diagram of the effect of exosomes derived from macrophages on hepatocellular carcinoma. The crosstalk of exosomes between macrophages and hepatocellular carcinoma (HCC) affects tumor progression. Both exosomes released by macrophages and HCC have unique intracellular components, including a variety of mRNAs, microRNAs, long non-coding RNAs, lipids, *etc.* These intracellular components are utilized as communicators to induce pathways that result in increased or inhibited cell proliferation, invasion, and other hallmarks of malignancy (M0: M0 macrophage; M1: M1 macrophage; M2: M2 macrophage; miR-92a-2-5p[82]; miR-15b[83]; miR-27a-3p[85]; miR-660-5p[86]; miR-628-5p[87]; miR-326[88]; miR-125a/b[90]). EVs: Extracellular vesicles; HCC: Hepatocellular carcinoma; miRNA: MicroRNA; lncRNA: Long non-coding RNA.

increased, which is conducive to exploring new treatments for CLD in the future.

CONCLUSION

The role and potential therapeutic value of exosomes in CLD have been the focus of research in recent years. Understanding the mechanism by which macrophage-derived exosomes affect liver diseases is critical for identifying their roles in liver disease pathogenesis and improving their therapeutic effects. In liver disease, macrophages can be activated and polarized. These activated macrophages secrete exosomes that carry various miRNAs and proteins, and these substances are encapsulated in exosomes and transferred from cell to cell. Macrophage-derived exosomes disrupt normal signaling between parenchymal and nonparenchymal cells in the liver, ultimately leading to liver damage and can act on specific targets to activate or inhibit signaling pathways and mediate related pathological processes. Thus, macrophage-derived exosomes are involved in the diagnosis of liver disease as biomarkers, and the signaling targets of these exosomes can also be used as potential therapeutic targets, providing more novel strategies for the diagnosis and treatment of liver disease. This article reviewed the mechanism and functions of macrophage-derived exosomes in liver diseases.

Currently, the link between macrophage-derived exosomes and liver diseases has mostly focused on a subset of liver diseases, such as NAFLD, HF, and HCC. However, the occurrence and development of CLDs are complicated processes involving multiple causes, stages, and links. In response to viral infection, alcohol intake, a high-fat diet, and drugs, liver inflammation and cellular degeneration first occur, which are accompanied by a series of adaptive events in the liver, including autophagy, aging, and the innate immune response, but these events can further aggravate liver damage, such as the activation of HSCs, leading to the accumulation of extracellular matrix and HF. Without early treatment, HF can further develop into cirrhosis, HCC, and even liver failure. Thus, some of the pathological stages of CLD overlap and further evolve. However, most current studies have only been conducted *in vivo* and *in vitro*, which limits the research object to a single liver disease while ignoring the role of macrophage-derived exosomes in the complex pathogenesis of CLD. Therefore, it is important to provide new ideas for subsequent studies so that researchers can focus on the role of macrophage-derived exosomes in different stages of CLD, which will be valuable to understanding the pathogenesis and treatment of many complex liver diseases.

Additionally, it has been found that different stimuli can cause macrophages to polarize into M1 and M2 cells and that M2 cells can then further differentiate into many subtypes. However, at present, there is still a lack of research on the mechanism by which these different isoforms release exosomes and the released exosome contents, which can be the focus of future research. In terms of clinical applications, techniques for extracting and purifying exosomes are improving, but determining how to amplify and change these extracted exosomes into a form that can be used in clinical settings requires cooperation and communication between different fields to develop a better treatment plan to reduce the global burden of CLD.

In the future, the development of exosomes is expected to shift from basic research to clinical applications. In recent years, exosomes have been recognized as potential biological treatments and drug delivery vehicles for the treatment of a variety of diseases. Compared with commonly used nanoparticles, macrophage-derived exosomes have the advantages of low immunogenicity and escape from macrophage phagocytosis. However, there are still gaps in clinical trials of macrophage-derived exosomes for the treatment of CLD. Before clinical translation, we urgently need to confirm which exosome components have profound diagnostic and therapeutic value, especially as accurate biomarkers that reflect disease status, target membrane segments, and critical cargo involved in the disease process. In addition, these systemically delivered exosomes tend to become trapped in nonspecific organs, particularly the liver, lungs, and spleen, resulting in inadequate target doses. Surface modifications for targeted delivery may provide an opportunity to enhance or expand the innate therapeutic value of exosomes. To improve the stability and delivery efficiency of natural exosomes, emerging biological nanotechnology provides a new option for precise material delivery. By designing exosome-like nanovesicles and membrane-camouflaging nanoparticles, the loading and delivery efficiency of effective substances of natural exosomes can be improved. In future clinical treatment of CLD, exosomes from macrophages can carry key effective substances and act as drug carriers after targeted modification or nanotechnology engineering and finally realize individualized targeted therapy, making great contributions to relieving the pressure of global liver diseases.

FOOTNOTES

Author contributions: Xiang SY performed the writing, prepared the figures and tables; Xiang SY and Deng KL designed the outline and coordinated the writing of the paper; Yang DX, Yang P, and Zhou YP provided review of the draft versions of the paper prior to submission of the final version; and all authors have read and approved the final manuscript.

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Global burden of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease, 1990-2019

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Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) has become the leading cause of cirrhosis and other chronic liver diseases (COCLDs).

AIM

To conduct a comprehensive and comparable updated analysis of the global, regional, and national burden of COCLDs due to NAFLD in 204 countries and territories from 1990 and 2019 by age, sex, and sociodemographic index.

METHODS

Data on COCLDs due to NAFLD were collected from the Global Burden of Diseases, Injuries, and Risk Factors Study 2019. Numbers and age-standardized prevalence, death, and disability-adjusted life years (DALYs) were estimated through a systematic analysis of modelled data from the Global Burden of Diseases, Injuries, and Risk Factors Study 2019. The estimated annual percentage change was used to determine the burden trend.

RESULTS

In 2019, the global age-standardized prevalence rate of COCLDs due to NAFLD was 15022.90 per 100000 population [95% uncertainty interval (UI): 13493.19-16764.24], which increased by 24.51% (22.63% to 26.08%) from 1990, with an estimated annual percentage change of 0.78 (95% confidence interval: 0.74-0.82). In the same year, however, the age-standardized death rate and age-standardized DALYs per 100000 population were 1.66 (95%UI: 1.20-2.17) and 43.69 (95%UI: 31.28-58.38), respectively. North Africa and the Middle East had the highest prevalence rates of COCLDs due to NAFLD. The death rate increased with age up to the 95+ age group for both sexes. Males had higher numbers of prevalence, death rate, and DALYs than females across all age groups before the 65-69 age

group. The sociodemographic index was negatively correlated with the age-standardized DALYs.

CONCLUSION

Globally, the age-standardized prevalence rate has increased during the past three decades. However, the age-standardized death rate and age-standardized DALYs decreased. There is geographical variation in the burden of COCLDs due to NAFLD. It is strongly recommended to improve the data quality of COCLDs due to NAFLD across all countries and regions to facilitate better monitoring of the burden of COCLDs due to NAFLD.

Key Words: Cirrhosis; Nonalcoholic fatty liver disease; Global burden of disease; Prevalence; Disability-adjusted life years; Death

Core Tip: Nonalcoholic fatty liver disease is the leading cause of cirrhosis and other chronic liver diseases. The global age-standardized prevalence rate of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease increased by 24.51% from 1990. The age-standardized death rate and age-standardized disability-adjusted life-years rate per 100000 population were 1.66 and 43.69, respectively. The highest prevalence rate was observed in North Africa and the Middle East. Males had a higher burden of prevalence, death, and disability-adjusted life-years lost than females before the 65-69 age group. Furthermore, there is a negative correlation between sociodemographic index and age-standardized death rate.

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INTRODUCTION

The incidence and prevalence of nonalcoholic fatty liver disease (NAFLD) have been rapidly increasing worldwide over the past few decades. Recent estimates suggest that approximately 25% of the world's population is affected by NAFLD, with projections indicating a potential 56% surge in the prevalence of nonalcoholic steatohepatitis (NASH) within the coming decade[1]. NAFLD encompasses a spectrum of liver damage, ranging from simple steatosis to NASH, fibrosis, cirrhosis, and even hepatocellular carcinoma[2]. It is noteworthy that NAFLD now stands as the fifth leading cause of mortality among young adults within the category of metabolic diseases. Alarming forecasts predict a staggering 158.4% increase in its death rate by the year 2050[3]. Conversely, another separate study demonstrated divergent trends, finding that the age-standardized prevalence rate (ASPR) of NAFLD increased while the age-standardized death rate (ASDR) and age-standardized disability-adjusted life-year (DALY) rate (ASDAR) decreased from 1990 to 2019[4].

Cirrhosis is the leading cause of liver-related morbidity, contributing to more than 1 million deaths annually worldwide. The mortality increase escalates markedly for individuals grappling with decompensated cirrhosis[5], and the deaths from cirrhosis increased by 47.15% globally from 1990 to 2017[6]. Beyond mortality statistics, cirrhosis imposes a significant public health burden globally, substantially compromising quality of life[7,8]. The etiologies of cirrhosis include alcoholic liver disease, hepatitis B virus, hepatitis C virus, and NAFLD[9,10]. Over the past few decades, universal hepatitis B virus vaccination initiatives, coupled with rising obesity rates and the prevalence of type 2 diabetes, have positioned NAFLD as a major etiological factor contributing to cirrhosis[11-14].

Chronic liver disease (CLD) is a disease that is characterized by decreased liver function resulting from chronic inflammation or injury to the liver, leading to fibrosis and cirrhosis that progresses for more than 6 mo[15,16]. This spectrum encompasses an array of liver pathologies, encompassing inflammation, cirrhosis, portal hypertension, and hepatorenal syndrome. Notably, the incidence of CLD is increasing yearly, and it is now the fifth leading cause of death in the United Kingdom. In Western regions, NAFLD has become the leading cause of CLD[17]. Today, the United States has nearly 4.5 million adults afflicted by cirrhosis and CLD, resulting in an overall death toll of 414731[16]. However, no studies have focused on the epidemiology of cirrhosis and other CLDs (COCLDs) due to NAFLD across the globe.

In the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2017, it was shown that the incidence of cases of liver cirrhosis caused by NASH increased by approximately 105.56%, and the age-standardized incidence rate increased by 1.35%. This study only included data from 195 countries, and data on prevalence, death, and DALYs were not provided[18]. Furthermore, no updated global studies on COCLDs due to NAFLD have been published since the 2017 estimates. Using the data from the GBD 2019, we conducted this comprehensive, updated analysis of the global, regional, and national levels of prevalence, death rate, and DALYs of COCLDs due to NAFLD with regard to age-standardized rates (ASRs) and raw numbers from 1990 to 2019, stratified by sex, age, and sociodemographic index (SDI).

MATERIALS AND METHODS

Overview

The GBD 2019 was conducted by the Institute of Health Metrics and Evaluation and analyzed approximately 369 diseases and injuries, 282 causes of death, and 84 risk factors from 204 countries/territories, 21 regions, and 7 superregions from 1990 to 2019[19]. Detailed methods for GBD 2019 regarding date inputs, analytical processes, and outputs have been described in previous publications[19,20]. Additional information on fatal and nonfatal estimates can be found at https://vizhub.healthdata.org/gbd-compare/ and http://ghdx.healthdata.org/gbd-results-tool. Our study complied with the Guidelines for Accurate and Transparent Health Estimates Reporting statement[21].

Case definition and data sources

NAFLD was defined as a range of liver conditions that mimic alcoholic liver disease but occur in people who drink little to no alcohol. It includes nonalcoholic fatty liver (characterized by fat deposition in liver cells), NASH (characterized by fat deposition and inflammation), and cirrhosis[19]. Cirrhosis is a CLD in which there is progressive destruction of functional hepatic cells and replacement with fibrosis (scarring) of the liver. In GBD 2019, COCLDs due to NAFLD were defined as COCLDs that were specifically caused by NAFLD, excluding all other potential etiologies[19]. All the GBD data used in this study are publicly available online at the Global Health Data Exchange.

Data processing and disease modelling

The Bayesian meta-regression tool DisMod-MR 2.1 was used to assess and model estimates of the burden of COCLDs due to NAFLD by pooling all the available epidemiological data. Prior settings included remission of 0 before the age of 15 years in the DisMod-MR 2.1 model. No prevalence of COCLDs due to NAFLD before the age of 15 years was assumed. The age range was restricted to ≥ 15 years and was divided into 17 5-year age groups.

The estimated annual percentage change (EAPC) values were calculated to reflect the change in ASRs over a specified period. EAPC values above or below 0 indicate that the ASR is increasing or decreasing, respectively. If the EAPC range includes 0, this means the ASR is stable during this period. ASPR, ASDR, ASDAR, and EAPC were used to quantify global trends of COCLDs due to NAFLD.

The SDI was used as a composite indicator of the development status in each country and territory. It was calculated based on lag-distributed income, the total fertility rate for individuals younger than 25 years, and average years of education in people older than 15 years. SDI ranged from 0 to 1, with a higher score indicating a higher level of development. The 204 countries and territories were categorized into five groups: low SDI, low-middle SDI, middle SDI, high-middle SDI, and high SDI.

Statistical analyses

Smoothing spline models were employed to examine the shape of the correlation curve between the burden index of COCLDs due to NAFLD and SDI according to the GBD estimates across 204 countries and 21 regions from 1990 to 2019. The 95% uncertainty intervals (UIs) were defined as the 2.5 to the 97.5 percentile of the ordered draws. R software version 3.6.3 was used for all statistical analyses and figures. A P value of < 0.05 was considered statistically significant.

RESULTS

Global level

Globally in 2019 there were 1235652879 (95%UI, 1109501987-1378481210) prevalent cases of COCLDs due to NAFLD. From 1990 to 2019, the global ASPR increased from 12065.15 (10779.06-13536.49) to 15022.90 (13493.19-16764.24) per 100000 population, with an EAPC of 0.78 (0.74-0.82) (Table 1, Figure 1). COCLDs due to NAFLD accounted for 134240 (96483-176920) deaths globally in 2019, which was a substantial increase of 76.73% (61.23%-94.75%) over that in 1990. The ASDR of COCLDs due to NAFLD decreased from 1.94 per 100000 population (1.39-2.59 per 100000) in 1990 to 1.66 per 100000 population (1.20-2.17 per 100000) in 2019, with an EAPC of 0.65 (-3.68-2.48) (Table 1, Supplementary Figure 1). In the same year, COCLDs due to NAFLD accounted for 3621471.92 (2585375.27-4862918.36) DALY cases at the global level with an ASR of 43.69 per 100000 population. The global ASDAR was reduced from 51.92 per 100000 population (37.23-69.19 per 100000) in 1990 to 43.69 per 100000 population (95%UI: 31.28-58.38) in 2019, with an EAPC of -0.73 (-1.33 to -0.13) (Table 1, Supplementary Figure 2).

SDI regions and 21 GBD region levels

The number of prevalence, deaths, and DALYs of COCLDs due to NAFLD increased in all five SDI regions from 1990 to 2019. Among them, the greatest increases in prevalence (1.54-fold), deaths (0.96-fold), and DALY (0.77-fold) cases were observed in low-SDI, middle-SDI, and low-middle-SDI regions, respectively (Figure 2, Supplementary Figure 3). All-age prevalence rates of COCLDs due to NAFLD increased across all SDI quintiles, with the most significant increase observed in the middle-SDI region (0.65-fold). Outside of the low-SDI quintile, all-age death rates and DALYs showed an increasing trend. The ASDR and ASDAR of COCLDs due to NAFLD exhibited a decreasing trend across all five SDI quintiles. The low-SDI quintile had the greatest absolute decreases in the ASDR (EAPC = -1.04; -3.48-1.45) and the ASDAR (EAPC = -1.14; -1.63 to -0.65). The ASPR of COCLDs due to NAFLD increased in all SDI regions from 1990 to 2019, with the highest increase observed in the high-SDI region (EAPC = 1.22; 1.17-1.26) (Table 1, Supplemen-

Table 1 Global burden of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease in 204 countries and territories from 1990 to 2019

Characteristics	Death (95%UI)			Prevalence (95%UI)			DALYs (95%UI)		
	2019	2019	1990-2019	2019	2019	1990-2019	2019	2019	1990-2019
	Death cases among COCLDs due to NAFLD	ASDR per 100000 population (95%UI)	EAPC (95%CI)	Prevalence cases among COCLDs due to NAFLD	ASPR per 100000 population (95%UI)	EAPC (95%CI)	DALY cases among COCLDs due to NAFLD	ASDAR per 100000 population (95%UI)	EAPC (95%CI)
Global	134240.56 (96483.10-176920.13)	1.66 (1.20-2.17)	-0.65 (-3.68-2.48)	1235652879.48 (1109501987.09-1378481210.81)	15022.90 (13493.19-16764.24)	0.78 (0.74-0.82)	3621471.92 (2585375.27-4862918.36)	43.69 (31.28-58.38)	-0.73 (-1.33 to -0.13)
Sex									
Male	72379.97 (51296.70-98029.91)	1.91 (1.36-2.54)	-0.62 (-3.46-2.31)	679261655.31 (610650033.21-753716195.72)	16789.38 (15136.45-18609.47)	0.77 (0.74-0.81)	2111194.29 (1490236.02-2905285.80)	52.39 (37.17-71.63)	-0.65 (-1.20 to -0.10)
Female	61860.58 (44641.50-81216.17)	1.42 (1.03-1.86)	-0.70 (-3.95-2.66)	556391224.17 (499050879.21-624989968.22)	13278.47 (11900.05-14925.95)	0.78 (0.74-0.82)	1510277.64 (1087564.54-1984959.30)	35.16 (25.35-46.24)	-0.84 (-1.49 to -0.18)
SDI									
Low SDI	12175.43 (8552.07-16892.87)	2.41 (1.65-3.38)	-1.04 (-3.48-1.45)	108176159.27 (95419333.67-122650081.72)	14278.53 (12741.85-16053.31)	0.34 (0.30-0.38)	367698.76 (255871.37-510346.73)	59.56 (41.46-82.97)	-1.14 (-1.63 to -0.65)
Low-middle SDI	28042.90 (20040.37-37459.68)	2.09 (1.52-2.77)	-0.73 (-3.47-2.08)	245742808.24 (219055120.10-276667118.34)	15232.77 (13662.46-17078.41)	0.56 (0.52-0.59)	826708.77 (584688.97-1130359.54)	54.77 (39.14-74.11)	-0.86 (-0.31 to -1.4)
Middle SDI	51854.16 (37473.84-68073.39)	2.23 (1.62-2.92)	-0.77 (-3.35-1.88)	464259979.22 (417290788.31-516020477.89)	17596.41 (15842.14-19525.24)	0.66 (0.62-0.69)	1347118.90 (956498.31-1772557.91)	52.41 (38.16-68.22)	-0.98 (-1.52 to -0.44)
Middle-high SDI	25074.30 (18164.41-33237.26)	1.27 (0.93-1.67)	-0.75 (-4.08-2.69)	278080918.57 (251203100.31-308471139.19)	15336.20 (13798.84-17095.00)	0.79 (0.75-0.83)	676567.46 (480709.11-915257.33)	34.81 (25.11-46.87)	-0.52 (-1.16-0.14)
High SDI	16984.00 (12138.02-22893.89)	0.95 (0.67-1.30)	-0.79 (-4.75-3.33)	138619942.87 (125499384.96-153306375.41)	10528.91 (9426.64-11724.43)	1.22 (1.17-1.26)	400520.00 (273854.75-550359.22)	25.46 (17.47-35.02)	-0.84 (-1.61 to -0.05)
Region									
Andean Latin America	2935.06 (2012.67-4051.53)	5.31 (3.62-7.30)	-0.23 (-1.96-1.54)	8442278.35 (7603147.20-9329779.19)	13689.71 (12376.75-15086.12)	0.50 (0.47-0.54)	70058.17 (47362.01-98970.21)	121.70 (82.37-172.61)	-0.69 (-1.05 to -0.34)
Australasia	413.12 (297.05-538.48)	0.87 (0.63-1.13)	-0.22 (-4.56-4.32)	3459804.59 (3121214.49-3821041.92)	9444.14 (8455.34-10478.02)	0.91 (0.86-0.95)	9514.38 (6661.05-12694.41)	22.52 (15.80-30.52)	-0.22 (-1.09-0.65)
Caribbean	1607.95 (1113.98-2236.69)	3.11(2.15-4.30)	-0.71 (-3.01-1.65)	8176172.43 (7382389.69-9017056.63)	16169.48 (14591.11-17866.53)	0.40 (0.37-0.43)	41576.95 (28066.48-60045.06)	80.64 (54.52-115.96)	-0.75 (-1.21 to -0.29)
Central Asia	2633.58 (1802.74-3693.53)	3.56 (2.51-4.95)	2.00 (-0.39-4.45)	12814865.72 (11452914.10-14330325.35)	14150.39 (12737.16-15748.23)	0.50 (0.46-0.54)	84039.15 (57215.03-120570.79)	97.76 (67.81-137.00)	1.97 (1.51-2.43)
Central Europe	2061.07 (1378.12-2972.76)	1.05 (0.71-1.49)	-0.36 (-4.12-	18627848.59 (16912570.59-	11894.85 (10754.82-	0.34 (0.30-	54731.91 (36055.44-	30.45 (19.95-43.92)	-0.35 (-1.06-

			3.56)	20480313.45)	13109.51)	0.38)	79057.76)		0.37)
Central Latin America	13972.52 (10310.77-18101.39)	5.90 (4.32-7.66)	-0.61 (-2.24-1.06)	42166216.88 (37817332.61-46840542.82)	16617.99 (14959.55-18388.64)	0.43 (0.40-0.46)	379421.91 (275350.09-504051.39)	154.08 (112.26-204.63)	-0.84 (-1.16 to -0.52)
Central Sub-Saharan Africa	1464.95 (913.12-2215.29)	2.71 (1.74-4.05)	-0.79 (-3.19-1.67)	11343938.83 (9886866.10-12995919.12)	13331.21 (11795.46-15097.36)	0.16 (0.13-0.20)	47553.59 (29400.33-74006.26)	70.04 (43.82-106.28)	-0.78 (-1.26 to -0.30)
East Asia	15620.62 (11027.12-21039.77)	0.80 (0.58-1.07)	-2.35 (-6.22-1.68)	303112259.13 (272363603.29-339481359.63)	15680.86 (14022.56-17552.19)	0.85 (0.81-0.89)	391872.94 (268774.96-535743.63)	18.91 (13.28-25.56)	-2.71 (-3.50 to -1.91)
Eastern Europe	7354.24 (5071.40-10094.04)	2.41 (1.67-3.30)	3.04 (0.05-6.12)	34110583.23 (30980982.64-37557624.89)	12295.07 (11098.49-13601.74)	0.39 (0.35-0.42)	245483.77 (166670.25-344145.21)	85.82 (58.40-120.98)	3.58 (3.06-4.11)
Eastern Sub-Saharan Africa	6094.93 (4211.94-8428.57)	3.98 (2.74-5.49)	-0.66 (-2.62-1.34)	36093305.19 (31609582.82-41132257.66)	13709.06 (12222.30-15370.26)	0.29 (0.26-0.33)	175459.64 (119576.20-250541.44)	91.96 (62.83-129.95)	-0.82 (-1.22-0.41)
High-income Asia Pacific	1462.94 (990.41-2089.17)	0.31 (0.21-0.42)	-3.18 (-8.97-2.97)	20933934.70 (18773194.85-23278277.91)	7671.66 (6829.94-8629.15)	0.56 (0.51-0.61)	26564.50 (18456.53-35825.79)	7.20 (4.90-9.83)	-3.36 (-4.57 to -2.14)
High-income North America	7014.45 (4867.08-9682.42)	1.20 (0.83-1.63)	0.34 (-3.56-4.39)	44312078.16 (39693504.66-49544059.79)	9395.90 (8403.86-10542.43)	0.98 (0.94-1.03)	178065.34 (120160.62-251423.01)	33.23 (22.97-46.98)	0.36 (-0.40-1.12)
North Africa and Middle East	11756.00 (7699.85-17161.92)	3.18 (2.07-4.60)	-0.35 (-2.59-1.94)	161456578.66 (146407605.69-177659074.34)	27748.49 (25409.87-30283.48)	0.47 (0.45-0.50)	265596.53 (171336.92-375177.75)	61.14 (39.58-87.12)	-0.31 (-0.83-0.21)
Oceania	87.79 (55.51-129.24)	1.13 (0.77-1.60)	-0.17 (-3.94-3.75)	1744101.91 (1541892.36-1965419.91)	16869.18 (15133.44-18726.79)	0.18 (0.15-0.21)	3194.63 (1975.61-4878.17)	33.00 (20.97-48.54)	-0.21 (-0.91-0.50)
South Asia	17739.51 (12596.43-24044.26)	1.28 (0.90-1.73)	-1.55 (-4.85-1.87)	241838470.86 (214848138.41-273682478.00)	14513.87 (12969.20-16400.60)	0.55 (0.51-0.58)	546376.19 (385933.87-757708.62)	34.54 (24.64-47.48)	-1.76 (-2.41 to -1.11)
Southeast Asia	19624.56 (13971.70-26356.88)	3.43 (2.46-4.62)	-0.23 (-2.40-1.99)	128065513.81 (114729541.70-142457456.46)	18299.21 (16508.63-20309.58)	0.46 (0.42-0.49)	539043.89 (379432.49-734864.03)	82.56 (58.82-110.61)	-0.58 (-1.01 to -0.14)
Southern Latin America	1429.48 (982.16-1989.97)	1.73 (1.18-2.41)	-0.18 (-3.30-3.02)	6483740.60 (5831421.14-7191963.86)	8602.52 (7719.35-9564.23)	0.90 (0.85-0.95)	34294.28 (22901.01-48792.78)	42.86 (28.61-61.04)	-0.52 (-1.14-0.11)
Southern Sub-Saharan Africa	888.05 (632.75-1213.53)	1.61 (1.14-2.18)	-0.53 (-3.41-2.44)	13161620.39 (11810250.76-14681736.21)	18075.91 (16347.92-19997.66)	0.41 (0.37-0.44)	26198.48 (18261.46-36311.83)	40.87 (28.84-55.60)	-0.69 (-1.26 to -0.12)
Tropical Latin America	4447.37 (3232.26-5827.27)	1.84 (1.35-2.40)	-0.44 (-3.42-2.63)	37986300.10 (34231442.55-41945927.58)	15241.19 (13723.17-16818.90)	0.49 (0.45-0.52)	120114.10 (85232.80-161026.13)	48.00 (34.37-63.83)	-0.60 (-1.18 to -0.02)
Western Europe	9604.81 (6833.82-12825.54)	1.09 (0.78-1.46)	-1.81 (-5.22-1.72)	59002155.13 (53307928.02-65208561.44)	9932.86 (8931.95-11044.15)	0.80 (0.76-0.85)	201120.00 (140612.13-273526.30)	26.97 (18.77-37.08)	-2.03 (-2.72 to -1.35)
Western Sub-Saharan Africa	6027.57 (4018.12-8572.80)	3.31 (2.21-4.67)	-0.67 (-2.78-1.48)	42321112.21 (37193511.05-48136808.11)	14283.82 (12756.86-15952.84)	0.22 (0.18-0.25)	181191.57 (116196.70-270466.76)	79.06 (51.99-113.80)	-0.70 (-1.13 to -0.26)

ASDAR: Age-standardized disability-adjusted life-years rate; ASDR: Age-standardized death rate; ASPR: Age-standardized prevalence rate; CI: Confidence interval; COCLD: Cirrhosis and other chronic liver diseases; DALYs: Disability-adjusted life-years; EAPC: Estimated annual percentage change; NAFLD: Nonalcoholic fatty liver disease; SDI: Sociodemographic index; UI: Uncertainty interval.

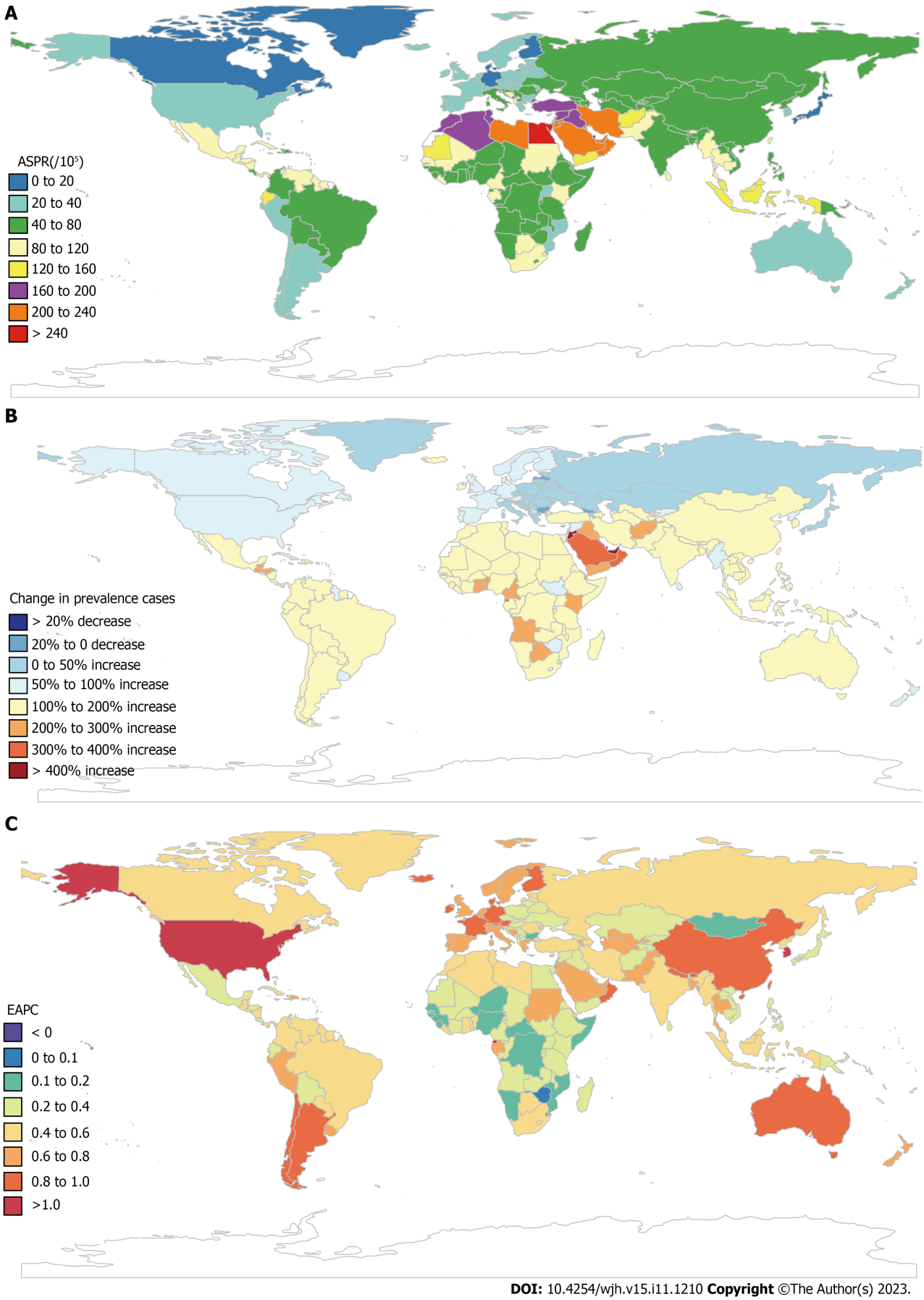

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Figure 1 Global disease burden of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease by countries and territories for both sexes combined. A: The age-standardized prevalence rate (ASPR) of cirrhosis and other chronic liver diseases (COCLDs) due to

nonalcoholic fatty liver disease (NAFLD) in 2019; B: The percentage change in prevalence cases of COCLDs due to NAFLD between 1990 and 2019; C: The estimated annual percentage change of COCLDs due to NAFLD ASPR from 1990 to 2019. EAPC: Estimated annual percentage change.

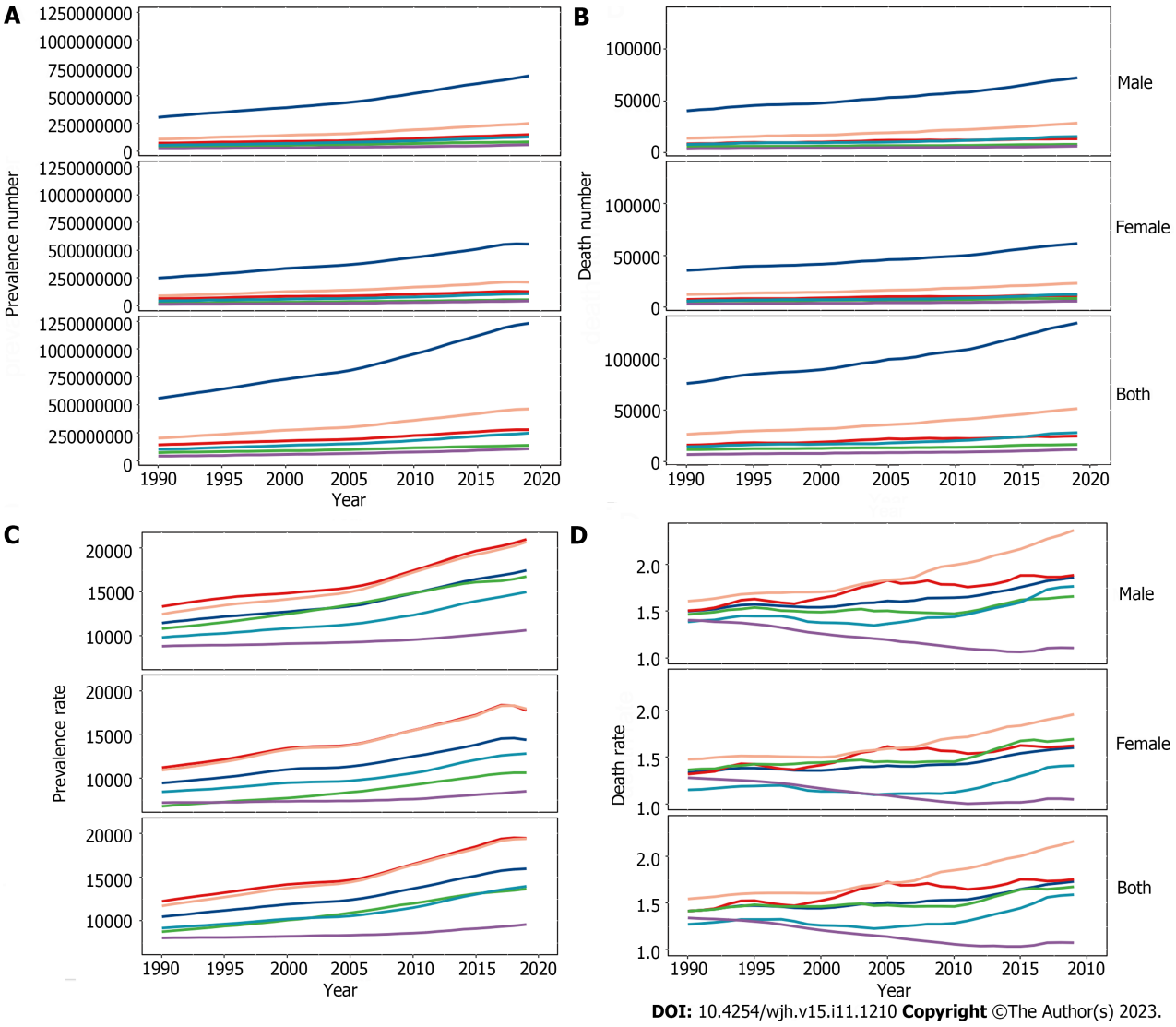

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Figure 2 Cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease at the global and regional levels from 1990 to 2019. A: Prevalence number; B: Death number; C: All-age prevalence rate; D: All-age death rate.

tary Figure 4).

Among the 21 GBD regions, both the highest ASDR and ASDAR were observed in Central Latin America in 2019. Both the lowest ASDR and ASDAR were observed in high-income Asia Pacific in 2019 (Supplementary Tables 1 and 2, Supplementary Figures 5 and 6). The highest increases in the ASDR (EAPC = 3.04; 0.05-6.12) and ASDAR (EAPC = 3.58; 3.06-4.11) were found in Eastern Europe, followed by Central Asia and high-income North America. In contrast, high-income Asia Pacific exhibited the most pronounced decreases in the ASDR (EAPC= -3.18; -8.97-2.97) and ASDAR (EAPC= -3.36; -4.57 to -2.14) (Table 1).

The highest ASPR of COCLDs due to NAFLD was found in North Africa and the Middle East regions, followed by Southeast Asia and Southern Sub-Saharan Africa (Figure 3, Supplementary Table 3). The highest increase in the ASPR was observed in high-income North America, followed by Australasia and Southern Latin America. Central Sub-Saharan Africa showed the lowest increases from 1990 to 2019 (Table 1).

National levels

At the national level, the ASPR of COCLDs due to NAFLD ranged from 6680.34 to 34515.88 per 100000 population in 2019. In that year, Egypt [34515.89 (31796.95-37253.06) per 100000] had the highest ASPR in 2019, followed by Qatar and Kuwait. Conversely, Finland, Canada, and Greenland had the lowest ASPR in 2019. The most pronounced changes in prevalent cases from 1990 to 2019 were seen in Qatar and Georgia. The largest increase in ASPRs was observed in the Republic of Korea [EAPC = 1.08; 95% confidence interval (CI): 1.03-1.13] and Equatorial Guinea between 1990 and 2019.

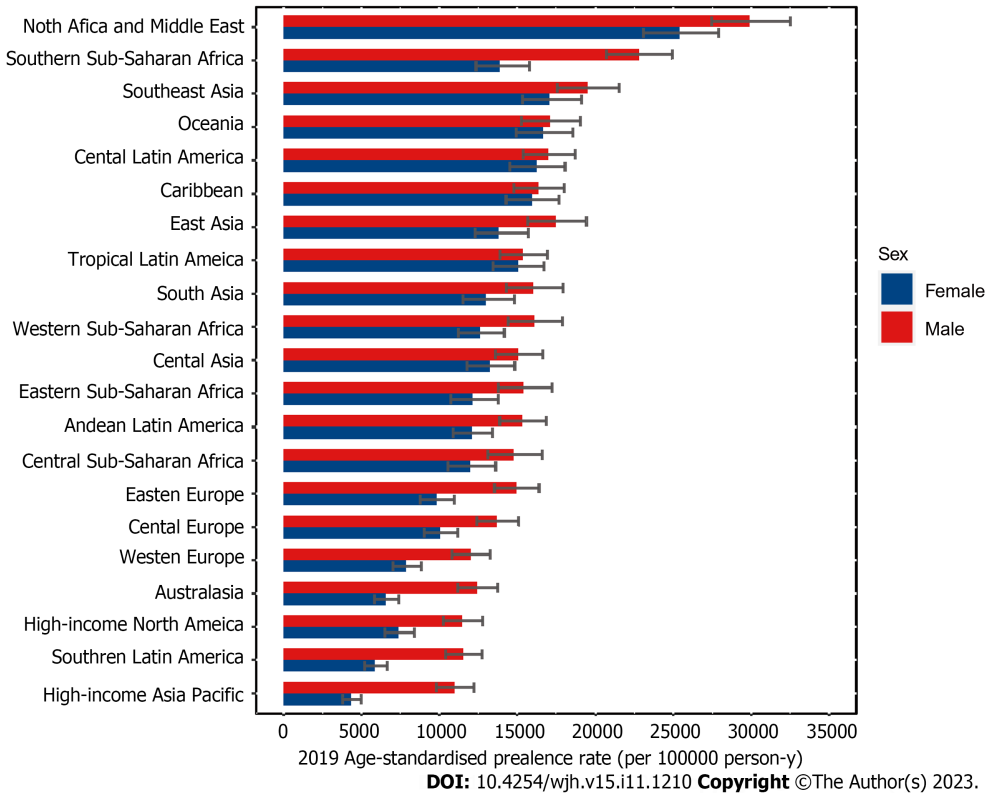

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Figure 3 Age-standardized prevalence rate for cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease by region and sex, 2019.

Only Zimbabwe (EAPC = -0.06; 95%CI: -0.10 to -0.03) showed a decreasing trend during this period (Supplementary Table 4, Figure 1C).

The highest ASDR of COCLDs due to NAFLD was observed in Egypt [14.02 (8.42-22.43)] in 2019, followed by Honduras and Guatemala. In contrast, Montenegro, Japan, and Singapore had the lowest ASDRs. Between 1990 and 2019, the United Arab Emirates [828.78% (368.37%-1461.06%)] showed the most significant increase in the number of deaths caused by COCLDs due to NAFLD, whereas Hungary showed a decrease of 39.94%. The largest increases in the ASDRs of COCLDs due to NAFLD were observed in Armenia (EAPC = 4.21; 95%CI: 0.98-7.54) and Kazakhstan (EAPC = 4.11; 95%CI: 1.33-6.97) from 1990 to 2019. In contrast, 143 countries or territories experienced decreasing trends, with the Republic of Korea presenting the largest decrease in ASDR during this period (EAPC = -4.19; 95%CI: -8.84 to -0.70) (Supplementary Table 5, Supplementary Figure 2).

The highest ASDAR of COCLDs due to NAFLD was observed in Guatemala [258.61 (170.42-329.98)] in 2019, followed by Honduras and Egypt. In contrast, Montenegro, Japan, and Singapore had the lowest ASDAR. Between 1990 and 2019, the United Arab Emirates [905.78% (402.13%-1627.41%)] showed the most significant increase in the number of DALYs from COCLDs due to NAFLD, whereas Hungary exhibited a decrease of 49.82% (-61.59% to -35.81%). The countries with the largest increases in ASDR during this period were Kazakhstan (EAPC = 4.02; 95%UI: 3.50-4.55) and Belarus. In contrast, the Republic of Korea experienced the largest decrease in ASDR, with the greatest reduction in ASDAR over the same period (Supplementary Table 6, Supplementary Figure 3).

Age and sex patterns

Globally, the prevalent number of COCLDs due to NAFLD exhibited an age-dependent pattern, reaching its peak at 45-49 years for males and 50-54 years for females. There was a declining trend in prevalence as age increased (Supplementary Figure 7). Similarly, the disease prevalence rate showed increasing and then decreasing trends with age in both sexes, with the highest prevalence rate observed in people aged 70-74 years, decreasing after this age group.

Globally, mortality rates increased with age and peaked at 65-69 years for males and 70-74 years for females before declining. The mortality rate steadily increased with age up to the 95+ age group for both sexes in 2019 (Figure 4). In the same year, the 50-54 age group for males and the 60-64 age group for females had the highest number of DALY cases, which decreased as age increased. The rate of DALYs peaked in the 80-84 age group, decreased in the 85-94 age group, and subsequently increased in the 95+ age group (Supplementary Figure 8). Among individuals under 70 years old, the numbers of prevalent cases, deaths, and DALYs lost was higher among males than females. However, among those aged 70 years and older, all three numbers were lower among males than among females (Figure 4, Supplementary Figures 7 and 8).

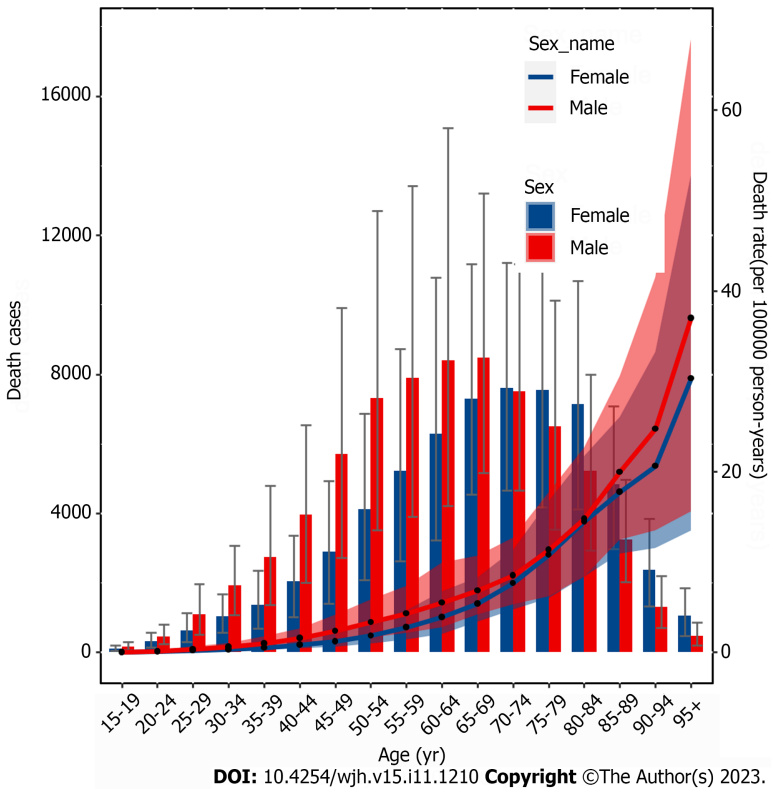

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Figure 4 All-age numbers and rates of deaths for cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease in 2019 are illustrated by sex for females and males.

Burden of COCLDs due to NAFLD by SDI

From 1990 to 2019, there was generally a negative correlation between SDI and global and regional ASDARs of COCLDs due to NAFLD. Globally, the observed burden of COCLDs due to NAFLD was lower than expected. In most regions, higher SDI values were associated with decreased ASDAR, except in Central Asia, high-income North America, and Eastern Europe, which showed an increasing trend during the study period. At the regional level, the observed burden of ASDAR of COCLDs due to NAFLD in Central Latin America, Andean Latin America, Eastern Sub-Saharan Africa, Western Sub-Saharan Africa, Southeast Asia, and Western Europe was found to be higher than the expected level based on the SDI from 1990 to 2019 (Figure 5). The link between the SDI and ASDR of COCLDs due to NAFLD had a similar pattern from 1990 to 2019 (Supplementary Figure 9). The predicted relationship between SDI and ASPR of COCLDs due to NAFLD exhibited an initial increasing trend, followed by a decreasing trend at an SDI value of 0.58 (Supplementary Figure 10).

At the national level, the ASDAR of COCLDs due to NAFLD in 2019 generally displayed a negative correlation with SDI. In numerous countries/territories, including Egypt, Guatemala, Honduras, and Mexico, the ASDAR was higher than the expected level based on SDI in 2019; conversely, in countries such as the Maldives, Bangladesh, Papua New Guinea, and Mozambique, the burden was lower than expected (Figure 6). Negative correlations between the national-level ASDR and ASPR of COCLDs due to NAFLD and SDI in 2019 were also found (Supplementary Figures 11 and 12).

DISCUSSION

This study comprehensively described the trends and patterns in prevalence, DALYs, and deaths caused by COCLDs due to NAFLD at the global, regional, and national levels over the past three decades. Globally, there were an estimated 123.56 million prevalent cases, 0.13 million deaths, and 3.62 million DALYs lost in 2019. Our findings indicated a substantial increase in the number of all-age deaths, prevalent cases, and DALYs. The global prevalence and ASPR of COCLDs due to NAFLD both showed increasing trends from 1990 to 2019. Although the ASDR and ASDAR of COCLDs due to NAFLD decreased between 1990 and 2019, the total number of deaths and DALYs experienced an increasing trend, which can be partly explained by population growth, longer life expectancy, and higher prevalence in older age groups. COCLDs due to NAFLD are an increasing threat to our population and place a strain on valuable health resources. As there is currently no effective treatment for NAFLD, this trend is likely to continue, driven by its increasing prevalence.

To highlight the vital role of metabolic dysfunction in the pathogenesis of fatty liver disease, the Asian Pacific Association for the study of the Liver proposed that NAFLD be renamed metabolic- or metabolic dysfunction-associated fatty liver disease (MAFLD) in 2020[22,23]. However, there exist slight discrepancies in the definitions of MAFLD and

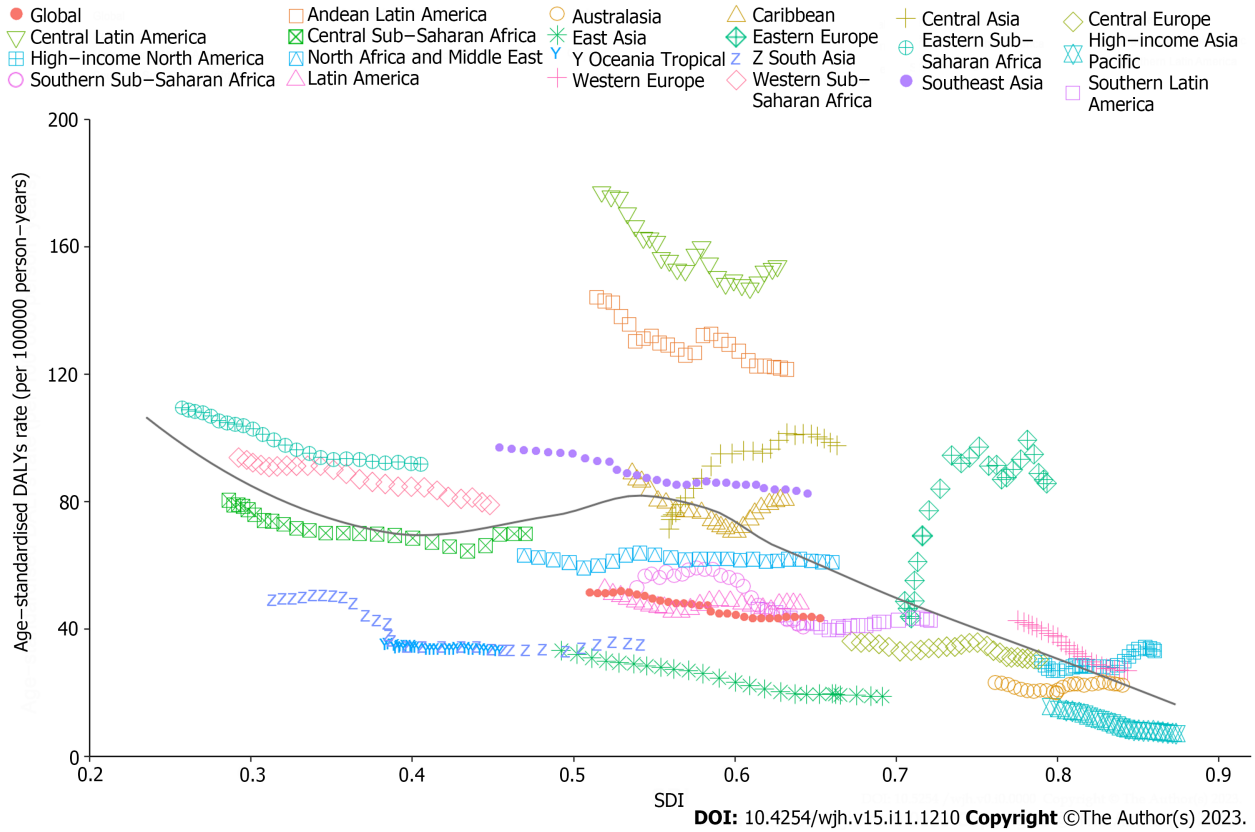

Figure 5 Age-standardized disability-adjusted life years rate of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease by sociodemographic index globally and in 21 regions, 1990-2019. SDI: Sociodemographic index; DALYs: Disability-adjusted life years.

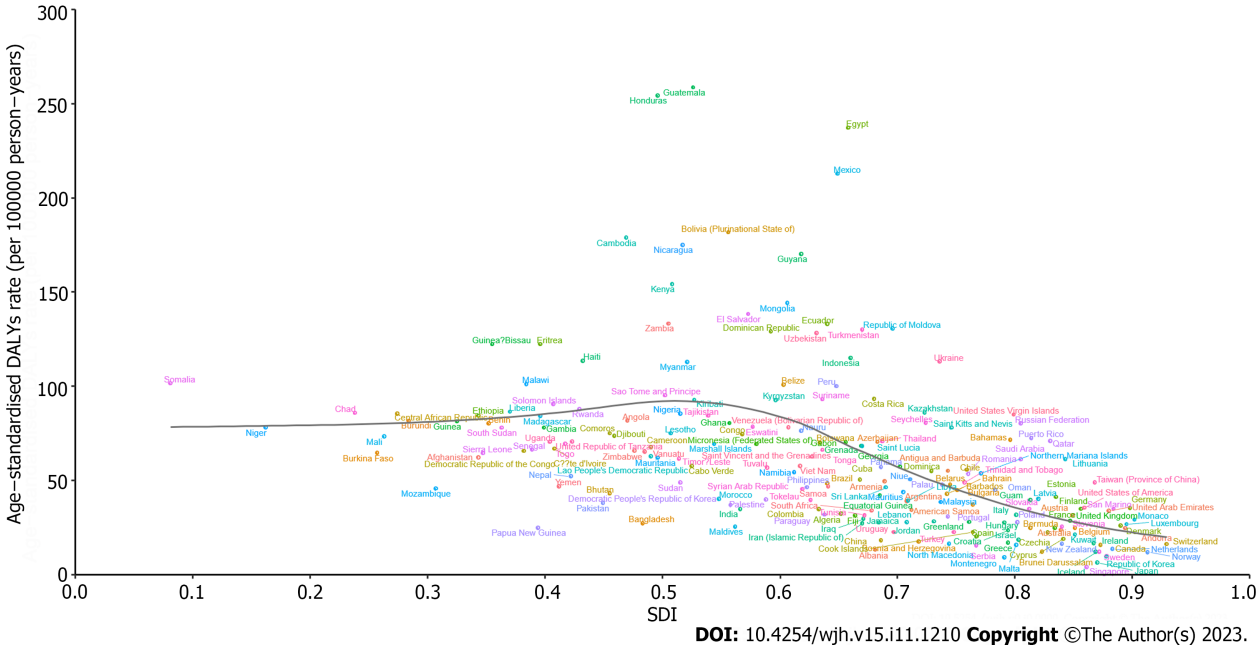

Figure 6 Age-standardized disability-adjusted life years rate of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease by sociodemographic index in 204 countries and territories, 2019. DALYs: Disability-adjusted life years; SDI: Sociodemographic index.

NAFLD among different populations. The key distinctions between NAFLD and MAFLD lie in the requirement for NAFLD to exclude alcohol consumption and other risk factors, such as chronic viral hepatitis, whereas the MAFLD diagnosis focuses on detecting fatty liver in conjunction with metabolic risk factors, without necessitating the exclusion of other liver disorders[24]. In addition, GBD 2019 only estimated the burden of COCLDs due to NAFLD, and the MAFLD burden was not estimated. Therefore, in this study, we focused on the burden of COCLDs due to NAFLD.

NAFLD is closely associated with overweight and obesity, affecting an estimated 25% of the general adult population. Obesity significantly elevates the risk of NAFLD development. In a previous study, obesity was found in approximately 81% of patients with NASH and 50% of patients with NAFLD[25]. Lifestyle modifications and bariatric surgery have significantly improved NAFLD activity scores[26,27]. Earlier epidemiological investigations projected that the worldwide count of obese or overweight individuals surpassed 2.1 billion, constituting a pervasive global health concern[28]. For 2019, we identified the prevalent count of COCLDs attributed to NAFLD as 1.2 billion individuals, which was slightly less than the number of obese individuals.

Interestingly, reports indicate that around 20% of Asian individuals diagnosed with NAFLD have body mass index and waist circumference measurements falling within the range designated as lean, despite the prevailing association of NAFLD with overweight or obesity[29]. Notably, regions with higher incomes, such as the United States, France, and Japan, tend to exhibit an elevated prevalence of lean MAFLD[30]. However, certain developing countries, such as India and Sri Lanka, have an even higher prevalence rate of lean MAFLD, which may be attributed to racial and dietary factors. United States-based studies also indicate that people with ancestry from Latin America have a higher prevalence rate of lean MAFLD, whereas African Americans have a lower prevalence rate. The suggested racial and ethnic variations are important risk factors for the prevalence of lean MAFLD[30,31]. In addition, lean MAFLD patients have worse long-term outcomes than healthy people and have a similar prognosis to overweight or obese MAFLD patients. Therefore, more attention should be focused on normal-weight MAFLD patients, and a 3%-5% weight reduction and improvement in diet quality are strongly recommended to improve lean MAFLD[30]. Beyond body weight, additional factors such as diabetes, ethnic disparities, and genetic variations could also contribute to the prevalence and outcomes of lean NAFLD[32]. Given the ongoing global epidemic of obesity and diabetes, the burden of COCLDs attributed to NAFLD is expected to rise in the coming years.

According to previous research, the number of deaths due to cirrhosis caused by NAFLD/NASH was 102615 in 2012 and 118030 in 2017. The annual percent change in the ASDR of cirrhosis due to NAFLD/NASH from 2012 to 2019 was 0.29[33]. In contrast, our study found that the EAPC of the ASDR of COCLDs due to NAFLD was -0.65 from 1990 to 2019, indicating a decreasing trend. This variation in findings can be ascribed to disparities in the time frames of participant enrolment: Our study encompassed data from 1990 to 2019, while the study by Paik *et al*[33] included data from 2012 to 2017. Notably, we also calculated the EAPC of the ASDR of COCLDs due to NAFLD from 2012 to 2019 and found a decreasing trend with a value of -0.05. This indicates that there may have been a decreasing trend from 2017 to 2019.

The global upsurge in type 2 diabetes and obesity has raised the prevalence of NAFLD in both developed and developing nations[4]. Our study found that the highest ASPRs of COCLDs due to NAFLD were in some developing regions, such as North Africa, the Middle East regions, and Southeast Asia, and in specific countries such as Egypt, Qatar, and Kuwait. Previous research reported an NAFLD prevalence of 42.04% in South Asia and 31.79% in the Middle East, which is consistent with our finding that these regions had the highest prevalence rates[25,34].

According to previous research, approximately half of the global burden of liver complications associated with NAFLD is concentrated in the Middle East, North Africa, and Asia[35]. The escalated prevalence in these geographical areas can be attributed to intricate interplays of lifestyle choices, economic conditions, and ethnic factors. The tandem epidemics of obesity and NAFLD are predominantly propelled by unhealthy dietary practices and sedentary habits characterized by consumption of calorie-dense foods and insufficient physical activity[34]. Studies have indicated that Middle East and North African countries have a high prevalence of overweight and obesity, with more than 30% of females and more than 20% of males being obese in most countries in the region. This trend is attributed to unhealthy diets and the lowest levels of physical activity worldwide[36]. Concurrently, the Middle East and North Africa register the highest prevalence rates of cirrhosis of the liver attributable to NAFLD. This underscores the critical need for effective interventions aimed at mitigating the escalating prevalence of obesity and diabetes to ameliorate the burden of COCLDs attributed to NAFLD [37].

According to our study, the prevalence, deaths, and DALYs lost due to COCLDs stemming from NAFLD were higher in males than in females across all age groups before the age of 65-69 years. Similarly, the rates of these occurrences were comparable when compared with their female counterparts across all age groups. This sex disparity is consistent with previous research demonstrating that from 1990 to 2017, the burden of cirrhosis in males was universally higher in males than in females[38]. Hormonal factors could underlie this pattern, where estrogen, acting as an antioxidant, mitigates the activity of stellate cells and the advancement of liver fibrosis[39]. After menopause, women lose the protective effect of estrogen[40], and physiological changes associated with hypoestrogenism, such as insulin resistance, dysglycemia, dyslipidemia, and visceral fat accumulation, may be associated with the higher prevalence of COCLDs due to NAFLD in postmenopausal women[41,42]. This may partly elucidate the higher prevalence in males older than 70 years in our study.

We found that the number of prevalent cases, deaths, and DALYs lost due to COCLDs due to NAFLD was the highest among middle-aged groups (approximately 45-69 years). Within this range, the 75-79 age group presented the highest prevalence rate, while the 95+ age group exhibited the highest mortality rate. The underlying reasons for these findings are multifactorial. One potential explanation involves the metabolic alterations that transpire in older age groups[43]. Another possible reason is the natural history of liver cirrhosis, which is characterized by a compensated phase that lasts significantly longer than the rapidly progressive decompensated phase, with a median survival time of more than 12 years. A large proportion of patients with cirrhosis die after transitioning from the compensated phase to the decompensated phase[5]. Given this context, the imperative need for accurate, noninvasive methodologies to enable early identification of COCLDs stemming from NAFLD becomes evident.

We found a negative correlation between the SDI and the ASDAR of COCLDs due to NAFLD in the 21 GBD regions from 1990 to 2019 and in 204 countries in 2019. Generally, regions with higher SDI had a lower burden of cirrhosis due to NAFLD, which may be attributed to accessible high-quality health care and enough safe spaces to exercise[44,45].

Conversely, low-SDI and low-middle-SDI regions tended to bear a higher burden. The Sub-Saharan African region showed a high ASDAR of COCLDs due to NAFLD in 2019. Some regions such as Western Sub-Saharan Africa, Southeast Asia, Central Latin America, Andean Latin America, and Eastern Sub-Saharan Africa, as well as some countries and territories, such as Egypt, Honduras, and Guatemala, had burdens higher than expected based on their SDIs, indicating that these regions and countries should receive more investment and public health programs.

The escalating prominence of COCLDs emanating from NAFLD has positioned it as a preeminent public health challenge. Notably, an absence of effective pharmaceutical interventions to fully eradicate NAFLD persists. Consequently, interventions targeting weight loss could be efficacious and cost-effective strategies to avert the progression of NAFLD to COCLDs. In addition, exercise interventions without significant weight loss have also had a beneficial effect on alleviating NAFLD[46]. Thus, it is imperative to emphasize the critical role of weight management and exercise within public health programs. Furthermore, an enhancement of noninvasive diagnostic methods and the development of effective treatment strategies will be pivotal in alleviating the burden of COCLDs stemming from NAFLD. Importantly, public awareness regarding NAFLD and its associated complications remains inadequate. A concerted effort to raise population awareness about the implications of NAFLD is paramount.

In this study, we pioneered a comprehensive analysis of the relative burden of COCLDs attributed to NAFLD on global, regional, and national scales spanning the period from 1990 to 2019. Nevertheless, several limitations warrant consideration. First, the data from GBD 2019 have the general limitations of the GBD approach that have been described. The GBD estimates depended on robust statistical methods and trends from neighboring countries to overcome data scarcity and low data quality in some countries. Second, liver biopsy remains the gold-standard diagnostic test for patients with COCLDs due to NAFLD, but its poor acceptability during compensation and sampling variability may lead to misdiagnosis and underdiagnosis[47]. A dearth of diagnostic techniques may cause an underestimation of COCLDs due to NAFLD, which may be more severe in regions with low SDIs. Third, patients admitted to hospitals mostly had decompensated cirrhosis. Therefore, the number of compensated cirrhosis cases may be underestimated. The underreporting of cirrhosis can bias the estimates. Fourth, GBD 2019 failed to adopt the new term MAFLD to replace NAFLD. NAFLD was defined only after the exclusion of other causes of hepatic steatosis, and there is an unclear differentiation between NAFLD and alcoholic liver disease owing to different adjustments for alcohol use. Finally, the exclusion of patients with cirrhosis with hepatocellular carcinoma from our study could result in an underestimation of the true mortality rate among individuals with liver cirrhosis due to NAFLD.

CONCLUSION

This study described the burden of COCLDs due to NAFLD in 204 countries and territories from 1990 to 2019 by age, sex, and SDI. COCLDs due to NAFLD are becoming a significant global public health concern. Over the past three decades, there has been a notable increase in the ASPR, while the ASDR and ASDAR have exhibited downward trends. Notably, substantial geographic disparities exist in the burden of COCLDs due to NAFLD, with the highest prevalence rates observed in North Africa and the Middle East. In 2019, males had a higher burden of prevalence, deaths, and DALYs lost than females before the 65-69 age group. Furthermore, there is a negative correlation between SDI values and ASDAR. We hope this study raises public awareness of COCLDs due to NAFLD and broadcasts the need for more effective prevention strategies to minimize the future health care burden.

ARTICLE HIGHLIGHTS

Research background

The incidence and prevalence of nonalcoholic fatty liver disease (NAFLD) have been rapidly increasing worldwide over the past few decades, leading to cirrhosis and other chronic liver diseases (COCLDs). Cirrhosis is the leading cause of liver-related morbidity and contributes to more than 1 million deaths annually worldwide. NAFLD has become the leading cause of COCLDs.

Research motivation

A previous study reported the burden of liver cirrhosis caused by nonalcoholic steatohepatitis. However, no studies have focused on the epidemiology of COCLDs due to NAFLD across the globe.

Research objectives

We conducted a comprehensive and comparable updated analysis of the global, regional, and national levels of prevalence, death, and disability-adjusted life-years (DALYs) of COCLDs due to NAFLD in regards to age-standardized rates and numbers from 1990 to 2019, stratified by sex, age, and sociodemographic index.

Research methods

Data on COCLDs due to NAFLD were collected from the Global Burden of Diseases, Injuries, and Risk Factors Study 2019. Numbers and age-standardized prevalence, death, and DALYs were estimated through a systematic analysis of modeled data from the Global Burden of Diseases, Injuries, and Risk Factors Study 2019. Estimated annual percentage

change was used to determine the burden trend.

Research results

We found that the global age-standardized prevalence rate of COCLDs due to NAFLD was 15022.90 per 100000 population in 2019, with an estimated annual percentage change of 0.78. The age-standardized death rate and age-standardized DALYs rate per 100000 population were 1.66 and 43.69 in 2019, respectively. The highest prevalence rate was observed in North Africa and the Middle East. The numbers of prevalent cases, deaths, and DALYs cases of COCLDs due to NAFLD were higher in males than in females across all age groups before the age of 65-69 years. There was a negative correlation between sociodemographic index and age-standardized DALYs rate.

Research conclusions

COCLDs due to NAFLD have emerged as a large and growing public health burden worldwide. Globally, the ASPR has increased during the past three decades, whereas the ASDR and age-standardized DALY rate have decreased. There is geographical variation in the burden of COCLDs due to NAFLD. It is strongly recommended to improve the quality of COCLDs due to NAFLD health data across all countries and regions to facilitate better monitoring of the burden of COCLDs due to NAFLD.

Research perspectives

We believe that the findings of this study will provide insight into the global disease burden of COCLDs due to NAFLD and assist policymakers in formulating effective policies to mitigate modifiable risk factors.

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FOOTNOTES

Co-first authors: Zhi-Peng Liu, Guo-Qing Ouyang.

Co-corresponding authors: Song-Qing He, Guan-Dou Yuan.

Author contributions: Liu ZP, Ouyang GQ, He SQ, and Yuan GD conceived and designed the study; Liu ZP, Ouyang GQ, Huang GZ, Wei J, and Dai L were involved in collection and interpretation of the data; Liu ZP, Ouyang GQ, and Yuan GD edited the manuscript; Liu ZP, Ouyang GQ, and He SQ revised the manuscript; All authors were involved in reading and approving the final manuscript. We designated co-corresponding authors because our research was a collaborative team effort, and the designation of co-corresponding authors helped to assign responsibilities and tasks related to the paper. This helped improve the quality and reliability of the paper. Second, He SQ and Yuan GD made equally important contributions throughout the study. The selection of co-corresponding authors recognizes this equal contribution. In conclusion, we believe that the designation of He SQ and Yuan GD as co-corresponding authors is an appropriate choice for our manuscript because it accurately reflects the collaborative spirit and diversity of our team. Liu ZP and Ouyang GQ contributed equally as co-first authors to this work; He SQ and Yuan GD contributed equally as co-corresponding authors to this work.

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Clinical trial registration statement: This study was an analysis of the burden of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease. The data were extracted from the Global Burden of Diseases, Injuries, and Risk Factors database, and clinical trial registration statements were not necessary.

Informed consent statement: This study was an analysis of the burden of cirrhosis and other chronic liver diseases due to nonalcoholic fatty liver disease. The data were extracted from the Global Burden of Diseases, Injuries, and Risk Factors database, and ethics approval and consent to participate were not necessary.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: Data are available from http://ghdx.healthdata.org/gbd-results-tool and can be acquired from the corresponding author at dr_hesongqing@163.com.

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ORIGINAL ARTICLE

Observational Study

Evaluation of a protocol for rifaximin discontinuation in critically ill patients with liver disease receiving broad-spectrum antibiotic therapy

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Abstract

BACKGROUND

Rifaximin is frequently administered to critically ill patients with liver disease and hepatic encephalopathy, but patients currently or recently treated with antibiotics were frequently excluded from studies of rifaximin efficacy. Due to overlapping spectrums of activity, combination therapy with broad-spectrum antibiotics and rifaximin may be unnecessary. A pharmacist-driven protocol was piloted to reduce potentially overlapping therapy in critically ill patients with liver disease. It was hypothesized that withholding rifaximin during broad-spectrum antibiotic therapy would be safe and reduce healthcare costs.

AIM

To determine the clinical, safety, and financial impact of discontinuing rifaximin during broad-spectrum antibiotic therapy in critically ill liver patients.

METHODS

This was a single-center, quasi-experimental, pre-post study based on a pilot pharmacist-driven protocol. Patients in the protocol group were prospectively identified *via* the medical intensive care unit (ICU) (MICU) protocol to have rifaximin withheld during broad-spectrum antibiotic treatment. These were compared to a historical cohort who received combination therapy with broad-spectrum antibiotics and rifaximin. All data were collected retrospectively. The primary outcome was days alive and free of delirium and coma (DAFD) to 14 d.

Safety outcomes included MICU length of stay, 48-h change in vasopressor dose, and ICU mortality. Secondary outcomes characterized rifaximin cost savings and protocol adherence. Multivariable analysis was utilized to evaluate the association between group assignment and the primary outcome while controlling for potential confounding factors.

RESULTS

Each group included 32 patients. The median number of delirium- and coma-free days was similar in the control and protocol groups [3 interquartile range (IQR 0, 8) *vs* 2 (IQR 0, 9.5), $P = 0.93$]. In multivariable analysis, group assignment was not associated with a reduced ratio of days alive and free of delirium or coma at 14 d. The protocol resulted in a reduced median duration of rifaximin use during broad-spectrum antibiotic therapy [6 d control (IQR 3, 9.5) *vs* 1 d protocol (IQR 0, 1); $P < 0.001$]. Rates of other secondary clinical and safety outcomes were similar including ICU mortality and 48-h change in vasopressor requirements. Overall adherence to the protocol was 91.4%. The median estimated total cost of rifaximin therapy per patient was reduced from $758.40 (IQR $379.20, $1200.80) to $126.40 (IQR $0, $126.40), $P < 0.01$.

CONCLUSION

The novel pharmacist-driven protocol for rifaximin discontinuation was associated with significant cost savings and no differences in safety outcomes including DAFD.

Key Words: Rifaximin; Hepatic encephalopathy; Critical illness; Antibiotics; Liver disease; Cirrhosis

Core Tip: Critically ill patients with liver disease receiving broad-spectrum antibiotic therapy have been frequently excluded from clinical trials of rifaximin efficacy. Therefore, despite overlapping spectrums of antibacterial activity, it is not known if rifaximin provides additional clinical benefit in these patients. In this study, pharmacist-guided rifaximin discontinuation during broad-spectrum antibiotic therapy resulted in significant cost savings and was not associated with negative short-term cognitive effects or adverse events.

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INTRODUCTION

Hepatic encephalopathy (HE) encompasses a spectrum of neurocognitive alterations in patients with liver dysfunction and/or porto-systemic shunting, and is associated with symptoms that range in severity from minimal neuropsychiatric manifestations to cerebral edema and coma in the most severe cases[1,2]. The pathogenesis of HE has not been fully elucidated and is likely multifactorial. Ammonia has been implicated as a contributing factor to the development of HE due to its association with direct neurotoxicity mediated by astrocyte swelling and modification of glutamine and gamma-amino-n-butyric acid systems[3]. Rifaximin is an oral, non-absorbable rifamycin derivative with antibiotic activity against ammonia-producing gram-positive, gram-negative, and anaerobic species[4,5]. Long-term rifaximin use is associated with clinically important reductions in infections, hospital re-admissions, durations of hospital stays, and overt HE recurrence[6-8]. The exact mechanism by which rifaximin exerts benefit remains unclear[9]. Previous hypotheses focused on the control of ammonia-producing enteric bacteria *via* antibiotic activity[10,11]. However, a growing body of evidence depicts increasingly understood mechanisms of rifaximin activity including decreased circulating endotoxin burden, decreased microbiota-derived systemic inflammation, and improvement in cirrhosis-related dysbiosis which suggests the presence of multi-factorial benefits of rifaximin in the pathobiology of cirrhosis[12]. Rifaximin is recommended by the American Association for the Study of Liver Diseases (AASLD) as adjunctive therapy for the prevention of overt HE recurrence (grade I, A, 1)[13]. Similarly, the European Association for the Study of the Liver (EASL) recommends rifaximin as an adjunct to lactulose as secondary prophylaxis following ≥ 1 additional episodes of overt HE within 6 mo of the first episode (LoE 2, strong)[14]. Use of rifaximin for the treatment of HE is not recommended in these guidelines; however, efficacy has been demonstrated in randomized controlled trials and use is common in clinical practice[15-18].

Patients receiving or recently treated with antibiotics were frequently excluded from these trials[15-17]. These studies, which included patients with both acute and chronic HE, excluded cases with precipitants or recent medication exposures which could interfere with HE or therapeutic effect monitoring. Infection is a frequent precipitant of overt HE in critically ill patients, the treatment of which commonly relies on broad-spectrum antibiotics[19]. In many cases of infection, patients also receive rifaximin either as a continuation of home therapy or newly initiated treatment. Because these

patients are infrequently studied, it remains unclear if rifaximin provides additional therapeutic benefit when combined with broad-spectrum antibiotics.

In 2018, Cleveland Clinic established the medical intensive liver unit (MILU), a specialty unit of the MICU supported by a multidisciplinary team including intensivists, hepatologists, and critical care clinical pharmacy specialists caring for patients with a variety of hepatic pathologies. Many patients admitted to the MILU are initiated on broad-spectrum antibiotics for empiric or targeted therapy for infections in addition to HE treatment with rifaximin. A pharmacist-driven pilot protocol was implemented to reduce potentially overlapping therapy through the discontinuation of rifaximin during broad-spectrum antibiotic treatment. Pharmacists were also responsible for the coordination of rifaximin re-initiation following antibiotic therapy narrowing or discontinuation. This study aimed to evaluate the impact of this protocol on clinical, safety, and financial outcomes.

MATERIALS AND METHODS

Study design

This was an Institutional Review Board-approved, quasi-experimental, pre-post study conducted at a large quaternary academic medical and liver transplant center in the United States. The pharmacist-driven protocol for rifaximin discontinuation was implemented beginning August 1, 2020. Adult patients in the medical intensive care unit (ICU) (MICU) were prospectively screened by clinical pharmacy specialists and eligible for the protocol if they had orders for rifaximin and a qualifying antibiotic regimen (Table 1). Discontinuation of rifaximin was recommended and recorded by the pharmacist and research team. Duration of antibiotic therapy was tracked and reviewed daily by a small group of critical care clinical pharmacy specialists to ensure re-initiation of rifaximin upon antibiotic narrowing or discontinuation. Before implementation, education was provided to all pharmacists who would manage or verify orders for MICU patients to reduce time to rifaximin discontinuation for patients admitted to the MICU during evenings and weekends. Physician leadership and medical teams were also involved in education about the protocol and its implementation.

Patient selection

Patients[3] 18 years old were eligible for study inclusion if they received at least 3 d of broad-spectrum antibiotics and had an order for rifaximin during MICU admission. Additional inclusion criteria for the control group were: (1) Admission to the MICU between August 1 and October 31, 2019 and (2) rifaximin therapy for ≥ 3 d or 75% of the antibiotic treatment duration during MICU admission, whichever was longer. In the protocol group inclusion criteria were (1) admission to the MICU between August 1, 2020 and January 31, 2021; and (2) ≥ 3 d of broad-spectrum antibiotics without rifaximin and concomitant rifaximin for ≤ 25% of the antibiotic duration during MICU admission. Any patient with a positive test for severe acute respiratory syndrome coronavirus 2 during admission was excluded.

Outcomes

The primary outcome was days alive and free of delirium and coma (DAFD) to day 14. Secondary outcomes were days alive and free of delirium to day 14, days alive and free of coma to day 14, ICU length of stay, ICU mortality, time to first extubation, rate of reintubation, days of combination therapy during MICU admission, rate of protocol adherence, time to rifaximin discontinuation in the protocol group, and the per-patient cost of rifaximin therapy during the follow-up period. The cost of rifaximin therapy was calculated using the average wholesaler price as of January 2023 to reflect the increase in rifaximin cost since initial protocol implementation[20]. The minimum cost of rifaximin was calculated based on one tablet given (control) or saved (protocol) per day of therapy while the maximum cost assumed two tablets given or saved per day. Changes in vasopressor requirements and Glasgow Coma Score (GCS) during the first 48 h of MICU combination therapy or withholding rifaximin were evaluated as additional safety measures.

Study definitions

Day one for the 14-d study period was defined as the first day during MICU admission on which patients received broad-spectrum antibiotics and rifaximin (control group) or broad-spectrum antibiotics without rifaximin (protocol group). Broad-spectrum antibiotic regimens were defined as providing gram-positive, gram-negative, and anaerobic coverage (Table 1). A day of therapy was defined as a 24-h period from midnight to 11:59 pm during which at least one-half of the scheduled doses of rifaximin and/or broad-spectrum antibiotics were received. Days were considered delirium-free if patients were alive and without a positive confusion assessment method for the ICU (CAM-ICU) assessment during the 24-h period and coma-free if patients were alive and with zero hours spent with a Richmond Agitation Sedation Scale (RASS) score of -4 or -5 or with GCS of 3 during the 24-h period. Days of mechanical ventilation were defined as the use of positive pressure ventilation during any one hour of the 24-h period from midnight to 11:59 pm for use in the multivariable model. All admission days in non-ICUs during which the patient was alive were considered to be free of delirium as brief CAM (bCAM) and West-Haven grades (WHG) were not routinely recorded. Protocol adherence was defined as the discontinuation of rifaximin occurring within 72-h of protocol-defined broad-spectrum antibiotic therapy initiation. All vasopressor doses were converted to norepinephrine equivalents according to the following equation: [norepinephrine (mcg/min) + (epinephrine (mcg/min)] + [(dopamine (mcg/kg/min) ÷ 2] + [(phenylephrine (mcg/min) ÷ 10] + [vasopressin (units/hour) × 8.33][21,22]. Sedative agents included propofol, dexmedetomidine, ketamine, lorazepam, or midazolam when administered as a continuous infusion. Ileus was defined as > 48 h with zero bowel movements or fecal management system output recorded. Orders for octreotide continuous infusions were used as a

Table 1 Protocol-defined broad-spectrum antibiotic regimens

Monotherapy	Gram-positive/negative	Gram-positive	Gram-negative	Anaerobic
Ampicillin-sulbactam	Cefazolin	Vancomycin	Aminoglycosides	Metronidazole
Amoxicillin-clavulanate	Cephalexin	Linezolid	Polymyxin B and colistin	Clindamycin
Piperacillin-tazobactam	Cefuroxime	Daptomycin	Aztreonam	
Cefoxitin	Cefdinir	Quinupristin-dalfopristin	Cefiderocol	
Meropenem +/- vaborbactam	Cefixime	Rifampin		
Imipinem-cilastatin +/- relebactam	Ceftazidime +/- avibactam	Rifabutin		
Ertapenem	Ceftriaxone	Penicillins		
Tigecycline	Cefepime			
Eravacycline	Ceftaroline			
	Ceftolozane-tazobactam			
	Ciprofloxacin			
	Levofloxacin			
	Doxycycline			
	Sulfamethoxazole-trimethoprim			

Broad-spectrum antibiotic therapy is defined as: (1) Any agent from "Monotherapy"; (2) one agent from each of "Gram-positive/negative" and "Anaerobic"; (3) one agent from each of "Gram-positive," "Gram-negative," and "Anaerobic."

surrogate to identify episodes of gastrointestinal bleeding according to routine institutional practice. Occurrences of *Clostridioides difficile* infection were recorded if the patient had either a positive polymerase chain reaction or enzyme-linked immunosorbent assay test.

Statistical analyses

Continuous data were assessed for normality by the Shapiro-Wilk test. Parametric continuous data are reported as mean ± SD and were analyzed using a two-sample *t*-test. Non-parametric continuous data are reported as median (IQR) and were analyzed using the Wilcoxon rank-sum test. Categorical data are presented as number (%) and were analyzed by chi-squared or Fisher's exact tests, based on sample size. The primary outcome of DAFD was compared using a one-sided Wilcoxon rank-sum test, assuming greater median DAFD in the control group. It was calculated that the inclusion of 32 patients in each group would provide 80% power to detect a 0.65-d difference in DAFD, with a one-sided significance level of 0.025. Multivariable analysis of the primary outcome was planned to include covariates of biologic plausibility (duration of mechanical ventilation, use of deep sedation, MELD-Na score, gastrointestinal bleeding) and those with a *P*-value of < 0.05 in univariable analysis. A negative binomial model was selected for the multivariable analysis to account for the observed over-dispersion of the primary outcome which violated foundational assumptions of a Poisson distribution that was attempted after similar violations of linear regression despite log-transformation of the variables. All variables included in the model were selected based on prior literature and biological plausibility to contribute to or interfere with the assessment of delirium and coma or to indicate a clinically significant baseline difference in illness severity between the groups. All analyses were performed based on an overall significance level of 0.05 using either SAS software (version 9.4, Cary, NC) or Stata/IC software, v.14 (StataCorp LP, College Station, TX). Data extracted from the electronic medical record were collected and managed using REDCap electronic data capture tools hosted by Cleveland Clinic[23,24].

RESULTS

Overall characteristics of patients

A total of 159 patients were screened for inclusion and 32 were included in both groups (Figure 1). The most common reason for exclusion in both groups was insufficient treatment duration. The two groups were well-balanced at baseline with the exception of norepinephrine requirements on the day of MICU admission, which were higher in the protocol group, and race (Table 2). There was no difference in high-grade HE at baseline; however, due to intubation and sedation on study day 1, many patients were unable to be assigned baseline WHG. Though none of these differences were statistically significant, deep sedation, paralysis, and gastrointestinal bleeding were more common among protocol patients while control patients more often received scheduled benzodiazepines and were admitted directly to the ICU.

Table 2 Baseline characteristics

Variable	Control (*n* = 32)	Protocol (*n* = 32)	*P* value
Male	19 (59.4)	18 (56.2)	0.21
Age (yr)	57.9 (± 12.9)	53.0 (± 12.7)	0.13
Race			0.01
White	26 (81.3)	24 (75)	
Unavailable	4 (12.5)	2 (6.3)	
Black	1 (3.1)	1 (3.1)	
American Indian/Alaskan native	1 (3.1)	0 (0)	
Asian	0 (0)	0 (0)	
Multiracial-Multicultural	0 (0)	0 (0)	
Weight (kg)	85.4 (± 23.1)	91.3 (± 29.2)	0.37
Direct Intensive Care Unit Admission	15 (46.9)	12 (37.5)	0.45
SOFA score	10.2 (± 3.0)	11 (± 3.2)	0.34
NE requirements, mcg/kg/min	0 (0, 0.136)	0.023 (0, 0.309)	0.02
MELD-Na	28.5 (± 8.5)	30.0 (± 8.2)	0.48
West-Haven grade			0.31
Unavailable	10 (31.3)	6 (18.8)	
0	6 (18.8)	8 (25)	
1	6 (18.8)	6 (18.8)	
2	4 (12.5)	6 (18.8)	
3	5 (15.6)	5 (15.6)	
4	1 (3.1)	1 (3.1)	
Glasgow Coma Score	9 (6, 14)	11 (7, 15)	0.69
Cirrhosis Etiology			0.21
Ethanol	17 (53.1)	21 (65.6)	
Non-alcoholic steatohepatitis	9 (28.1)	5 (15.6)	
Primary biliary cholangitis	2 (6.3)	1 (3.1)	
Autoimmune hepatitis	2 (6.3)	1 (3.1)	
Primary sclerosing cholangitis	0 (0)	1 (3.1)	
Unknown	2 (6.3)	0 (0)	
Other	0 (0)	3 (9.4)	
Pre-ICU rifaximin treatment	12 (37.5)	17 (48.6)	0.21
Antibiotic Indication			0.80
Empiric; source unknown	20 (62.5)	23 (71.9)	
Pneumonia	6 (18.8)	3 (9.4)	
Intraabdominal	4 (12.5)	4 (12.5)	
Bloodstream infection	1 (3.1)	1 (3.1)	
Skin and soft tissue	1 (3.1)	0 (0)	
Bone and joint infection	0 (0)	1 (3.1)	
Rifaximin regimen			0.37
550 mg BID	31 (96.9)	31 (96.9)	
400 mg BID	1 (3.1)	0 (0)	

200 mg BID	0 (0)	1 (3.1)	
Deep sedation use	5 (15.6)	9 (28.1)	0.38
Benzodiazepine use	3 (9.4)	0 (0)	0.29
Continuous neuromuscular blockade use	2 (6.3)	3 (9.4)	1.00
Polyethylene glycol use	9 (28.1)	13 (40.6)	0.29
Lactulose use	29 (90.6)	31 (96.9)	0.30
Gastrointestinal bleeding treatment	8 (25)	14 (43.8)	0.11
Alcohol withdrawal diagnosis	1 (3.1)	0 (0)	1.00
Ileus	6 (18.8)	9 (28.1)	0.56
C. difficile infection	1 (3.1)	2 (6.3)	1.00

All values are presented as mean ± SD, median (interquartile range), or N (%). BID: bis in die; ICU: Intensive care units; MELD-Na: Model for End-Stage Liver Disease Sodium score; NE: Norepinephrine; SOFA: Sequential Organ Failure Assessment score.

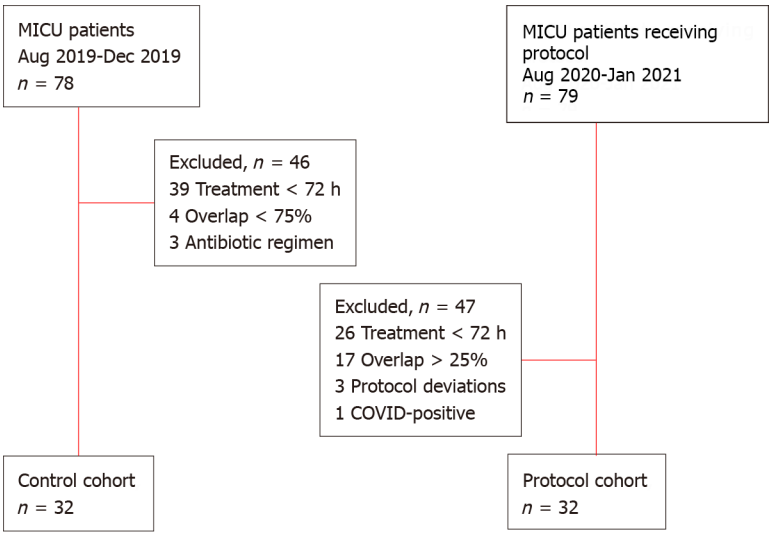

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Figure 1 Consort diagram.

Primary outcome

No significant differences were observed in the primary outcome (DAFD to day 14), between the control and protocol groups [3 (interquartile range (IQR 0, 8) *vs* 2 (IQR 0, 9.5); $P = 0.93$] (Table 3). After adjustment for deep-sedation, gastrointestinal bleeding treatment, MELD-Na score, and duration of mechanical ventilation in a negative binomial regression there remained no significant difference in the primary outcome between the control and protocol groups (ratio 0.78, 95% confidence interval 0.39-1.56, $P = 0.48$) (Table 4).

Secondary outcomes including safety analyses

The observed ICU mortality rate was high in both groups [control 13 (40.6%) *vs* protocol 15 (46.9%); $P = 0.61$]. Days alive and free of either delirium or coma, ICU length of stay, and time to extubation were similar between the groups. Similarly, no significant differences in vasopressor requirements and GCSs in the 48 h following rifaximin discontinuation were observed between groups (Table 3). For patients included in the protocol group, the median time to rifaximin discontinuation was approximately 24 h from MICU admission. Protocol adherence was 91.4% with the most common reason for non-adherence being rifaximin discontinuation during antibiotic therapy not meeting the protocol definition of broad-spectrum. Days of rifaximin therapy during broad-spectrum antibiotics were significantly reduced in the protocol group [6 (IQR 3-9.5) *vs* 1 (IQR 0-1); $P < 0.001$]. The cost of rifaximin therapy was also significantly reduced in the protocol group with an estimated cost savings of $316.00 [United States dollar (USD)] to $632.00 (USD) per patient.

Table 3 Clinical, safety, and cost outcomes

Outcome	Control (*n* = 32)	Protocol (*n* = 32)	*P* value
Primary outcome			
Days alive and free of delirium and coma to day 14	3 (0, 8)	2 (0, 9.5)	0.93
Secondary outcomes			
Days alive and free of delirium	3 (0, 8.5)	2 (0, 9)	0.85
Days alive and free of coma (RASS)	6 (3.5, 12)	8 (4, 11)	0.81
Days alive and free of coma (GCS)	8 (5.5, 13)	7.5 (5.5, 9)	0.21
Glasgow Coma Score 48-h change	0 (-3, 1.5)	0 (-1, 0.5)	0.43
ICU mortality (%)	13 (40.6)	15 (46.9)	0.61
ICU length of stay	10 (4.5, 20.5)	11 (7, 17)	0.73
Time to first extubation from day 1 of intubation	6 (4, 14) (*n* = 23)	5 (4, 9) (*n* = 26)	0.49
Rate of reintubation (%)	4 (17.4)	3 (11.5)	0.56
IVVP requirement 48-h change, NE mcg/kg/min equivalents	0 (0, 0.12)	0 (0, 0.01)	0.45
Days of MICU combination therapy	6 (3, 9.5)	1 (0, 1)	< 0.01
Protocol adherence	-	91.4%	-
Time to rifaximin discontinuation, days	-	1 (0, 1)	-
Cost of rifaximin therapy per patient to day 14, USD			
Minimum	379.20 (189.60, 600.40)	63.20 (0, 63.20)	< 0.01
Maximum	758.40 (379.20, 1200.80)	126.40 (0, 126.40)	< 0.01

GCS: Glasgow Coma Score; ICU: Intensive care unit; IVVP: Intravenous vasopressor; MICU: Medical intensive care unit; NE: Norepinephrine; RASS: Richmond Agitation-Sedation Scale; USD: United States dollar.

Table 4 Negative binomial multivariable model

(*n* = 60)	DAFD ratio (95%CI)
Group assignment (protocol)	0.78 (0.39, 1.56)
Deep sedation (yes)	0.89 (0.39, 2.02)
Gastrointestinal bleeding (yes)	0.65 (0.32, 1.31)
MELD-Na (per unit increase)	0.97 (0.94, 1.01)
Mechanical ventilation duration (per day)	0.79 (0.72, 0.87)

Reference value listed in parentheses. DAFD: Days alive and free of delirium and coma to day 14; MELD-Na: Model for End-Stage Liver Disease Sodium score; CI: Confidence interval.

DISCUSSION

The addition of rifaximin to broad-spectrum antibiotic therapy may provide overlapping antibacterial activity without additional therapeutic benefit in critically ill patients with HE. Notably, patients on broad-spectrum antibiotics have been generally excluded from studies of rifaximin efficacy in HE. This gap in the literature represents a need to better understand the role of rifaximin in this unique patient population, as ICU hospitalizations for patients with HE are typically characterized by severe disease and increased morbidity and mortality. This study is the first to evaluate the feasibility and safety of rifaximin discontinuation during broad-spectrum antibiotic therapy.

In our pilot investigation, rifaximin discontinuation during broad-spectrum antibiotic therapy in critically ill patients with liver disease was not associated with higher rates of delirium or coma. This result was robust to adjustment in multivariable analysis. As demonstrated in Table 3, no negative associations were observed between rifaximin discontinuation and short-term cognitive outcomes. Neither was rifaximin discontinuation associated with increased adverse effects, which adds support to the hypothesis that withholding rifaximin during broad-spectrum antibiotic therapy is

safe. The lack of observed differences in cognitive outcomes is an important contribution to the existing understanding of the interaction between treatment with rifaximin and other broad-spectrum antibiotics.

Much of the promising data for the benefit of rifaximin therapy in patients with cirrhosis, including reduced infections and hospitalizations, have been produced in the outpatient setting and reflect chronic use (> 30 d in most cases)[6-8]. In the present study, rifaximin was held for a limited time in hospitalized, critically ill patients during concomitant broad-spectrum antibiotic treatment. Few trials of rifaximin efficacy have included or focused on a critically ill population, however, the very limited data available suggest potential for harm with rifaximin discontinuation[19,25,26]. Sharma *et al* [18] published the largest single cohort evaluating rifaximin efficacy including critically ill patients. The authors reported a decrease in mortality among patients treated with rifaximin and lactulose compared with those treated with lactulose and placebo. Importantly, this finding is limited by the high rate of sepsis-related mortality in the placebo group. In contrast to the study by Sharma *et al*[18], all patients included in the present study had known or suspected infection but observed mortality rates were similar among patients who continued rifaximin during broad-spectrum antibiotic therapy and those who did not. In an open-label single-center study including 15 patients, Kalambokis *et al*[24] demonstrated that rifaximin therapy was associated with increased systemic vascular resistance after four weeks. This finding raised a question about the potential impact sudden discontinuation of therapy might have in critically ill patients predisposed to clinically significant vasodilation exacerbated in the setting of active infection. To address this question, vasopressor requirements in the first 48-h after rifaximin discontinuation (protocol) or antibiotic initiation (control) were compared. In the current larger cohort, rifaximin discontinuation was not associated with changes in vasopressor requirements despite severe and progressive illness in many included patients. Finally, in a single-center retrospective cohort analysis of mechanically ventilated critically ill patients with decompensated cirrhosis, rifaximin administration within the first 24-h post-intubation was associated with shorter time to extubation [hazard ratios 1.74 (1.21-2.50)], although pre-intubation rifaximin and lactulose administration was associated with a delayed time to extubation[26]. Rifaximin discontinuation was not associated with delays in extubation or rates of reintubation in the present study, though the sample size was smaller. In summary, although several studies in critically ill patients had demonstrated potential associations with mortality, vasopressor requirements, and duration of mechanical ventilation, the current study evaluated several potential safety concerns in a highly vulnerable patient population and did not reveal any negative signals associated with rifaximin discontinuation.

Notably, the present study demonstrates the feasibility and benefit of a pharmacist-driven, manually applied protocol with multidisciplinary support aimed at antimicrobial stewardship in a critically ill population. Though likely to be applicable to many centers in the United States, opportunities to optimize protocol execution exist including streamlining patient identification and enrollment, minimizing delays in rifaximin discontinuation, and ensuring rifaximin re-initiation after broad-spectrum antibiotic therapy completion or narrowing.

Several limitations exist within this evaluation. First, this was a single-center study with retrospective data collection. Second acute (overt) HE was not a requirement for inclusion, nor was the chronicity of HE episodes able to be quantified. Patients were stratified according to WHG and/or GCS in order to describe clinical status and align with available guideline recommendations for HE assessment. Similarly, it was not possible to definitively identify the specific indication for rifaximin for every patient nor to confirm the prescription of rifaximin prior to hospital admission with the limited available insurance claim records. Though based on available records, pre-hospital rifaximin therapy was prescribed at a similar rate in both the control and protocol groups, 37.5% *vs* 48.6%, respectively. Third, the high frequency of missing WHG due to retrospective clinical assessment in the setting of critical illness requiring mechanical ventilation and sedation necessitated the use of multiple surrogate endpoints. The primary outcome of DAFD was selected based on previously published studies evaluating critically ill patients' level of awareness[27,28]. While CAM scoring is routine in ICUs at our institution, delirium assessments (*i.e.* bCAM) are not routine in non-ICU care areas. The decision to consider all patients in non-ICU care areas delirium-free was based on an understanding of the clinical improvements required to support ICU discharge and the lack of routinely available validated scores to collect for the endpoint. This may have led to an over-estimation of the days free of delirium, however, this is likely balanced by the high average proportion of time spent in the ICU compared to non-ICU care areas. The 48-h change in GCS was also collected as a sensitive marker for any negative cognitive effect rifaximin discontinuation may have exerted. GCS was selected as current AASLD guidelines recommend this score as an alternative measure to the WHG for the diagnosis of HE (grade II-2, B, 1)[13]. The EASL guidelines recommend the addition of GCS to West-Haven criteria in patients with impaired consciousness, including those treated in an ICU (LoE 5, strong)[14]. Several outcomes of interest were unable to be assessed due to the absence of baseline WHG including the achievement and time to resolution or improvement of HE. Despite a robust effort to characterize the patient population and describe the severity and extent of illness, there are illness-specific variables and outcomes that were unable to be feasibly assessed, including indication for MICU admission and infection resolution which may have contributed to cognitive and clinical outcomes. Additionally, this study was not designed to evaluate long-term outcomes or impact of withheld rifaximin therapy. Despite these limitations, no differences were found in the available and utilized markers of cognitive outcomes between patients who did or did not have rifaximin discontinued during broad-spectrum antimicrobial therapy. Finally, the few baseline differences observed in the two cohorts may have been smaller or eliminated in a larger sample size. However, the authors anticipate the strong left skew in the primary outcome with a predominance of zero or minimal days spent alive and free of delirium and coma to persist, even in a larger sample, given the tenuous nature of critically ill patients with liver disease. Similarly, rates of HE resolution in a comparable population would be expected to be very low. These data characteristics would likely render future non-inferiority trials difficult or impossible to complete.

CONCLUSION

In conclusion, no significant differences were noted between the control and protocol groups in key clinical or safety outcomes. The robustness of the primary outcome to multivariable analysis strengthens the conclusion that rifaximin discontinuation during broad-spectrum antibiotic therapy does not appear to negatively impact the cognitive status of critically ill liver patients. This study demonstrates the feasibility of a pharmacist-driven protocol to reduce combination therapy in critically ill patients with liver disease treated with rifaximin and broad-spectrum antibiotics. Given the significant cost savings achieved during ICU and hospital admission, a prospective, multi-center evaluation of a similar protocol in a larger sample is warranted, including investigation into longer-term outcomes. Investigation of the impact of this type of protocol in non-critically ill liver patients should likewise be considered.

ARTICLE HIGHLIGHTS

Research background

Rifaximin is frequently administered to critically ill patients with liver disease and hepatic encephalopathy (HE). However, data supporting the use of rifaximin in this population, particularly in combination with broad-spectrum antibiotics, are extremely limited. Due to the overlapping spectrums of antibiotic activity, it was hypothesized that withholding rifaximin during broad-spectrum antibiotic therapy would be safe and reduce healthcare costs. The present study is the first to evaluate the feasibility and safety of rifaximin discontinuation during broad-spectrum antibiotic therapy and represents a highly vulnerable patient population.

Research motivation

The gap in available evidence demonstrates the need to better understand the role of rifaximin in this unique population, as intensive care unit (ICU) hospitalizations for patients with HE are typically characterized by severe disease and increased morbidity and mortality. Therefore, after protocol development the need to assess clinical and safety outcomes was clear. Additionally, given the opportunity to reduce healthcare expenditures with decreased use of rifaximin during ICU admission, costs of therapy were quantified. This proof-of-concept evaluation also provides a foundation for future, larger-scale, well-controlled studies to confirm and expand on the findings.

Research objectives

The present study aimed to evaluate the safety, efficacy, and financial impact of discontinuing rifaximin during broad-spectrum antibiotic use. The efficacy of withholding rifaximin was evaluated using a surrogate marker for short-term cognitive impact, days alive and free of delirium and coma. Multiple, robust safety outcomes were considered including mortality, ICU length of stay, 48-h change in vasopressor requirements, duration of mechanical ventilation, and successful extubation. Cost avoidance was evaluated by comparing rifaximin drug costs during the observation period pre- and post-protocol. The outcomes utilized provided an initial, comprehensive assessment of the pilot protocol that could be replicated in further investigations.

Research methods

This was a single-center, quasi-experimental study evaluating outcomes pre- and post-implementation of a pharmacist-driven protocol for rifaximin discontinuation in critically ill liver patients being treated in a medical ICU. To address potential sources of bias, multivariable analysis of the primary outcome was performed with characteristics selected based on biological plausibility and univariate screening. Inferential statistics were performed in the usual fashion based on data type and distribution. The study achieved 80% power to detect a 0.65 d difference in the primary outcome.

Research results

In this pilot investigation, rifaximin discontinuation during broad-spectrum antibiotic therapy in critically ill patients with liver disease was not associated with more days of delirium or coma [3 (0, 8) *vs* 2 (0, 9.5); $P = 0.93$]. Protocol application was associated with a high rate of adherence (91.4%) and resulted in a significant reduction in days of combination therapy [6 (3-9.5), 1 (0-1); $P < 0.001$] and medication expenditures (estimated per patient cost avoidance $316.00 to $632.00 USD). No signals of harm were detected in any safety endpoint. The results of this study support the safety and feasibility of a protocolized discontinuation of rifaximin during broad-spectrum antibiotic therapy. Due to the limited sample size and retrospective nature of the present study, future evaluations should prioritize larger sample sizes and prospective designs to the greatest extent possible. Many questions remain regarding the optimal use of rifaximin among patients being treated with broad-spectrum antibiotics, including non-critically ill patients and those receiving long courses of therapy.

Research conclusions

This was a novel evaluation that provides new insight about the potential safety of discontinuing rifaximin during short-term, broad-spectrum antibiotic therapy in critically ill patients with liver disease which has not yet been investigated in the literature. The safety, efficacy, and cost-saving results of this study warrant confirmation in an investigation with a larger sample size and prospective, well-controlled methods which could lead to broader application of a similar protocol. Finally, this study provides further support that pharmacists may be leveraged to assist with antimicrobial

stewardship efforts in specific and dynamic patient populations.

Research perspectives

Future research related to this question should focus on: Confirmation of the reported findings; longer-term outcomes of withholding rifaximin therapy, particularly during prolonged courses of broad-spectrum antibiotics; the impact of a similar protocol among non-critically ill patients; and opportunities to optimize protocol application.

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FOOTNOTES

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Informed consent statement: The requirement for informed consent for this study was waived due to the minimal risk posed to included patients.

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Basic Study

Metabolomics in chronic hepatitis C: Decoding fibrosis grading and underlying pathways

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Abstract

BACKGROUND

Chronic Hepatitis C (CHC) affects 71 million people globally and leads to liver issues such as fibrosis, cirrhosis, cancer, and death. A better understanding and prognosis of liver involvement are vital to reduce morbidity and mortality. The accurate identification of the fibrosis stage is crucial for making treatment decisions and predicting outcomes. Tests used to grade fibrosis include histological analysis and imaging but have limitations. Blood markers such as molecular biomarkers can offer valuable insights into fibrosis.

AIM

To identify potential biomarkers that might stratify these lesions and add information about the molecular mechanisms involved in the disease.

METHODS

Plasma samples were collected from 46 patients with hepatitis C and classified into fibrosis grades F1 (n = 13), F2 (n = 12), F3 (n = 6), and F4 (n = 15). To ensure that the identified biomarkers were exclusive to liver lesions (CHC fibrosis), healthy volunteer participants (n = 50) were also included. An untargeted metabolomic technique was used to analyze the plasma metabolites using mass spectrometry and database verification. Statistical analyses were performed to identify differential biomarkers among groups.

RESULTS
Six differential metabolites were identified in each grade of fibrosis. This six-metabolite profile was able to establish a clustering tendency in patients with the same grade of fibrosis; thus, they showed greater efficiency in discriminating grades.

CONCLUSION
This study suggests that some of the observed biomarkers, once validated, have the potential to be applied as prognostic biomarkers. Furthermore, it suggests that liquid biopsy analyses of plasma metabolites are a good source of molecular biomarkers capable of stratifying patients with CHC according to fibrosis grade.

Key Words: Chronic Hepatitis C; Fibrosis; Metabolome; Biomarkers; Plasma; Liquid biopsy

Core Tip: Chronic Hepatitis C affects 71 million people globally and leads to liver fibrosis, cirrhosis, cancer, and death. The accurate staging of fibrosis is crucial for treatment decisions and outcome prediction. Blood markers are a relevant source of information, and various molecular biomarkers have been investigated to characterize liver fibrosis. We analyzed plasma metabolites by mass spectrometry in 50 healthy participants, and in 46 patients with hepatitis C and classified them into fibrosis grades F1-F4. Six differential metabolites were identified in each grade of fibrosis; their biochemical pathways were analyzed and suggests molecular mechanisms involved in the disease.

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INTRODUCTION

Chronic hepatitis C (CHC) is an infectious disease caused by the hepatitis C virus (HCV) and is a serious public health problem, affecting an estimated 71 million people worldwide[1-3].

Approximately 50%-80% of HCV-infected individuals develop CHC, which can trigger a chronic inflammatory disease process leading to liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and death[4].

Natural progression of CHC occurs with sustained inflammation due to repetitive liver injury, followed by the activation of hepatic stellate cells, deposition of fibrillar collagen in the extracellular matrix (ECM), and progressive fibrosis[5,6]. These progressive processes may result in ECM degradation and, consequently, vascular and architectural alterations, leading to cirrhosis (10%-20% of patients)[7] or HCC (1%-5%)[8].

Early diagnosis and treatment can prevent liver cirrhosis and HCC, especially with screening and recent advances in CHC treatment based on direct-acting antiviral therapy. However, effective reduction of disease morbidity and mortality requires better characterization of liver involvement, more accurate prognosis, and follow-up[9]. Under this scenario, accurate identification of the liver fibrosis stage is critical for the clinical management of HCC, guiding therapeutic options and helping to predict prognosis[10]. However, this approach is challenging. Tests used to stage fibrosis include histological analysis of liver biopsies and imaging tests. Liver biopsy is considered the "gold standard" for the diagnosis and staging of liver fibrosis. However, it is an invasive and uncomfortable procedure with a risk of minor (10%-20%) or serious (0.5%-1%) complications[7,11]. In addition, the interpretation of histological results is subject to sampling errors and inter-observer subjectivity in the interpretation of histological results[7,12,13]. For staging the grades of fibrosis in biopsied liver tissue, the 0-4 scale of the Metavir classification system[14] is commonly used; however, the main limitations are related to the representativeness of liver samples and histopathological interpretation. Conventional imaging tests include ultrasonography, computed tomography, and magnetic resonance imaging. Although they represent important tools for detecting cirrhosis, nodules on the liver surface, and splenomegaly, they present low sensitivity for moderate or even advanced fibrosis. Newer acoustic technologies, such as hepatic elastography, can increase the accuracy of imaging techniques. For these tests, acoustic vibrations are applied to the abdomen and, according to how quickly these vibrations are transmitted along the liver tissue, the stiffness (fibrosis) of the liver is indicated. However, conditions other than fibrosis also increase liver stiffness[7], which requires further study and standardization. Another important limitation is the cost of the equipment[15], which is unaffordable in places with limited financial resources. In clinical practice, blood markers should be considered a relevant source of information. Current approaches are limited to combining commonly available tests (*e.g.*, aspartate transaminase, alanine aminotransferase, albumin, serum bilirubin, and international normalized ratio) with clinical information (*e.g.*, age, body mass index, and diabetes) and, in some cases, direct markers of liver function. However, this approach is most useful in distinguishing between two levels of fibrosis: Absent to minimal *vs* moderate to severe and fails to stratify the grades.

Undeniably, the search for blood biomarkers is a less invasive method for diagnosis and prognosis, and as blood circulates through most tissues, it can be a relevant source of information about diseases. Therefore, different molecular biomarkers, particularly those with easier and more accessible analytical methodologies, have been investigated for the characterization of liver fibrosis[16-20].

The present study focused on analyzing the plasma metabolome of patients with CHC with different grades of fibrosis aiming to identify potential biomarkers for stratifying these lesions. The metabolome is the set of endogenously synthesized metabolites in a specific physiological condition and may represent the final product of gene expression. Thus, as a secondary aim of this study, we analyzed the pathways linked to the main metabolites detected, contributing information about the molecular mechanisms involved in the disease.

MATERIALS AND METHODS

Study participants

This study was approved by the Ethics Committee on Research of São Paulo State University in accordance with the provisions of the Declaration of Helsinki. Plasma samples from 46 volunteer participants diagnosed with hepatitis C were obtained from peripheral blood. The inclusion criteria were as follows: Patients > 18 years, unrelated, diagnosed by detection of HCV RNA, with identification of HCV genotype, naïve patients (with no previous hepatitis C treatment), and patients with a known fibrosis stage or clinical diagnosis of cirrhosis by imaging. The exclusion criteria were as follows: Volunteers with a history of liver transplantation, hepatic steatosis unrelated to chronic hepatitis C and other liver diseases. To ensure that the biomarkers identified were exclusive to liver lesions (hepatitis C fibrosis), 50 healthy volunteer blood bank donors [healthy control group (CG)] were included in this study. Participants were recruited from the Viral Hepatitis Outpatient Clinic of Botucatu Medical School, UNESP, Brazil. The demographic and clinical characteristics of the study participants are summarized in Table 1.

Fibrosis was classified based on the Metavir score[14]. Liver samples were collected by percutaneous biopsy before treatment and analyzed histologically. Peripheral blood was collected at the same time as the liver biopsy.

Sample preparation

Samples were collected in tubes with ethylenediamine tetraacetic acid anticoagulant, followed by centrifugation to separate the plasma, which was stored at -80 ℃ until metabolite extraction. At the time of extraction, 20 μL of blood plasma was solubilized in 200 μL of tetrahydrofuran, vortexed, and centrifuged at 3200 rpm for 5 min. Then, the collected supernatant was solubilized in 780 μL of methanol and again centrifuged as above. Afterward, 50 μL of this supernatant was solubilized in 500 μL methanol q.s., homogenized, and subjected to chemical ionization with 0.1% formic acid.

Mass spectrometry

For mass spectrometry analysis, the ionized solution was directly injected into an LTQ Mass Spectrometer (ESI-LTQ-XL Discovery, Thermo Fisher Scientific, Waltham, MA, United States) using electrospray ionization. Ten replicates were used for each biological replicate. The parameters for analysis were set as the following configuration: Sample flow rate of 10 μL/min, capillary temperature of 180 ℃, 7 kV spray voltage, and carrier gas of 2 arbitrary units. After direct injection, the samples were analyzed in the positive ion mode in the mass range of 100-1400 (mass-to-charge ratio), and the signal intensity was detected, which resulted in a set of ions *m/z* for each sample. XCalibur software (v. 2.4, Thermo Scientific) was used to acquire and process the spectrometer data, which were submitted for statistical analysis.

Statistical analyses

Statistical analysis was performed using MetaboAnalyst 4.0 platform[21], in which raw data were evaluated using partial least squares discriminant analysis (PLS-DA). As a result, a list of markers was generated according to the intensity of the most differential and important markers for each group evaluated; that is, the variable importance score (VIP score) was obtained. From this, six ions with the highest VIP score for each grade of fibrosis, with scores > 2.0, were selected. The accuracy of the identified biomarkers was assessed by receiver operating characteristic (ROC) curve analysis.

Identification of biomarkers

From the selected biomarkers, a search was performed using the METLIN online metabolomics database (http://metlin.scripps.edu) to identify molecules compatible with the mass/charge values selected for each grade of fibrosis. The molecules of interest were added to the candidate list and fragmented in silico using the MassFrontier tool (v. 6.0, Thermo Fisher Scientific). After the fragmentation in silico, the molecules whose fragments were compatible with those generated experimentally were selected.

RESULTS

Selection of biomarkers

Based on the PLS-DA, the ions were grouped according to the signal intensity profile within each staging grade, making it possible to analyze the separation between fibrosis grades, as represented in the PLS-DA score plot (Figure 1).

Table 1 Demographic and clinical characteristics of all study participants, distributed by fibrosis grade (Metavir score)

Variables	Healthy control	Fibrosis grade (Metavir)[1]			
		F1	F2	F3	F4
Age (yr)	44 ± 12.2	50 ± 10.78	49 ± 8.6	55 ± 8.8	54 ± 11.22
Sex					
Male	24 (65.0)	8 (61.5)	7 (58.3)	5 (100)	9 (60.0)
Female	26 (52.0)	5 (38.5)	5 (41.7)	0	6 (40.0)
BMI (kg/m^2)	22.9 ± 2.98	28.3 ± 8.89	26.5 ± 6.26	26.9 ± 3.60	27.4 ± 7.14
HCV genotype					
1[2]	-	10 (77.0)	8 (66.7)	3 (50.0)	12 (80.0)
Not 1[3]	-	3 (23.0)	4 (33.3)	3 (50.0)	3 (20.0)

[1]Metavir score to assess the fibrosis grade by histopathological examination of a liver biopsy.
[2]HCV 1: Hepatitis C Virus genotype 1.
[3]HCV not 1: Hepatitis C Virus other genotypes.
BMI: Body mass index; HCV: Hepatitis C virus.

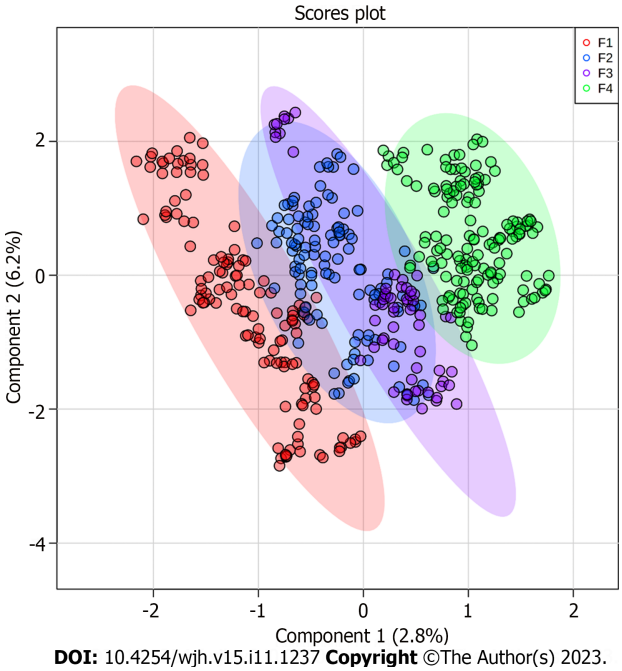

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Figure 1 Score plot generated from partial least squares discriminant analysis comparing fibrosis grades (Metavir). Each plasma metabolite profile is highlighted by different colors: F1 (red dots), F2 (blue dots), F3 (purple dots), and F4 (green dots). The shaded regions around the points represent the 95% confidence interval for each group.

To identify the biomarkers responsible for the separation between the groups (Figure 1), a VIP score was used in the projection. This score allows visualization of the relevance of each marker within each grade analyzed according to the mass/charge ratios of the metabolites[22]. Considering a VIP score of > 2.0 (Figure 2), the six most important ions were selected for each group (Table 2).

To ensure that the identified biomarkers were exclusive to liver lesions (fibrosis, CHC), CG were included. The plasma samples from the two groups (CHC *vs* CG) were compared and this analysis showed that the fibrosis biomarkers (Table 2) were not detected in CG. The PLS-DA and VIP score graphs comparing the two groups are shown in Figures 3 and 4, respectively.

Identification of biomarkers

The most relevant biomarkers, represented by *m/z* values, were identified according to fibrosis grade, as shown in Table 2.

Table 2 Metabolites identified as important in discriminating grades of fibrosis (Metavir) in chronic hepatitis C

Class	Molecule	Ion	VIP score	Formula	Adducts	MSMS	Metlin ID
F1							
Sterols	18:0 Cholesteryl ester	671	2.3	$C_{45}H_{80}O_2$	$[M+NH_4]^+$	303-369-583-437-147-161-135-109-95	83955
	20:5 Cholesteryl ester	672	2.35	$C_{47}H_{74}O_2$	$[M+H]^+$		41710
Glycerolipids	DG(44:12)[1]	695	2.9	$C_{47}H_{68}O_5$	$[M+H-H_2O]^+$	311-119-95-81-69-57-97-113-339	4681
	DG(42:7)[1]			$C_{45}H_{74}O_5$	$[M+H]^+$	311-119-95-81-69-57-97-113-339-437	4605/59181
Coenzyme A	cis,cis-3,6-Dodecadienoyl-CoA	928	2.35	$C_{33}H_{54}N_7O_{17}P_3S$	$[M+H-H_2O]^+$	95-112-119-720-184	58193
Polypeptide	Angiotensin III	931	2.4	$C_{46}H_{66}N_{12}O_9$	$[M+H]^+$	400-311-112-113-96-97-437-659-720-146-147	58017
Unknown	-	1297	2.55	-	-	-	-
F2							
Methyladenosine	N6-Methyladenosine	265	2.18	$C_{11}H_{15}N_5O_4$	$[M+H-H_2O]^+$	81-85-63-99-117-135-149-163-177	58196
	3'-O-Methyladenosine					81-85-63-117-135-163	58340
	1-Methyladenosine					81-84-63-99-117-135-163	6888
	2'-O-Methyladenosine					81-85-63-99-117	58235
	O6-Methyl-2'-deoxyguanosine					81-85-63-99-149-163-177	66286
Eicosanoids	8,15-diHPETE	369	2.08	$C_{20}H_{32}O_6$	$[M+H]^+$	95-81-109-147-161-135-69-93-107	75001
	5S,15S-diHPETE						75023
	5,15-diHPETE						75000
	15S-hydroperoxy-PGD2						74985
	20-hydroxy-PGD2						74981
Sphingolipids	Cer (42:1)[1]	673	2.15	$C_{42}H_{83}NO_3$	$[M+Na]^+$	303-370-95-81-109-60-69-93-107	41569
Unknown	-	828	2.05	-	-	-	0
F3							
Aminoacid derivative	(S)-2,3,4,5-Tetrahydropiperidine-2-carboxylate	150	2.1	$C_6H_9NO_2$	$[M+Na]^+$	106-134-84-61-56-52-105-120	62803
Prenol lipid	Farnesylcysteine	365	2.05	$C_{18}H_{31}NO_2S$	$[M+K]^+$	203-185-112-81-71-307	62388
Glycerophospholipid	PE(34:5)[1]	732	2.17	$C_{39}H_{68}NO_8P$	$[M+Na]^+$	184-437-438-660-83-113-133-97	60844
Coenzyme A	S-2-Octenoyl CoA	914	2.18	$C_{29}H_{48}N_7O_{17}P_3S$	$[M+Na]^+$	86-80-119-112-95-67-104-184-720	58140
Unknown	-	158	2.2	-	-	-	-
		920	2.1				
F4							
Glycerophospholipids	PE(36:1)[1]	731	3	$C_{41}H_{80}NO_7P$	$[M+H]^+$	184-659-437-393-113	62180
	PE(34:6)[1]			$C_{39}H_{66}NO_8P$	$[M+Na]^+$	184-659-437-393-316-113	77367
	PE(O-36:1)[1]	733	2.6	$C_{41}H_{82}NO_7P$	$[M+H]^+$	184-97-304-437-369	77526

	PE(P-36:0)[1]						77623
	PE(34:5)[1]			$C_{39}H_{68}NO_8P$	$[M+Na]^+$	184-97-304-437-675-369	60812
Coenzyme A	CoA(22:2)[1]	1118	2.8	$C_{43}H_{74}N_7O_{17}P_3S$	$[M+CH_3OH+H]^+$	453-703-437-338-113-780-675-799	75415
Acyl-carnitines	Malonylcarnitine	266	2.7	$C_{10}H_{17}NO_6$	$[M+NH_4]^+$	172-94-95-116-57-90-204	6484
Unknown	-	1257	2.85	-	-	-	-
		1296	2.95				

[1]Carbon number: Double bond number. MSMS: In tandem mass spectrometry; DG: Diacylglycerol; CoA: Coenzyme A; HPETE: Hydroxy/hydroperoxyeicosatetraenoic acids; Cer: Ceramide; PE: Phosphoethanolamine; PGD: Prostaglandin; VIP score: Variable importance score.

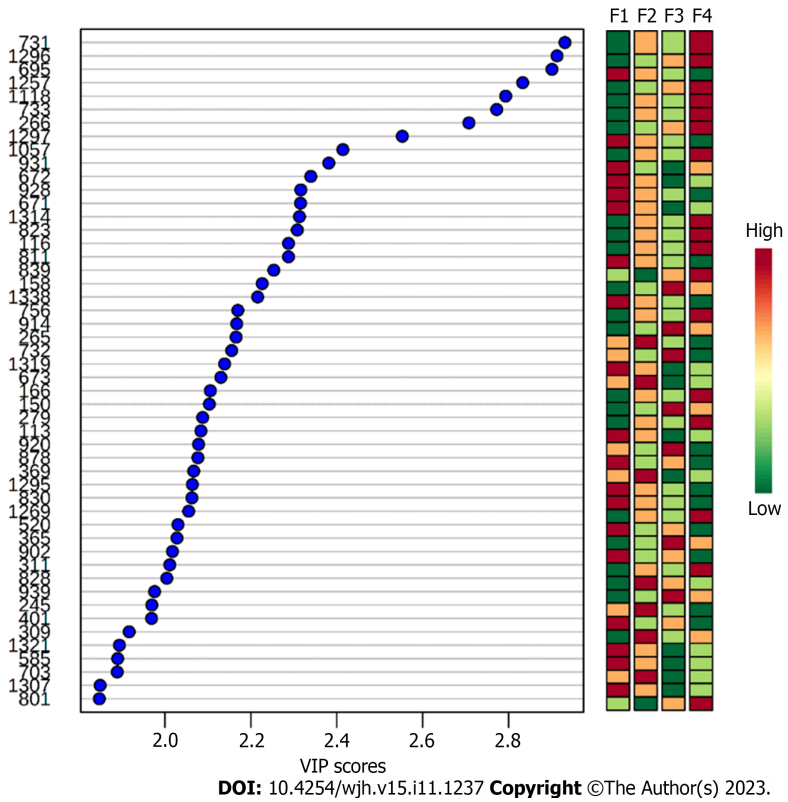

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Figure 2 Variable importance score of the biomarkers identified for grades F1, F2, F3, and F4. The Y axis represents the metabolites *m/z* ratio. The X axis indicates the importance of projection for each biomarker. Laterally to the right, the relevance of each specific marker within the group analyzed is represented according to the color gradations of each biomarker for each grade of fibrosis. The red color represents the most relevant biomarkers; as the red intensity decreases and approaches the green color, the relevance of the biomarkers reduces. Red = up-regulation; Green = down-regulation. VIP Score: Variable importance score.

ROC curve analysis

The accuracy of the biomarkers was assessed using the ROC curve analysis of the sets of metabolites identified for each fibrosis grade (Figure 5). ROC curves were used to analyze the sensitivity, specificity, and area under the curve (AUC) of each group of metabolites identified at each grade of fibrosis. The ROC curve of the selected metabolites for F1 (AUC = 0.806) was plotted with a sensitivity of 82% and a specificity of 68%, and the other selected metabolite groups for F2 (AUC = 0. 652), F3 (AUC = 0.807), and F4 (AUC = 0.864) showed sensitivities of 62%, 82%, and 83% and specificities of 57%, 74%, and 76%, respectively.

DISCUSSION

The metabolome was analyzed to identify new prognostic and diagnostic biomarkers. Thus, the present study investigated the differential metabolites in blood plasma as potential biomarkers of fibrosis stages. Our analysis identified potential biomarkers for each grade of liver fibrosis, which will increase our knowledge about the progression of CHC

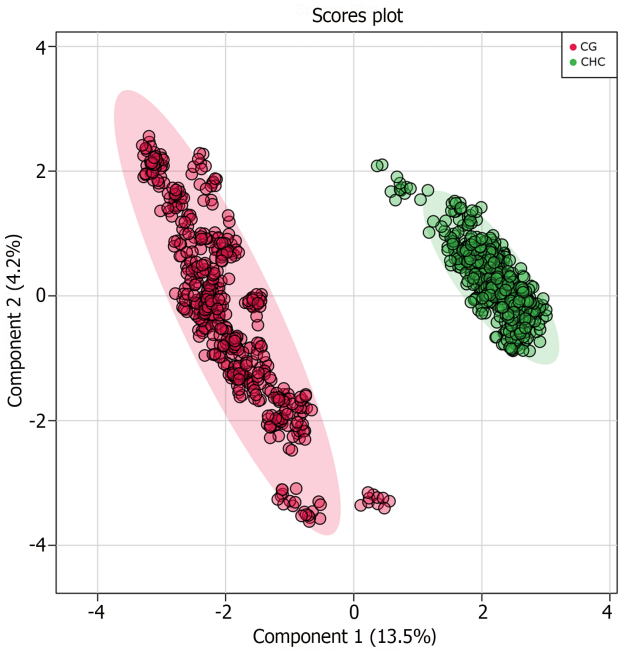

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Figure 3 Score plot generated from partial least squares discriminant analysis comparing the chronic hepatitis C *vs* healthy control groups. Each group is highlighted by different colors: Healthy control groups (red dots) and chronic hepatitis C (green dots), where each dot represents one analytical replicate. The shaded regions around the points represent the 95% confidence interval for each group.

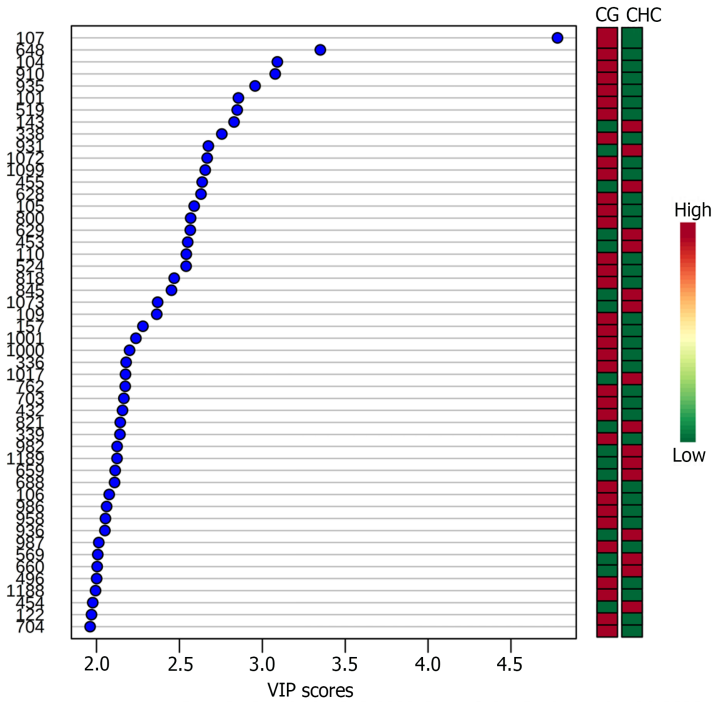

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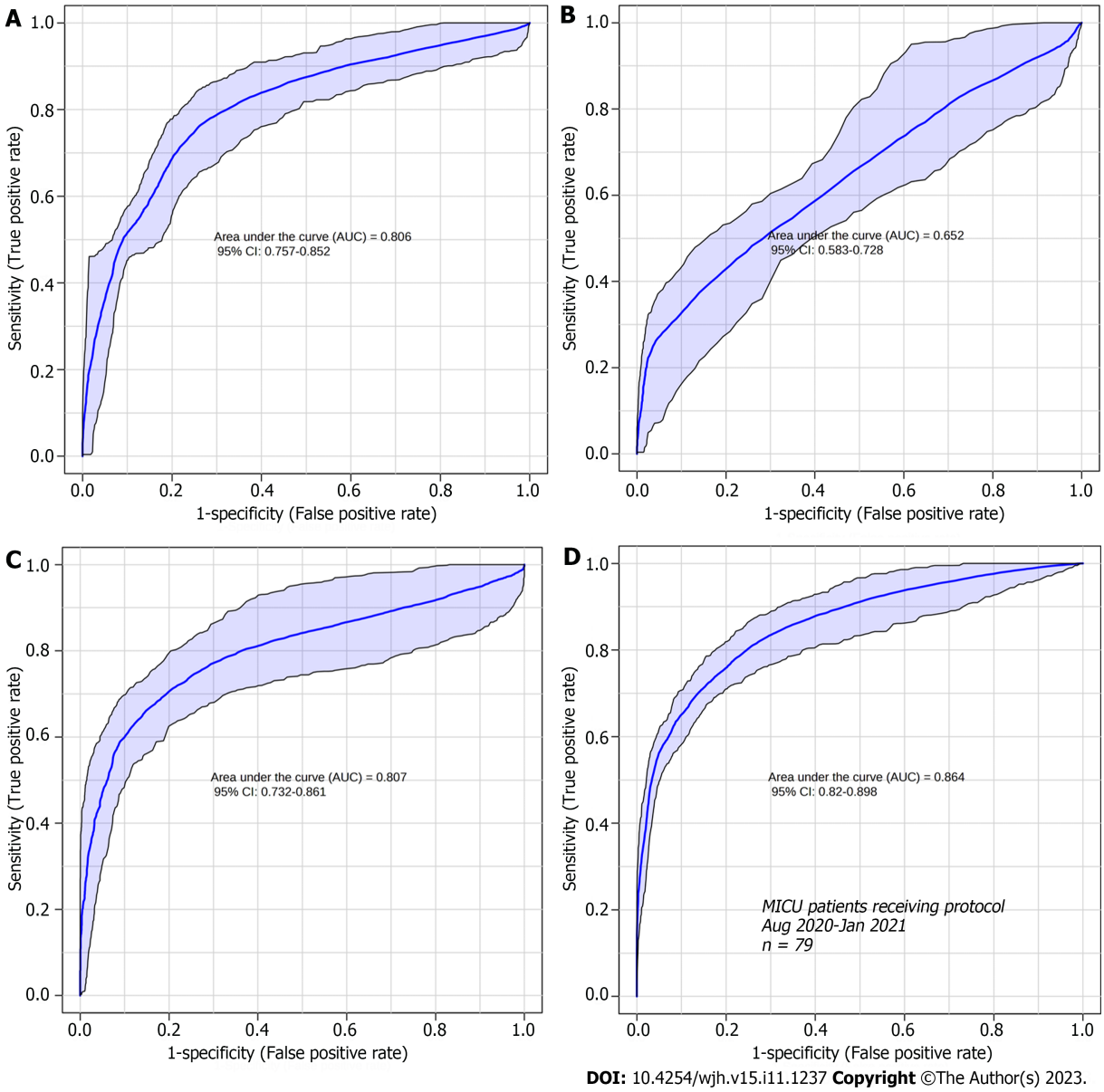
Figure 4 Variable importance score of the biomarkers identified when comparing the chronic hepatitis C *vs* healthy control groups. The Y axis represents the metabolites *m/z* ratio. The X axis indicates the importance in projection for each biomarker. Laterally to the right, the relevance of each specific marker within the group analyzed is represented according to the color gradations of each biomarker for each grade of fibrosis. The red color represents the most relevant biomarkers; as the red intensity decreases and approaches the green color, the relevance of the biomarkers reduces. Red = up-regulation; Green = down-regulation. VIP Score: Variable importance score.



DOI: 10.4254/wjh.v15.i11.1237

Figure 5 Receiver operating characteristic curve analysis of the sets of metabolites identified at each fibrosis grade. A: Receiver operating characteristic (ROC) curve analysis of the set of differential metabolites identified in F1 compared to the other grades (F2, F3, and F4); B: ROC curve analysis of the set of differential metabolites identified in F2 compared to the other grades (F1, F3, and F4); C: ROC curve analysis of the set of differential metabolites identified in F3 compared to the other grades (F1, F2, and F4); D: ROC curve analysis of the set of differential metabolites identified in F4 compared to the other grades (F1, F2, and F3).

and highlight targets for further investigation. The identified biomarkers were able to establish a clustering tendency in patients with the same grade of fibrosis despite some overlap. The score plot analysis showed greater efficiency in discriminating between the extreme grades (F1 and F4), with an overlap in grades F2 and F3. This result may be related to the analytical bias of histological classification, as the formation of groups was based on this criterion-Metavir[12-14], which is subject to some bias related to inadequate sample acquisition, incorrect sample representation or inter-observer variability[7,12,13]. To ensure that the identified biomarkers were exclusive to liver lesions caused by CHC, we compared them with the plasma samples of healthy donors (Figures 3 and 4). None of the biomarkers found in the patient within the CHC group were detected in the plasma of healthy controls, which reinforces their potential as biomarkers exclusive to the disease.

Analysis of the accuracy of the most relevant metabolites in each grade showed that the sets associated with grades F1, F3, and F4 were good biomarkers (AUC 0.806, 0.807, and 0.864, respectively; Figure 5) and had good sensitivity and specificity scores. However, the metabolites identified as grade F2 were less specific and showed poor sensitivity. Such findings could be useful for distinguishing grades F1, F3, and F4, where uncertainty exists when the analyses are based solely on histology. Some serum markers of fibrosis validated in patients with hepatitis C and correlated with liver biopsy as a reference standard showed a mean AUC suitable for clinical practice (> 0.80)[23]; however, an overlap was also observed between adjacent grades of liver fibrosis, particularly the lower grade[24].

Despite the histological bias, our analysis identified different metabolites from diverse chemical classes, including sterols, fatty acids, lipids, and coenzymes. However, for each grade of fibrosis, a metabolite profile has been identified, and as observed in Figure 2, the relevance of each molecule changes according to the fibrosis grade and may intensify or decrease during the disease.

Some studies have demonstrated the potential of metabolomics analyses for different scenarios in diverse diseases, particularly in cancer management[25]. One of the great achievements of metabolomics is the assessment of therapeutic responses and tumor progression, as shown by Rattner *et al*[26], in which serum blood metabolites indicated positive or negative responses to chemotherapy using gas chromatography-mass spectrometry. In addition, some methods for metabolomics analysis, such as nuclear magnetic resonance and multisegment injection-capillary electrophoresis-mass spectrometry, have also shown impressive results, and have also been used to evaluate the metabolome of serum samples from patients with CHC with fibrosis of different grades[27]. This study identified markers for the highest grades of fibrosis, which are compatible with our results, such as glycerophospholipid and acyl-carnitine markers. Therefore, the use of metabolomics approaches for liquid biopsies show promise as diagnostic, prognostic, and therapeutic monitoring tools.

In the context of viral infection, viruses are known to synthesize fatty acids by benefitting from their intermediate products. HCV alters the expression of lipid-related genes associated with cholesterol biosynthesis[28,29]. Interestingly, some metabolites found in different grades of fibrosis are associated with lipid alterations[30-32].

For grade F1, biomarkers that may be more related to HCV infection than to the development of fibrosis were observed when compared to patients with more advanced fibrosis. Thus, the first molecule identified in F1 belonged to the sterol class, with specific signatures for cholesterol ester (CE) (m/z = 671 and m/z = 672). Previous studies have pointed out that CE is a critical component of lipoviral particles whose synthesis has been linked to HCV infection in vitro when cholesterol and triglyceride accumulation is observed[29]. In agreement with our results, we suggest that HCV may modulate the environment, promoting a higher density and infectivity of viral particles and viral spread in the hepatic tissue, which intensifies infection[28,33,34].

Considering lipid metabolism and accumulation, it was possible to identify the sphingolipid class in intermediate-grade F2, represented by ceramide (m/z = 673). It is a central molecule in sphingolipid metabolism with anti-proliferative and pro-apoptotic effects[30]. In the context of HCV infection, lipid accumulation and, consequently, ceramide accumulation occur and may lead to steatosis[35], which may contribute to the development of liver fibrosis[5,35-37].

In addition, a glycerolipid was also identified in F1, specified as diacylglycerol (DG) (m/z = 695). Recent studies have shown that the conversion of DG to phosphatidic acid (mediated by diacylglycerokinases) results in lysophosphatidic acid production, which is involved in many chronic inflammatory diseases, including fibrosis and cancer[38,39]. Therefore, the present study highlights a potential relationship between high levels of DG and a less fibrotic state (low-grade fibrosis) compared to F4, where fibrosis is accentuated.

Another lipid class, glycerophospholipids, was identified in intermediate-grade F3 and advanced-grade F4, in which the biomarkers were identified as phosphoethanolamines (PE) (m/z = 731, m/z = 732, and m/z = 733). Some studies have suggested that PE gradually increases according to the grade of liver fibrosis and acts as a potential marker of carcinogenesis[40,41]. This finding suggests that patients diagnosed with F3 could be at the beginning of the carcinogenesis process; however, this hypothesis needs to be further investigated.

Other biomarkers related to changes in lipid signaling pathways have also been identified. One of these belongs to the eicosanoid class (m/z = 369) identified in F2. This molecule is a biologically active lipid that has several implications in biological processes and is a potent mediator of inflammation in infectious diseases and HCC[42,43]. In addition, it is associated with liver fibrosis staging and is a potential biomarker[44-46]. Another class of lipids, prenol lipids, was identified as F3, represented by farnesylcysteine (m/z = 365). This marker participates in the process of liver carcinogenesis by directly acting on the activity of oncogenic rat sarcoma virus protein[47,48]. Thus, these results encourage investigations into the use of this metabolite as a potential biomarker of the risk of tumor development.

Different intermediate metabolites of the coenzyme A (CoA) class have also been identified, and they are typically involved in the β-oxidation of medium- and long-chain fatty acids to acyl-CoA, a key intermediate in lipid metabolism. Some studies suggest the existence of a disruption in fatty acid lipid metabolic pathways during HCV infection[49,50]. This process results in the accumulation of acyl-CoA and fatty acid metabolic intermediates, such as the three molecules identified in the present study, described as follows. The cis,cis-3,6-dodecadienoyl-CoA (m/z = 928) was identified in the F1 cases in our study. For F3, the marker S-2-octenoyl CoA (m/z = 914) was found[51,52], and in advanced grade (F4), a CoA metabolite (m/z = 1118) was identified. Because different acyl-CoAs isoenzymes are expressed in the liver, some of which are overexpressed in activated hepatic stellate cells[51,53], the results of the present study indicate that there is a disruption in lipid metabolism throughout the infection; however, this is unclear and requires further investigation. Considering the presence of acyl-CoAs in three different fibrosis grades, these molecules are not good candidates for the classification of fibrosis stages but highlight their importance in CHC.

Another marker involved in β-oxidation was found in patients with F4, represented by malonyl carnitine (m/z = 266). Tumors require more energy for cell proliferation, which may lead to dysregulation of energy-supplying metabolic pathways, such as β-oxidation of fatty acids[54,55]. In the context of HCC, alterations in the metabolism of acylcarnitine are directly related to the worsening of the disease and to alterations of β-oxidation[56], which results in the accumulation of Acyl-CoA[57], as discussed previously. Thus, malonylcarnitine can be considered a potential HCC biomarker; however, further studies are needed to validate this hypothesis.

In addition to the lipid biomarkers, the polypeptide angiotensin III (Ang III) (m/z = 931) was identified in F1, which, according to some studies, exhibits physiologically relevant effects similar to those of angiotensin II. In the context of CHC and liver fibrosis, Ang III participates in the increase in collagen production through its interaction with the angiotensin type 2 receptor[58,59] Therefore, this pathway may be involved at the beginning of the fibrotic process once

Ang III is identified in F1.

The last two metabolites were identified as intermediate grades: methyladenosine (*m/z* = 265) in F2 and (S)-2,3,4,5-tetrahydropiperidine-2-carboxylate (*m/z* = 150) in F3. Adenosine methylation is a post-transcriptional modification of mRNAs that affects various biological functions[60-62]. In HCV infection, methyladenosine may represent an RNA modification that enhances the production of infectious particles by interacting with viral proteins[62-64]. These findings suggest that these modifications are involved in the progression of infections and liver fibrosis. Finally, (S)-2,3,4,5-tetrahydropiperidine-2-carboxylate identified in F3 may be related to the degradation of enzymatically inactive proteins and viral assembly[65]. Although this study related amino acid residues to the progression of infection and consequent worsening of fibrosis staging, further studies are necessary to clarify the actions of these protein residues in the viral cycle.

The main limitation of this research was the sample size. This study covered a regional sample and were limited to a single center, which may limit external generalization. However, the results encourage further research with a larger casuistry and the application of this methodology to other liver diseases.

The current study has innovative potential for the detection of markers in plasma, an easily accessible biological fluid. Besides, liquid biopsy could be used side by side with the other noninvasive tests (like elastography) for achieving more accuracy in predicting prognosis.

CONCLUSION

In conclusion, the results from this study suggest that some of the observed biomarkers, once validated, have the potential to be applied as prognostic biomarkers. In addition, they suggest that liquid biopsy analyses of plasma metabolites are a good source of molecular biomarkers capable of stratifying patients with CHC according to fibrosis grade.

ARTICLE HIGHLIGHTS

Research background

Chronic hepatitis C (CHC) is an infectious disease caused by the hepatitis C virus, leading to liver issues like fibrosis, cirrhosis, cancer, and death. The accurate fibrosis stage identification is crucial for treatment decisions and predicting outcomes. Thus, blood markers are a source of relevant information on the staging of fibrosis, in a less invasive and representative way, compared to percutaneous biopsies.

Research motivation

Currently, approaches to staging fibrosis are invasive, subject to sampling errors and subjectivity between observers. In clinical routine, blood markers should be considered a relevant source of information. However, current approaches are limited to routine biochemical tests associated with clinical information, which is not very informative. Analyses based on liquid biopsy are less invasive, and blood plasma, since it circulates throughout the body, can provide information on pathologies that have not yet manifested themselves clinically, positively impacting on prognosis.

Research objectives

Analyze the plasmatic metabolome of CHC patients, looking for potential biomarkers to stratify these lesions.

Research methods

Plasma metabolites from hepatitis C patients and 50 healthy volunteer participants were analyzed using the LTQ Mass Spectrometer. The sample and the control group were classified into Fibrosis grades was classified using the Metavir score. Liver samples were collected by percutaneous biopsy before any treatment and then analyzed histologically. The most relevant metabolites were categorized using the METLIN online metabolomics database. The molecules of interest were added to a list of candidates and subsequently fragmented in silico using the MassFrontier tool. Molecules compatible with those generated experimentally were then selected for functional analysis.

Research results

For each degree of fibrosis, six differential metabolites were identified that were able to establish an interesting grouping trend among patients with the same degree of fibrosis.

Research conclusions

The results of this study suggest that liquid biopsy analyzes of plasma metabolites are a good source of molecular biomarkers capable of stratifying patients with CHC according to their fibrosis grade.

Research perspectives

Some of the observed biomarkers, once validated, have the potential for application as prognostic biomarkers. This study has innovative potential regarding the detection of pre-clinical biomarkers in easily accessible plasma using minimally

invasive methods.

FOOTNOTES

Author contributions: Ferrasi AC, Lima EO, Delafiori J and Dias-Audibert FL performed mass spectrometry experiments; Ferrasi AC, Galvani AF, Santos DB, Silva GF, and Praxedes RR performed biofluid collection and selection and provided clinical support; Ferrasi AC, Almeida DTM, Lima EO, Praxedes RR, and Lima SVG analyzed the data and prepared the Figures; Ferrasi AC and Lima SVG wrote the manuscript; Lima EO, Silva GF, and Catharino RR revised the manuscript; Catharino RR provided the infrastructure and methodological support; Ferrasi AC and Lima EO designed, managed, and supervised the study; All authors approved the final version of the article.

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LETTER TO THE EDITOR

Letter to editor 'Non-invasive model for predicting high-risk esophageal varices based on liver and spleen stiffness'

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Abstract

This letter to the editor relates to the study entitled "Non-invasive model for predicting high-risk esophageal varices based on liver and spleen stiffness". Acute bleeding caused by esophageal varices is a life-threatening complication in patients with liver cirrhosis. Due to the discomfort, contraindications, and associated complications of upper gastrointestinal endoscopy screening, it is crucial to identify an imaging-based non-invasive model for predicting high-risk esophageal varices in patients with cirrhosis.

Key Words: Cirrhosis; High-risk esophageal varices; Non-invasive prediction model; Spleen stiffness measurement; Liver stiffness measurement; Upper gastrointestinal endoscopy

Core Tip: Liver cirrhosis is the end of chronic liver disease. Rupture of esophageal varices (EVs) is a common and potentially fatal complication in patients with cirrhosis. In clinical practice, prophylactic treatment is primarily used to prevent events of esophageal venous bleeding, however, this strategy requires invasive and expensive upper gastrointestinal endoscopy testing, leading to poor patient adherence. In recent years, several studies have demonstrated an association between EVs and liver stiffness measurement (LSM) as well as spleen stiffness measurement (SSM). The main objectives of this paper are to elucidate the differences between EVs, SSM, and LSM and explore the feasibility of using LSM and SSM to develop a non-invasive model for predicting high-risk esophageal varices.

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TO THE EDITOR

We read with interest the retrospective study by Yang *et al*[1], which is titled "Non-invasive model for predicting high-risk esophageal varices based on liver and spleen stiffness". The results of this study highlight the potential use of liver stiffness measurement (LSM) and spleen stiffness measurement (SSM) for predicting high-risk esophageal varices (HEVs) in patients with cirrhosis.

Portal hypertension (PH) is a common and significant complication in patients with cirrhosis, leading to esophageal varices (EVs)[2]. Hepatic venous pressure gradient and upper gastrointestinal endoscopy (UGE) are considered the gold standard for assessing the severity of PH and the risk of EV bleeding. However, due to their invasiveness, discomfort, and high cost, it is crucial to identify non-invasive markers for screening HEVs in cirrhotic patients[3,4]. In recent years, LSM and SSM using transient elastography (TE), acoustic radiation force impulse elastography, two-dimensional shear wave elastography, and magnetic resonance elastography have been proven to be accurate diagnostic tools for evaluating chronic liver disease with liver fibrosis as well as predicting the presence or absence of HEVs[5].

We want to emphasize a few points about this study: In this study, the authors used Baveno VI as a comparator but did not include the more comprehensive Baveno VII as a comparator[6]. At the same time, the authors did not distinguish between the M and XL models of the FibroScan probe when measuring LSM and SSM, which may have an impact on the comprehensiveness and accuracy of the results[7]. Second, patients with current/past clinical cirrhosis were included in this study, but the proportion of patients with decompensated cirrhosis in this cohort is unclear, since the non-invasive measures used here were primarily used for endoscopic triage of patients with compensated cirrhosis. No guidelines recommend its use in patients with clinical decompensation, for whom screening by UGE is recommended. Additionally, while all subjects included in this study had viral hepatitis cirrhosis, they did not consider the possible effect of antiviral treatment on TE measurements. Furthermore, the effect of alcoholic and nonalcoholic steatohepatitis on cirrhosis has been underrepresented, which may limit the external validity of our findings across diverse populations and settings. To enhance the reliability of the conclusions of this study, we recommend a study with a larger sample size, especially in patients with nonalcoholic steatohepatitis and alcohol-induced cirrhosis, to verify the validity of the model in patients with different types of cirrhosis. Such a study would help improve the convenience and operability of clinical practice and more accurately assess the condition of patients.

The highlight of this study is that all enrolled patients completed UGE testing. Additionally, when SSM is unavailable or unsuccessful, the Baveno VI criterion can be used as a reasonable alternative according to Yang *et al*[1] Moreover, a screening strategy based on LSM and SSM could reduce the workload of endoscopy and optimize the use of health care resources while minimizing risk and patient discomfort. In summary, we acknowledge the efforts and contributions made by the authors. Furthermore, we recommend further prospective validation to facilitate future research on this topic.

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FOOTNOTES

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