ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2024 February 27; 16(2): 112-299

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

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The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

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The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (ESCI), Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJH* as 2.4.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

PUBLICATION DATE
February 27, 2024

PUBLISHING PARTNER
Department of Infectious Diseases, the Second Affiliated Hospital of Xi'an Jiaotong University

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

Shuang-Suo Dang

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

PUBLISHING PARTNER's OFFICIAL WEBSITE
http://2yuan.xjtu.edu.cn/Html/Departments/Main/Index_21148.html

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 112-114

DOI: 10.4254/wjh.v16.i2.112

ISSN 1948-5182 (online)

EDITORIAL

New markers of fibrosis in hepatitis C: A step towards the Holy Grail?

Konstantinos John Dabos

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gao S, China; Wang XN, China

Received: November 15, 2023
Peer-review started: November 15, 2023
First decision: December 5, 2023
Revised: December 12, 2023
Accepted: January 12, 2024
Article in press: January 12, 2024
Published online: February 27, 2024

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Abstract

In the present issue of the *World Journal of Hepatology*, Ferrassi *et al* examine the problem of liver fibrosis staging in chronic hepatitis C. They identify novel biomarkers in an effort to predict accurate fibrosis staging with the aid of the metabolome of Hepatitis C patients. Overall I think Ferrassi *et al* took a different approach in identifying fibrosis biomarkers, by looking at the patients' metabolome. Their biomarkers clearly separate patients from controls. They can also separate out, patients with minimal fibrosis (F0-F1 stage) and patients with cirrhosis (F4 stage). Obviously, if these biomarkers were to be widely used, tests for all the important metabolites would need to be readily available for use in hospitals or outpatient setting and that may prove difficult and above all, costly. Nevertheless, this step could eventually lead to a metabolomic approach for novel biomarkers of Fibrosis. Obviously, it would need to be validated, but could represent a step towards the Holy Grail of Hepatology.

Key Words: Hepatitis C metabolomics; Fibrosis; Non invasive markers; Metavir

Core Tip: A novel approach for identifying non-invasive biomarkers as a step towards an accurate serological tool for fibrosis staging in hepatitis C.

Citation: Dabos KJ. New markers of fibrosis in hepatitis C: A step towards the Holy Grail? *World J Hepatol* 2024; 16(2): 112-114
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/112.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.112

INTRODUCTION

Hepatology is, relatively speaking, a newcomer amongst the medical specialities. Hepatologists have tirelessly worked towards better treatments for patients with liver disease and have achieved great goals resulting in transforming the lives of millions of people with liver disease. However, the ability to accurately estimate the amount of fibrosis in the liver without the need for a liver biopsy, which can be described as the Holy Grail of Hepatology remains unobtainable.

In contrast, one of the achievable goals in the near future is hopefully the elimination of hepatitis C[1]. Since the advent of Direct Acting Antivirals at the beginning of this century, we have been able to cure patients with hepatitis C with great efficacy. The goal of eliminating hepatitis C by 2030 is still a target the community strives towards.

Greatly reducing the numbers of patients with hepatitis C does not necessarily mean that patients with fibrosis and cirrhosis due to previous Hep C infection would not need any follow up[2]. There is still a risk of progression of their existing disease. Hepatologists would ideally like to be able to accurately predict at any point the possibility of progression of liver fibrosis in patient with hepatitis C.

There are already plenty of non invasive fibrosis assessment tests in Chronic Hepatitis C (CHC), which can be classified into physical and serological ones. The most common physical test used in the West is Transient Elastography (Fibroscan, Echosens)[3]. By measuring the liver elasticity it gives a pretty good approximation of the fibrosis stage in CHC. However, very expensive equipment is required and many resource strapped countries cannot rely on it for a comprehensive assessment of the affected population. Acoustic radiation force impulse elastography and magnetic resonance enterography (a 2D gradient recalled Echo) have also been used but are not widely available[4].

Many serological tests are available using direct and indirect biomarkers. Direct biomarkers such as Hyaluronic Acid, European Liver fibrosis panel, Procollagen II, (aspartate amino transferase) to platelets ratio and Non- alcoholic fatty liver diaseas fibrosis score can now be used routinely in clinical practice[5-8].

Indirect biomarkers, like red cell distribution width to platelets ratio, FIB-4 and the Forns index have been used with some success, as index tests, mainly to assess the probability of fibrosis in an individual[9-11]. Tests that combine direct and indirect biomarkers like the Fibro test and the Fibro meter index have also been used as well as combinations of serological and physical tests. The plethora of available tests indicates the lack of confidence in the Hepatology community that any one test alone can accurately predict a patient's liver fibrosis stage[4].

In the present issue of the *World Journal of Hepatology*, Ferrassi *et al*[12] examine the problem of liver fibrosis staging in CHC. They identify novel biomarkers in an effort to predict accurate fibrosis staging with the aid of the metabolome of hepatitis C patients

The authors collected plasma from 46 Patients with hepatitis C who had biopsy proven fibrosis staging, graded by the METAVIR score[12] to F1-F4 grades of fibrosis. They then used an untargeted metabolomic technique to analyse plasma metabolites, using mass spectrometry.

Their analysis found potential metabolites specific for each grade of fibrosis that showed a clustering tendency.those metabolites' clusters were more efficient in distinguishing stage F1 and stage F4 fibrosis on the METAVIR score as between F2 and F3 stages there was an overlap.

They also analysed the accuracy of the sets of metabolites specific for each grade and found that F2 markers were less specific but the sets for the other three grades showed good sensitivity and specificity scores.

The metabolites identified were sterols, fatty acids, lipids and coenzymes .In their discussion the authors point out that markers for F1 fibrosis are linked to the viral replication of the Hep C virus Furthermore, molecules identified as biomarkers in F2 fibrosis stage (*i.e.* ceramide) could be specifically produced in the context of CHC infection. These results make it impossible to generalise the observations to other chronic liver diseases.

CONCLUSION

Overall I think Ferrassi *et al*[11] took a different approach in identifying fibrosis biomarkers, by looking at the patients' metabolome. Their biomarkers clearly separate patients from controls. They can also separate out, patients with minimal fibrosis (F0-F1 stage) and patients with cirrhosis (F4 stage). Obviously, if these biomarkers were to be widely used, tests for all the important metabolites would need to be readily available for use in hospitals or outpatient setting and that may prove difficult and above all , costly. Nevertheless, this step could eventually lead to a metabolomic approach for novel biomarkers of Fibrosis. Obviously, it would need to be validated, but could represent a step towards the Holy Grail of Hepatology.

ACKNOWLEDGEMENTS

I am grateful to Sophia Douka, MA in Linguistics for final language polishing.

FOOTNOTES

Author contributions: Dabos KJ wrote the manuscript.

Conflict-of-interest statement: Konstantinos Dabos declares no conflicts of interest.

Country/Territory of origin: United Kingdom

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S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 115-119

DOI: 10.4254/wjh.v16.i2.115

ISSN 1948-5182 (online)

EDITORIAL

Can rifaximin for hepatic encephalopathy be discontinued during broad-spectrum antibiotic treatment?

Chien-Hao Huang, Piero Amodio

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Perazzo JC, Argentina

Received: November 19, 2023
Peer-review started: November 19, 2023
First decision: December 8, 2023
Revised: January 3, 2024
Accepted: January 24, 2024
Article in press: January 24, 2024
Published online: February 27, 2024

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Abstract

Hepatic encephalopathy (HE) is a formidable complication in patients with decompensated cirrhosis, often necessitating the administration of rifaximin (RFX) for effective management. RFX, is a gut-restricted, poorly-absorbable oral rifamycin derived antibiotic that can be used in addition to lactulose for the secondary prophylaxis of HE. It has shown notable reductions in infection, hospital readmission, duration of hospital stay, and mortality. However, limited data exist about the concurrent use of RFX with broad-spectrum antibiotics, because the patients are typically excluded from studies assessing RFX efficacy in HE. A pharmacist-driven quasi-experimental pilot study was done to address this gap. They argue against the necessity of RFX in HE during broad-spectrum antibiotic treatment, particularly in critically ill patients in intensive care unit (ICU). The potential for safe RFX discontinuation without adverse effects is clearly illuminated and valuable insight into the optimization of therapeutic strategies is offered. The findings also indicate that RFX discontinuation during broad-spectrum antibiotic therapy was not associated with higher rates of delirium or coma, and this result remained robust after adjustment in multivariate analysis. Furthermore, rates of other secondary clinical and safety outcomes, including ICU mortality and 48-hour changes in vasopressor requirements, were comparable. However, since the activity of RFX is mainly confined to the modulation of gut microbiota, its potential utility in patients undergoing extensive systemic antibiotic therapy is debatable, given the overlapping antibiotic activity. Further, this suggests that the action of RFX on HE is class-specific (related to its activity on gut microbiota), rather than drug-specific. A recent double-blind randomized controlled (ARiE) trial provided further evidence-based support for RFX withdrawal in critically ill cirrhotic ICU patients receiving broad-spectrum antibiotics. Both studies prompt further discussion about optimal therapeutic strategy for patients facing the dual challenge of HE and systemic infections.

Despite these compelling results, both studies have limitations. A prospective, multi-center evaluation of a larger sample, with placebo control, and comprehensive neurologic evaluation of HE is warranted. It should include an exploration of longer-term outcome and the impact of this protocol in non-critically ill liver disease patients.

Key Words: Rifaximin discontinuation; Hepatic encephalopathy; Broad-spectrum antibiotics; Crit-ically ill; Medical intensive care unit; Pharmacist-driven protocol

Core Tip: Rifaximin (RFX) is a gut-restricted adjunct to lactulose that minimizes hepatic encephalopathy (HE) recurrence with minimal systemic absorption. Despite established benefits, limited Food and Drug Administration approval for acute HE raises concern about its use in treating acute overt HE. Recent evidence challenges the routine use of RFX with broad-spectrum antibiotics, emphasizing their class-specific effects in critically ill patients. The study sheds light on the safety of discontinuing RFX during broad-spectrum antibiotic therapy in intensive care unit patients with liver disease and HE, and also prompts reevaluation of the role of RFX amid the overlapping antibiotic activity. This evidence underscores the need for further investigations to optimize the management of both HE and systemic infections in patients with liver disease, including those who are not critically ill.

Citation: Huang CH, Amodio P. Can rifaximin for hepatic encephalopathy be discontinued during broad-spectrum antibiotic treatment? *World J Hepatol* 2024; 16(2): 115-119
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/115.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.115

INTRODUCTION

Hepatic encephalopathy (HE), a neuropsychiatric complication of decompensated cirrhosis, manifests across a clinical spectrum from minimal cognitive dysfunction to lethargy and coma[1,2]. It carries substantial mortality and recurrence risks[3] and impacts the health-related quality of life in cirrhotic patients to a significant degree[4]. Despite an incomplete understanding of the comprehensive mechanisms[3], pseudo-neurotransmitters like gamma-aminobutyric acid (GABA), ammonia, and indole are well-established neurotoxins implicated in HE[5,6]. It has been suggested that neurotoxins, such as the ammonia produced by colonic bacteria and enterocytes that metabolize proteins, enter the systemic circulation through the portal vein and blood-brain barrier, and contribute to neurologic dysfunction and HE under cirrhotic conditions[7]. The gastrointestinal tract serves as the primary source of ammonia, with growing a recognition of gut microbiota as another significant contributor. Current drug therapies for HE, such as lactulose and rifaximin (RFX), focus on the reduction of plasma ammonia levels and the improvement of gut dysbiosis.

RFX, which has very low systemic absorption, is used as an adjunctive to lactulose to prevent the recurrence of HE[3,8]. This gut-restricted, non-absorbable rifamycin derived oral antibiotic, acts against ammonia-producing gram-positive, gram-negative, and anaerobic bacteria[9,10]. RFX demonstrates efficacy in preventing overt HE (OHE) recurrence and is linked to significant reduction in infections, hospital readmissions, and durations of hospital stay[11,12]. RFX actively improves the quality of life[13] and single-center studies suggest potential survival benefit for HE patients treated with RFX[12]. However, despite being frequently used for the prevention or treatment of acute HE[12], RFX stills awaits Food and Drug Administration approval for the treatment of acute OHE due to the limited amount of supportive evidence[14].

Antibiotics, by acting on gut microbiota, reduce indole which can potentially be converted into the neurotoxic substance oxindole in the brain[15]. They also attenuate the inflammatory response driven by pathogen-associated molecular pattern molecules (PAMPS)/endotoxins[16] and may prevent the binding of GABA in the central nervous system[17]. Notably, proinflammatory interleukins, produced during infection or an inflammatory state interact with ammonia, and can contribute to alterations in brain function[18].

In the medical intensive care unit (MICU), where infection prevalence is high[19] and infection commonly acts as a precipitant of OHE in critically ill patients[20], individuals frequently receive RFX alongside broad-spectrum antibiotics. This practice persists despite the absence of endorsement in current practice guidelines for treating OHE in the presence of infection[14]. Therefore, Ward *et al*[21] conducted a study that addressed this crucial knowledge gap; they demonstrated that RFX can be safely discontinued without adverse effect in patients with severe liver disease and HE undergoing extensive broad-spectrum antibiotic treatment in the MICU. The implicit rationale of the study suggests that the beneficial effects of RFX in HE were mainly class-specific, not drug-specific.

A plethora of antibiotic treatments has demonstrated efficacy in treating HE, with the initial recognition of tetracyclines' utility in hepatic coma[22]. Subsequent investigations highlighted the crucial role of reducing microbiota activity to reverse HE, leading to the exploration of metronidazole[23] and other poorly absorbed antibiotics like vancomycin[24], neomycin[25], and RFX[26]. These agents were preferred due to their capacity to diminish or avoid unnecessary systemic effects, considering the frequent associated systemic toxicity. Particularly noteworthy is RFX, which, among other poorly absorbed oral antibiotics, exhibits a safer profile than aminoglycosides[27]. Importantly, the

development of bacterial resistance to RFX does not compromise the activity of other antibiotics[28].

Thus, the effect of antibiotics in HE is a class action[29] that is shared by the antibiotic RFX, which was found to have a profound activity on gut microbiota in the first study[30]. Later studies based on microbiome analysis found no remarkable RFX effect[31] and suggested that the effect could have been a modulation of gut microbiota metabolism. However, conclusions were driven by the analysis of the relative abundance of bacteria. Changes in the absolute microbiota burden, which is the determinant of microbiota metabolic effect, might have yielded different results. A recent elegant study by Eriksen *et al*[32] showed that RFX+ lactulose administration reduces the systemic inflow of ammonia by 20%, while the ammonia plasma level was poorly changed, thus suggesting that a single measure of fasting ammonia may be less accurate in estimating gut ammonia production in cirrhosis[32].

Considering that the mechanism of RFX action is mainly related to its positive modulation of the distorted gut microbiota profile and burden (bacterial overgrowth) in advanced cirrhosis, the possibility that its use might be useful in subjects undergoing massive systemic antibiotic use is not evident a priori, and overlapping spectra of antibiotic activity with broad-spectrum antibiotics are expected.

The Ward *et al*[21] study has several limitations. As a single-center study, its generalizability may be limited, and variations in patient populations across centers may impact external validity. Second, the retrospective data collection introduces inherent limitations, including incomplete or missing data, recall bias, and an inability to control for all confounding variables. Third, despite including both acute and chronic HE, the absence of subgroup analysis may limit result interpretation. Fourth, reliance on surrogate endpoints such as days alive and freedom from delirium and coma, instead of West-Haven grades, may introduce variability and might not fully capture the impact, especially in assessing acute HE episodes. Fifth, the study did not assess long-term outcomes or the sustained effects of withheld RFX therapy, which could underestimate the beneficial role of RFX. Sixth, despite generally balanced groups, observed baseline differences, such as norepinephrine requirements and race, may potentially confound results. Seventh, the study enrolled critically ill patients with liver disease, representing a spectrum from compensated cirrhosis to decompensated liver cirrhosis without analyzing their Model for end stage liver disease scores, which greatly impact prognoses.

CONCLUSION

At any rate, data supporting the combination use of RFX with broad-spectrum antibiotics are limited, as patients on broad-spectrum antibiotics have generally been excluded from studies on RFX efficacy in HE. Therefore, the Ward *et al* [21] study has great merit, since it provides an evidence-based argument to justify RFX withdrawal in patients with cirrhosis who receive broad-spectrum antibiotic treatment. Further, this is in agreement with the recent article by Kulkarni *et al*[14], which does not show any advantage on HE in patients with or without RFX that are treated with other antibiotics. Thus, both articles provide empirical evidence as to what could have been assumed only *a priori* based on antibiotic action. Despite these compelling results, it is crucial to acknowledge limitations in these studies. A prospective, multi-center evaluation in a larger sample, with placebo control and comprehensive neurologic evaluations for HE, might be useful. Additionally, exploring the impact of this protocol in non-critically ill liver disease patients should be considered.

FOOTNOTES

Author contributions: Huang CH wrote the original manuscript; Amodio P reviewed and revised the manuscript.

Supported by the Chang Gung Medical Research Project, No. CMRPG3M1931-1932; the National Science and Technology Council, No. MOST 110-2314-B-182A-093- and No. NMRPG3L0331.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Li L
L-Editor: A
P-Editor: Li L

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 120-122

DOI: 10.4254/wjh.v16.i2.120

ISSN 1948-5182 (online)

EDITORIAL

Insights into skullcap herb-induced liver injury

Jonathan Soldera

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Zhou S, China

Received: November 26, 2023
Peer-review started: November 26, 2023
First decision: January 9, 2024
Revised: January 14, 2024
Accepted: January 29, 2024
Article in press: January 29, 2024
Published online: February 27, 2024

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Abstract

This editorial addresses the growing concern of herb-induced liver injury (HILI), focusing on a unique case of Skullcap-induced HILI report. This editorial underscore the significant mortality rate linked to Skullcap-induced HILI, emphasizing the importance of vigilant monitoring and intervention. As herbal supplement usage rises, collaboration among clinicians and researchers is crucial to comprehend and address the complexities of HILI, particularly those involving Skullcap.

Key Words: Herb-induced liver injury; Drug induced liver injury; Dietary supplements; Herbal hepatotoxicity; Liver transplantation

Core Tip: This study presents a comprehensive analysis of herb-induced liver injury (HILI), focusing on a unique case report of drug-induced autoimmune hepatitis due to Skullcap supplements and a systematic review/meta-analysis of 936 HILI cases associated with 79 herbs. Notably, Skullcap-induced HILI demonstrated hepatocellular patterns and mild-to-moderate severity, emphasizing the importance of recognizing potential adverse events associated with herbal dietary supplements. The study's findings underscore the need for increased awareness and vigilance in monitoring HILI, particularly in the context of rising herbal supplement usage.

Citation: Soldera J. Insights into skullcap herb-induced liver injury. *World J Hepatol* 2024; 16(2): 120-122
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/120.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.120

INTRODUCTION

The growing popularity of traditional herbal dietary supplements (HDS) has prompted discussions regarding their potential association with liver injury, currently named Herb Induced Liver Injury (HILI). A case study reported by Thakral *et al*[1] published in the *World Journal of Hepatology* highlights a distinctive occurrence of autoimmune hepatitis induced by Skullcap supplements. This specific case underscores the significance of identifying potential adverse events associated with the utilization of HDS, particularly considering the rising prevalence of their use among the general population.

The reported case involves a middle-aged woman with no prior health issues, who developed sudden jaundice after four months of consuming Skullcap mushroom supplements. The case report indicates that despite testing negative for multiple chronic liver diseases, the first liver biopsy detected severe drug-induced liver injury in resolution. The patient's condition worsened, leading to a subsequent diagnosis of autoimmune hepatitis, with a positive response to discontinuation of the supplement.

In parallel, a comprehensive systematic review and meta-analysis by Ballotin *et al*[2] aimed to identify herbal products associated with HILI. The study, published in the World Journal of Clinical Cases, analyzed 936 cases reported in 446 references, identifying 79 types of herbs related to HILI. Skullcap-induced HILI typically manifested in individuals using the dried leaves and stems of Scutellaria spp. for various purposes, including the treatment of anxiety, stress, and insomnia. The onset of symptoms and jaundice occurred within one week to three months, with a pattern of typically hepatocellular liver injury, with a few cases of mixed hepatocellular and cholestatic liver injury. The dose that was ingested varied greatly Skullcap, ranging from 400 mg to 16 g daily. Skullcap-induced HLI was more prevalent among females and the mean age was 54 years-old. Common symptoms encompass nausea and choluria, with affected patients frequently reporting osteoarthritis and hypertension. Generally, HILI induced by Skullcap exhibits mild-to-moderate severity, resolving promptly upon discontinuation of the herb. This cessation typically results in the normalization of liver function tests within a period of 12 wk. However, in the systematic review by Ballotin *et al*[2] reveals a considerable mortality rate of 14.2% was described, and in some severe cases, liver transplantation was necessary, potentially influenced by publication bias favoring the reporting of more severe outcomes.

Both studies underscore the generally positive prognosis of HILI following discontinuation of the implicated herbal product. However, they also elucidate the potential for severity, emphasizing the need for vigilant monitoring and intervention. This severity is reflected in a significant mortality rate associated with Skullcap-induced HILI.

CONCLUSION

In summary, there is an urgent need for increased awareness concerning the potential hepatotoxic effects of herbal supplements, with a specific focus on Skullcap. These cases emphasize the critical importance of recognizing and comprehending the patterns and outcomes linked to Skullcap-induced liver injury and other forms of HILI. As the usage of herbal supplements continues to escalate, fostering collaboration between clinicians and researchers becomes imperative to unravel the intricate nature of herb-induced liver injuries and guarantee the safe utilization of these products.

FOOTNOTES

Author contributions: Soldera J contributed to writing and reviewing the final draft of the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Li L
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 123-125

DOI: 10.4254/wjh.v16.i2.123

ISSN 1948-5182 (online)

EDITORIAL

Non-invasive assessment of esophageal varices: Status of today

Tarana Gupta

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ding HG, China; Ma L, China

Received: November 27, 2023
Peer-review started: November 27, 2023
First decision: December 25, 2023
Revised: December 29, 2023
Accepted: January 17, 2024
Article in press: January 17, 2024
Published online: February 27, 2024

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Abstract

With increasing burden of compensated cirrhosis, we desperately need non-invasive methods for assessment of clinically significant portal hypertension. The use of liver and spleen stiffness measurement helps in deferring unnecessary endoscopies for low risk esophageal varices. This would reduce cost and patient discomfort. However, these special techniques may not be feasible at remote areas where still we need only biochemical parameters. More prospective studies validating the non-invasive risk prediction models are definitely needed.

Key Words: Compensated cirrhosis; Spleen stiffness measurement; Liver stiffness measurement; High-risk esophageal varices; Clinically significant portal hypertension

Core Tip: The liver stiffness measurement is time tested tool for assessing liver fibrosis. The new application of spleen stiffness has again supplemented for assessment of portal hypertension and has alleviated the need for unnecessary endoscopies. The novel spleen dedicated stiffness measurement @100 Hz has further improved the screening of high-risk esophageal varices.

Citation: Gupta T. Non-invasive assessment of esophageal varices: Status of today. *World J Hepatol* 2024; 16(2): 123-125
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/123.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.123

INTRODUCTION

We read with great interest "Non-invasive model for predicting high-risk esophageal varices (HEVs) based on liver and spleen stiffness" by Yang *et al*[1]. In view of risk,

discomfort and cost associated with invasive procedures like liver biopsy, endoscopy *etc.* it has become essential to validate the non-invasive model for predicting clinically significant portal hypertension (CSPH). Yang *et al*[1] created a model using spleen and liver stiffness measurement (SSM and LSM). The initial models LSPS (LS-spleen diameter to platelet ratio score), Baveno VI, improved Baveno VII have already given better understanding of worsening portal hypertension to CSPH[2]. Baveno VII criteria proposed additional use of SSM cut-off 40 kPa in patients where Baveno VI criteria were not met to rule out HEVs (high risk esophageal varices). This prevented more unnecessary endoscopies in clinical practice. The proposed RESIST-HCV criteria in the SIMPLE study included platelet count and serum albumin for predicting the development of HEVs in future in patients with HCV-related compensated cirrhosis with LEV[3].

The increasing burden of alcohol and metabolic dysfunction-associated steatotic liver disease (MASLD), viral cirrhosis with their long follow-up warrants the use of non-invasive strategies to risk stratify these patients. Follow-up of the compensated advanced chronic liver disease requires close monitoring to limit future complications as well as health care burden. The LSM is affected by hepatic inflammation, cholestasis, congestion due to right heart failure, infiltrative diseases *etc.* and may be spuriously high (false positive). In these settings, it may not be truly reflective of portal hemodynamics *i.e.* portal hypertension. On the other hand, spleen stiffness is affected by splenomegaly, increased blood pooling in spleen, associated splenic fibrosis and all these factors are reflective of portal hypertension[4]. However, patients with small spleen having < 4 cm thickness may not be suitable candidates for elastography measurement. Studies have shown magnetic resonance (MR) based spleen stiffness of 7.23 kPa indicative of presence of esophageal varices[5]. The assessment of SSM has been done by both 50 Hz and 100 Hz probes. One recent study demonstrated endoscopy spare rate by SSM at 100 Hz to be significantly better than SSM at 50 Hz (38.9% *vs* 26.5%; $P < 0.001$) respectively[6].

Yang *et al*[1] derived model based upon LSM and SSM; however, it is essential to note the shortcomings of this particular model. First, it requires specialized equipment to measure elastography and may not be feasible in remote areas. Second, as already mentioned there is a subset of patient population with right heart failure, chronic kidney disease with fluid overload, infiltrative and cholestatic liver diseases where elastography is not accurate and may fallaciously give high values. Though here we are focusing on cirrhosis related portal hypertension, but in clinical practice, this may not be reproducible in non-cirrhotic portal hypertension. Third, we need to address the issues related to heterogeneity in etiology of liver disease such as MASLD where the corresponding LSM values for F3-F4 fibrosis are higher than in viral and alcohol-related cirrhosis. The authors have taken predominant virus related cirrhosis and patients of alcohol and MASLD have been excluded. Fourth, they have used Baveno VI for comparing their model whereas Baveno VII has already included SSM in assessing portal hypertension. Finally, they have not mentioned as to whether endoscopist doing the evaluation of EVs as HEV/LEV was aware of LSM and SSM values. As this may lead to additional bias in reporting.

CONCLUSION

Therefore, the current model may not be generalizable to all etiologies. But nonetheless, these models are the need of hour for addressing long-term complications in these patients. In future, we shall need more studies including adequate number of patients from every etiology to validate the current model.

FOOTNOTES

Author contributions: Gupta T did collection of data, review of literature, wrote the manuscript, critically analysed and did final drafting.

Conflict-of-interest statement: Tarana Gupta have no conflict of interest.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zhang YL

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 126-134

DOI: 10.4254/wjh.v16.i2.126

ISSN 1948-5182 (online)

EDITORIAL

Contemporary concepts of prevention and management of gastroesophageal variceal bleeding in liver cirrhosis patients

Dmitry Victorovich Garbuzenko

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Aydin S, Turkey; Fan X, China

Received: November 30, 2023
Peer-review started: November 30, 2023
First decision: December 23, 2023
Revised: December 31, 2023
Accepted: January 29, 2024
Article in press: January 29, 2024
Published online: February 27, 2024

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Abstract

This editorial describes the contemporary concepts of prevention and management of gastroesophageal variceal bleeding in liver cirrhosis (LC) patients according to the current guidelines. Gastroesophageal variceal bleeding is the most dangerous complication of portal hypertension in LC patients. Risk stratification and determination of an individual approach to the choice of therapeutic measures aimed at their prevention and management has emerged as one of the top concerns in modern hepatology. According to the current guidelines, in the absence of clinically significant portal hypertension, etiological and non-etiological therapies of LC is advisable for the primary preventing gastroesophageal variceal bleeding, whereas its presence serves as an indication for the administration of non-selective β-blockers, among which carvedilol is the drug of choice. Non-selective β-blockers, as well as endoscopic variceal ligation and transjugular intrahepatic portosystemic shunt can be used to prevent recurrence of gastroesophageal variceal bleeding. Pharmacotherapy with vasoactive drugs (terlipressin, somatostatin, octreotide), endoscopic variceal ligation, endovascular techniques and transjugular intrahepatic portosystemic shunt are recommended for the treatment of acute gastroesophageal variceal bleeding. Objective and accurate risk stratification of gastroesophageal variceal bleeding will allow developing individual strategies for their prevention and management, avoiding the first and further decompensation in LC, which will improve the prognosis and survival of patients suffering from it.

Key Words: Liver cirrhosis; Portal hypertension; Gastroesophageal variceal bleeding; Prevention; Management

Core Tip: Given that gastroesophageal variceal bleeding is the most dangerous complication of portal hypertension, objective and accurate risk stratification will allow developing individual strategies for their prevention and management, avoiding the first and further decompensation in liver cirrhosis, which will improve the prognosis and survival of patients suffering from it. This editorial describes the contemporary concepts of prevention and management of gastroesophageal variceal bleeding in liver cirrhosis patients according to the current guidelines.

Citation: Garbuzenko DV. Contemporary concepts of prevention and management of gastroesophageal variceal bleeding in liver cirrhosis patients. *World J Hepatol* 2024; 16(2): 126-134
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/126.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.126

INTRODUCTION

Liver cirrhosis (LC) is the final stage of many chronic liver diseases and has long been considered a static, irreversible pathological process. However, research in recent years has refuted this well-established notion. LC is now considered as a dynamic, potentially reversible disorder, where there are a compensated stage (without or with clinically significant portal hypertension (CSPH), which is characterized by hepatic venous pressure gradient (HVPG) values ≥ 10 mmHg and gastroesophageal varices (GEV) forming) and a decompensated stage, often accompanied by fatal complications associated with portal hypertension (PH) and/or liver failure[1]. Hence, LC decompensation is the most important stratification variable of a bad prognosis[2]. After the first decompensation, a progressive growth in portal pressure increases a likelihood of further decompensation, a resistance to treatment, a risk of death, and the need for liver transplantation[3].

Based on the modern concept of the natural history of LC, at the Baveno VI consensus workshop, held in 2015, recognized the use of "advanced chronic liver disease" as a term equivalent to LC to refer to cases of chronic liver disease with a risk of complications[4]. Since LC is an exclusively histological term, the approval of the new concept expanded the spectrum of the clinical course of the disease, allowing non-invasive methods to be used for its diagnosis and staging. Now LC patients can be stratified by a risk of complications, and therapeutic approaches are individualized. In 2021, at the Baveno VII consensus workshop, the main provisions adopted at the Baveno VI workshop were approved, and given the latest achievements, practical recommendations for personalized care for PH were developed[5].

This editorial describes the contemporary concepts of prevention and management of gastroesophageal variceal bleeding in LC patients according to the current guidelines.

DIAGNOSIS OF CLINICALLY SIGNIFICANT PORTAL HYPERTENSION

The development of CSPH in compensated LC patients is an important prognostic factor, since it leads to an increased risk of first decompensation, one of the clinical manifestations of which is gastroesophageal variceal bleeding[1]. The gold standard of its diagnosis is HVPG measurement[6]. The normal range of HVPG is 1-5 mmHg, whereas a values of ≥ 10 mmHg indicates the presence of CSPH[7]. It should be recognized that until now, HVPG measurement is possible only in specialized centers. In addition, the procedure invasiveness and the need for repeated measurements increases a risk of possible complications and raises costs.

These limitations have contributed to the development of non-invasive methods for assessment of advanced chronic liver disease. One of them is the liver stiffness measurement by transient elastography. This is a fast, simple to perform, and are well tolerated procedure by patients with immediately available results[8]. According to the current guidelines, liver stiffness values by transient elastography of less than 10 kPa in the absence of other known clinical/imaging signs excludes LC, a values between 10 and 15 kPa suggest of it, and a values of more than 15 kPa indicates the presence of LC with a high probability[9]. At the Baveno VII consensus workshop, criteria were established for the exclusion or identification of CSPH by liver stiffness values that, combined with platelet count. According to them, liver stiffness values by transient elastography of more than 25 kPa indicates the presence of CSPH, whereas with liver stiffness values of less than 15 kPa and a normal platelet count, it is unlikely. LC patients with liver stiffness values between 20 and 25 kPa and platelet count less than 150×10^9/L or with liver stiffness values between 15 and 20 kPa and platelet count less than 110×10^9/L have a risk of developing CSPH with a probability of about 60%. They need additional screening[5]. For example, esophagogastroduodenoscopy (EGDS) has traditionally been used to detect GEV, the degree of dilation of which correlates with HVPG and, accordingly, with a risk of bleeding[10].

PREVENTING FIRST LIVER CIRRHOSIS DECOMPENSATION

Primary preventing gastroesophageal variceal bleeding

Therapeutic measures in compensated LC patients with or without CSPH, should be aimed at preventing first decompensation, in particular, primary preventing gastroesophageal variceal bleeding (Figure 1). In the absence of

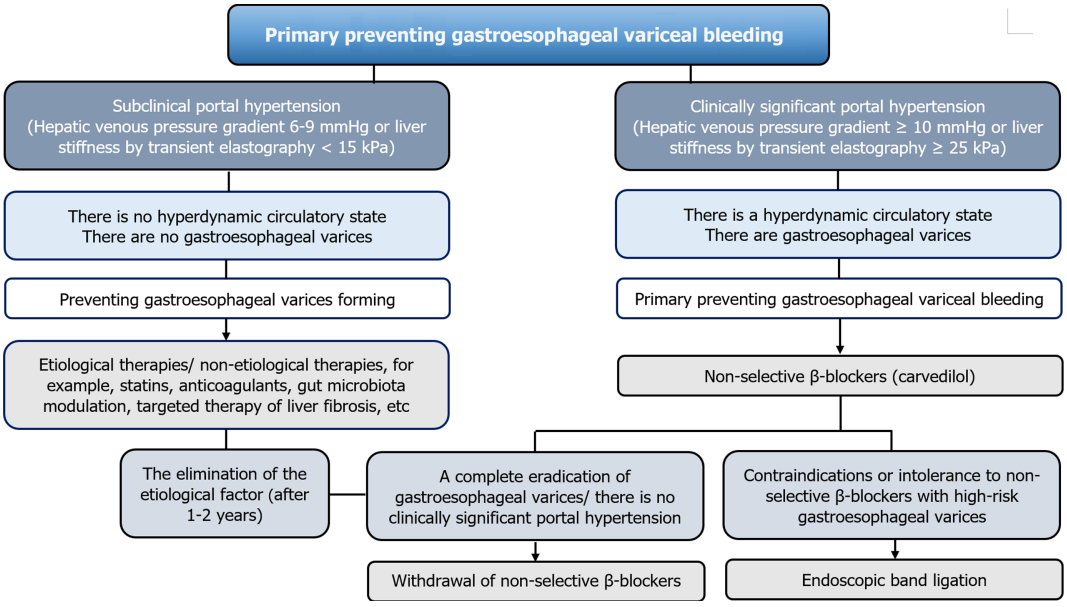

Figure 1 Algorithm for primary preventing gastroesophageal variceal bleeding in liver cirrhosis patients.

hyperdynamic circulatory state, etiological and non-etiological therapies of LC may be beneficial for preventing GEV forming[11]. Indeed, it has been found that alcohol abstinence positively affects the prognosis in alcohol-related LC, including patients with CSPH[12], and a sustained virologic response in LC associated with chronic HBV and HCV infection improves liver morphology and reduces HVPG[13]. The use of statins[14], anticoagulants[15], gut microbiota modulation[16] and targeted therapy of liver fibrosis[17] seems promising as a non-etiological treatment.

The drugs of choice for the primary preventing gastroesophageal variceal bleeding are non-selective β-blockers (NSBB), which is associated with their positive effect on the hyperdynamic circulatory state in CSPH. They can both reduce heart rate and cardiac output by blocking β1-adrenergic receptors, and decrease portal inflow as a result of splanchnic vasoconstriction caused by an endogenous α-adrenergic effect against the background of the blocking vasodilating β2-adrenergic receptors. In addition, NSBBs suppress the small intestinal bacterial overgrowth and prevent bacterial translocation contributing to systemic inflammation characteristic of LC, by accelerating orocecal transit[18].

Liver stiffness values of more than 25 kPa in compensated LC patients, which indicates the presence of CSPH, may be a criterion for prescribing NSBBs[19]. In the PREDESCI trial, the administration of NSBBs (propranolol and carvedilol) reduced HVPG and improved hyperdynamic circulatory state, which contributed to lowering the risk of first decompensation in compensated LC patients with CSPH[20]. The results of the PREDESCI trial were confirmed by quantifying the benefits of NSBBs in preventing LC decompensation[21]. A meta-analysis of 15 studies has shown that a reduction in portal pressure by NSBBs contribute to lowering the risk of complications, death or liver transplantation in LC patients[22]. Thus, the indication for prescribing NSBBs in compensated LC patients is a CSPH in the presence of GEV [5].

The aim of PH pharmacotherapy with NSBBs should be HVPG reduction to less than 12 mmHg or 20% of the baseline, without allowing significant arterial hypotension and other adverse effects. However, since HVPG measurement is not widely available, and a decrease in heart rate does not correlate with HVPG reduction, the dose of NSBBs is adjusted to the maximum tolerated doses[23]. According to the current guidelines, NSBBs should be prescribed at a dose that decrease heart rate at rest by 25% or up to 55 beats per minute at the initial bradycardia. Daily doses of propranolol can vary from 20 mg orally (initial) to 320 mg (maximum) and should be individually determined according to clinical response. The dose of carvedilol should be titrated from the initial daily dose of 6.25 mg. The maximum dose is 25 mg/d [24]. In some systematic reviews and meta-analyses, it has been shown that correctly determined therapeutic dosages of carvedilol more significantly reduce HVPG compared to propranolol, making it more effective in preventing gastroesophageal variceal bleeding in LC patients[25,26]. In the majority of responders to carvedilol therapy, the HVPG-response is maintained over a long period, which improves the clinical outcome[27]. As a consequence, at the Baveno VII consensus workshop, carvedilol was recommended as the drug of choice for preventing first decompensation in compensated LC patients with CSPH[5].

In compensated LC patients after the start of NSBBs therapy, there is no need to monitor the presence and dynamics of GEV during follow-up due to the lack of influence of the results of EGDS on therapeutic tactics. An exception may be in the case of a decision to withdrawal of NSBBs with the effectiveness of etiological treatment. In particular, their withdrawal is possible in patients who, 1-2 years after the elimination of the etiological factor, had a complete eradication of GEV and, according to transient elastography or HVPG, there are no signs of CSPH[28].

Endoscopic band ligation (EBL) is recommended in compensated LC patients with contraindications or intolerance to NSBBs with high-risk GEV for preventing first bleeding[5].

PREVENTING FURTHER LIVER CIRRHOSIS DECOMPENSATION

Further LC decompensation is an unfavorable prognostic stage associated with an even higher mortality rate than during the first decompensation, therefore, patients with it are candidates for liver transplantation. Further LC decompensation is characterized by recurrent gastroesophageal variceal bleeding, refractory ascites (requires > 3 large volume paracentesis within 1 year), recurrent encephalopathy, the development of spontaneous bacterial peritonitis, hepatorenal syndrome/acute kidney injury, as well as jaundice[5].

Preventing recurrent gastroesophageal variceal bleeding (secondary prophylaxis)

The combined use of NSBBs and EBL is the treatment of choice for secondary prophylaxis of gastroesophageal variceal bleeding[5]. This approach proved to be more effective than the use of each technique separately, both in preventing recurrent bleeding[29] and in improving survival[30]. With regard to the isolated use of NSBBs in secondary prophylaxis of gastroesophageal variceal bleeding, a recent systematic review showed the advantages of carvedilol over propranolol, which was accompanied by lower rates of recurrent bleeding, liver-related death, and further nonbleeding decompensation[31].

In recent years, the issue of the expediency of prescribing NSBBs to decompensated LC patients with ascites has been discussed due to their ability to reduce increased cardiac output, which is a compensatory reaction to hypovolemia to maintain systemic and renal perfusion[32]. In this regard, at the Baveno VII consensus workshop, it was recommended that in decompensated LC patients with ascites, in case of persistently low blood pressure (systolic blood pressure < 90 mmHg or mean arterial pressure < 65 mmHg) and/or the presence of hepatorenal syndrome/acute kidney injury, the dose of NSBBs should be reduced or they should be completely canceled. Once blood pressure returns to baseline and/or after eliminating signs of hepatorenal syndrome/acute kidney injury, NSBBs can be re-initiated or re-titrated initially at a dose lower than when discontinuation. If patients remain intolerant to NSBBs, EBL is recommended to prevent gastroesophageal variceal bleeding[5].

Transjugular intrahepatic portosystemic shunt (TIPS) is the method of choice for preventing recurrent gastroesophageal variceal bleeding with the ineffectiveness of the combined use of NSBBs and EBL taking into account rebleeding severity and other complications of PH, with careful patient selection to minimize hepatic encephalopathy[33]. Compared to the combined use of NSBBs and EBL, polytetrafluoroethylene (PTFE)-covered TIPS have a significant benefit of preventing gastroesophageal variceal rebleeding[34], decrease the threat of further decompensation[35], and postoperative reduction of HVPG below 12 mmHg can contribute to recompensation[36].

Managing acute gastroesophageal variceal bleeding

At the Baveno VII consensus workshop, a number of changes and additions were made to the previously adopted recommendations for the management of LC patients with acute gastroesophageal variceal bleeding[5], although the general principles remained the same. If possible, all LC patients with acute gastroesophageal variceal bleeding should be hospitalized in the intensive care unit for resuscitation measures aimed at preserving tissue perfusion. It is important to quickly begin restoration of circulating blood volume to ensure and maintain hemodynamic stability. The threshold for red blood cell transfusion should be a hemoglobin level of 7-8 g/dL, taking into account factors such as cardiovascular diseases, age, hemodynamic status and the presence of ongoing bleeding. If there is a suspicion of acute gastroesophageal variceal bleeding, as early as possible, ideally before the EGDS, vasoactive drugs should be administered: terlipressin (under the control of serum sodium levels), somatostatin, octreotide for at least 5 d[37].

Terlipressin is usually administered 2 mg IV immediately, then 1-2 mg every 4-6 h until hemostasis achieved, or for 3 to 5 d. Somatostatin is administered 250 mg bolus IV initially, followed by 250 mg/h IV infusion for 3 to 5 d. Octreotide is administered 50 mcg bolus IV initially, followed by 50 mcg/h IV infusion until hemostasis achieved or for 3 to 5 d[38]. In a systematic review and meta-analysis, vasoactive drugs had similar indicators of mortality risk, control of acute gastroesophageal variceal bleeding, its early and late recurrence, need for transfusion of red blood cells and hospitalization duration. However, the use of terlipressin was accompanied by a higher risk of adverse events[39]. At the same time, the administration of proton pump inhibitors started before EGDS, after the diagnosis of acute gastroesophageal variceal bleeding in the absence of strict indications, should be stopped immediately, since their use in LC patients increases the likelihood of spontaneous bacterial peritonitis and other infectious complications[40].

Given the risk of bacterial infection primarily in decompensated LC patients with acute gastroesophageal variceal bleeding, antibiotic prophylaxis is an integral part for therapy. It should be prescribed from the moment of admission by IV administration of ceftriaxone at a dose of 1 g/d. Antibiotic prophylaxis should always be in accordance with local resistance patterns and antimicrobial policies. Antibiotic prophylaxis in LC patients with acute gastroesophageal variceal bleeding significantly reduces the frequency of bacterial infections, all-cause mortality, bacterial infection mortality, rebleeding events and hospitalization duration[41].

Malnutrition in LC patients with acute gastroesophageal variceal bleeding increases the risk of adverse outcomes, therefore, their feeding should be resumed 48-72 h after achieving hemostasis. Because of the lower cost and the lack of complications, enteral nutrition is always preferable to parenteral. If it is carried out through a nasogastric probe, manipulations with it should be performed with extreme caution should be performed with caution due to the risk of pulmonary infection[42].

Correction of hepatic encephalopathy in LC patients with acute gastroesophageal variceal bleeding is carried out by rapid removal of blood from the gastrointestinal tract by lactulose (through a nasogastric probe or in the form of enemas)[43].

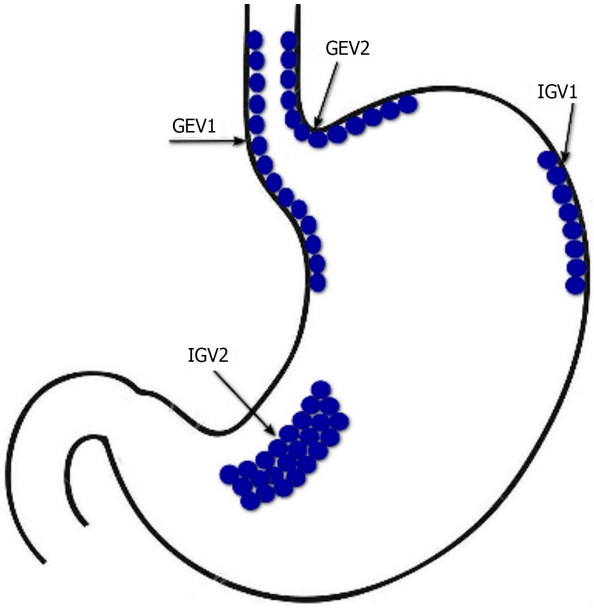

Figure 2 Sarin's classification of gastric varices. GEV1: Type 1 gastroesophageal varices; GEV2: type 2 gastroesophageal varices; IGV1: Type 1 isolated gastric varices; IGV2: Type 2 isolated gastric varices.

Against the background of resuscitation measures in LC patients with acute upper gastrointestinal bleeding, EGDS should be performed within 12 h from the moment of admission. If the patient's condition is unstable, it is carried out as soon as possible, as far as it is safe. The diagnosis of gastroesophageal variceal bleeding is established by the presence of its active manifestations. In the absence of bleeding, indirect signs of the complication are the "white nipple sign" and blood clots on varices, as well as blood in the lumen of the esophagus and/or stomach if other possible causes have been ruled out[44]. Tracheal intubation before EGDS is recommended in patients with impaired consciousness and/or active vomiting blood. They are extubated immediately after the procedure is completed. In the absence of contraindications (QT interval prolongation), administration of intravenous erythromycin at 250 mg for 30-120 min before EGDS to improve mucosa visualization by enhancing gastric motility is considered appropriate[45].

In LC patients with acute gastroesophageal variceal bleeding, the method of choice is EBL[5]. An additional 5-d course of pharmacotherapy with vasoactive drugs can significantly reduce the risk of their recurrence[46]. Endoscopic therapy with tissue adhesives (*e.g.* histoacryl (N-butyl-2-cyanoacrylate)/thrombin) is recommended for acute bleeding from type 1 GEV (GEV1) and type 2 GEV (GEV2) that extend beyond the cardia, and from type 1 and type 2 isolated gastric varices (IGV1 and IGV2) (Figure 2)[47]. In case of refractory gastroesophageal variceal bleeding despite combined pharmacotherapy with vasoactive drugs and EBL, esophageal balloon tamponade with a Sengstaken–Blakemore tube or the installation of a dedicated self-expandable, covered esophageal metal stent should be resorted to. Ideally, this should serve as a bridge to rescue PTFE-covered TIPS[48]. PTFE-covered TIPS is recommended in LC patients with uncontrolled acute gastroesophageal variceal bleeding at EGDS or who have successfully undergone EBL but who rebleed at any time during admission (after endoscopy). In addition, select LC patients Child-Turcotte-Pugh (CTP) class B or C with active gastroesophageal variceal bleeding at EGDS are at highest risk for rebleeding and may benefit from early or pre-emptive PTFE-covered TIPS within 72 h of admission to improve survival[49]. It has been shown that among selected advanced LC patients (CTP class B or C) with acute gastroesophageal variceal bleeding, PTFE-covered TIPS is superior to pharmacotherapy with vasoactive drugs plus EBL in improving transplantation-free survival, reducing failure to control bleeding, without increasing the risk of overt hepatic encephalopathy[50]. At the same time, TIPS may be useless in LC patients CTP class C with > 14 points, or with a MELD score > 30 and a lactate level > 12 mmol/L, if liver transplantation is not planned in the short term[5]. In patients with bleeding from GEV2 and from IGV1 and IGV2 balloon-occluded retrograde transvenous obliteration (BRTO) is possible as an alternative to endoscopic treatment or TIPS, provided that this is feasible (type and diameter of gastrorenal shunts) and there is experience in its use[51]. The combined use of TIPS and BRTO is possible both to control acute gastric variceal bleeding and to reduce the risk of their recurrence, particularly in cases when, despite a reduction in HVPG, portal flow remains diverted to gastrorenal shunts[47].

Since the cause of gastroesophageal variceal bleeding is PH, it is obvious that the basis of their treatment should be a reduction in portal pressure, and not correction of blood clotting disorders. Moreover, conventional coagulation screening parameters, for example, prothrombin time/international normalized ratio and activated partial thromboplastin time, reflect the hemostasis state in LC patients is not quite correct[52]. Therefore, fresh frozen plasma transfusion is not recommended in gastroesophageal variceal bleeding, since it will not correct coagulopathy and may lead to volume overload and worsening of PH[53]. This postulate was, in particular, confirmed in a multicentre cohort study, where fresh frozen plasma transfusion in acute gastroesophageal variceal bleeding was independently associated with poor clinical outcomes[54]. There is also no evidence that platelet count and fibrinogen levels are correlated with the risk of failure to control gastroesophageal variceal bleeding or rebleeding. In addition, the use of recombinant factor VIIa and tranexamic acid are not recommended in gastroesophageal variceal bleeding. At the same time, if pharmacotherapy with vasoactive

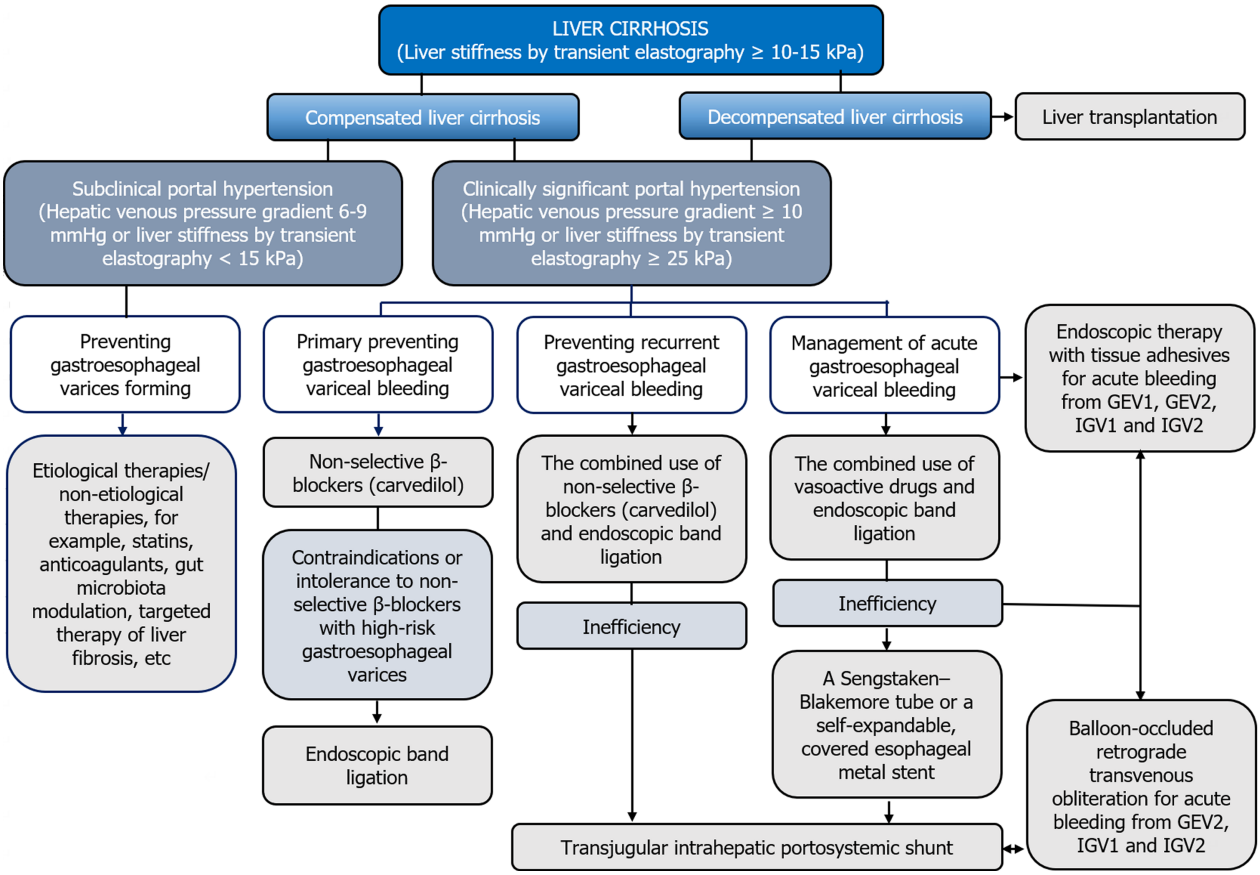

Figure 3 Algorithm for preventing and managing gastroesophageal variceal bleeding in liver cirrhosis patients. GEV1: Type 1 gastroesophageal varices; GEV2: Type 2 gastroesophageal varices; IGV1: Type 1 isolated gastric varices; IGV2: Type 2 isolated gastric varices.

drugs and/or EBL is ineffective, the decision to eliminate blood clotting disorders should be considered individually[53].

CONCLUSION

PH is the most important event of the natural history of LC, since it can be associated with its first and further decompensation and is responsible for the development of severe, often fatal complications, such as gastroesophageal variceal bleeding. The dynamic character and potential reversibility of LC requires the improvement of invasive and non-invasive methods of its diagnosis, as well as the identification of CSPH. This will allow to objectively and accurately stratify the risk of gastroesophageal variceal bleeding, develop individual strategies for their prevention and management, avoid the first and further decompensation in LC, which will improve the prognosis and survival of patients suffering from it (Figure 3).

FOOTNOTES

Author contributions: Garbuzenko DV contributed to the conception, design, acquisition, analysis, interpretation of data, wrote the manuscript and approved the final version.

Conflict-of-interest statement: Author have no conflict of interest to declare.

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S-Editor: Liu JH

L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 135-139

DOI: 10.4254/wjh.v16.i2.135

ISSN 1948-5182 (online)

EDITORIAL

Advancements in autoimmune hepatitis management: Perspectives for future guidelines

Marcos Mucenic

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Mohammadi F, Iran

Received: December 3, 2023
Peer-review started: December 3, 2023
First decision: December 15, 2023
Revised: January 4, 2024
Accepted: January 23, 2024
Article in press: January 23, 2024
Published online: February 27, 2024

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Abstract

The first-line treatment for autoimmune hepatitis involves the use of prednisone or prednisolone either as monotherapy or in combination with azathioprine (AZA). Budesonide has shown promise in inducing a complete biochemical response (CBR) with fewer adverse effects and is considered an optional first-line treatment, particularly for patients without cirrhosis; however, it is worth noting that the design of that study favored budesonide. A recent real-life study revealed higher CBR rates with prednisone when equivalent initial doses were administered. Current guidelines recommend mycophenolate mofetil (MMF) for patients who are intolerant to AZA. It is important to mention that the evidence supporting this recommendation is weak, primarily consisting of case series. Nevertheless, MMF has demonstrated superiority to AZA in the context of renal transplant. Recent comparative studies have shown higher CBR rates, lower therapeutic failure rates, and reduced intolerance in the MMF group. These findings may influence future guidelines, potentially leading to a significant modification in the first-line treatment of autoimmune hepatitis. Until recently, the only alternative to corticosteroids was lifelong maintenance treatment with AZA, which comes with notable risks, such as skin cancer and lymphoma. Prospective trials are essential for a more comprehensive assessment of treatment suspension strategies, whether relying on histological criteria, strict biochemical criteria, or a combination of both. Single-center studies using chloroquine diphosphate have shown promising results in significantly reducing relapse rates compared to placebo. However, these interesting findings have yet to be replicated by other research groups. Additionally, second-line drugs, such as tacrolimus, rituximab, and infliximab, should be subjected to controlled trials for further evaluation.

Key Words: Autoimmune hepatitis; Treatment; Immunosuppression; Relapse; Remission induction

Core Tip: Autoimmune hepatitis guidelines consider corticosteroids as first-line treatment, including budesonide as an option in patients without cirrhosis. Azathioprine is recommended to reduce corticosteroid doses and side effects. Nevertheless, there are concerns regarding its long-term malignancy risks. Recent publications suggest that these guidelines may be outdated. The efficacy of budesonide can be limited to patients with lower aminotransferases levels. The potential superiority of mycophenolate mofetil to azathioprine is under scrutiny. Additionally, there are controversies regarding treatment suspension, with a potential role for chloroquine for long-term maintenance treatment. Other therapeutic agents are still in the initial stages of research.

Citation: Mucenic M. Advancements in autoimmune hepatitis management: Perspectives for future guidelines. *World J Hepatol* 2024; 16(2): 135-139
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/135.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.135

INTRODUCTION

Autoimmune hepatitis (AIH) was the first chronic liver disease for which a treatment benefit was demonstrated in randomized studies, marking over 50 years since those pivotal clinical trials. Patients under treatment exhibited a survival rate of 85%-95%, a stark contrast to the 33%-44% observed with a placebo after 2.0 years to 3.5 years of follow-up [1-3].

The first-line treatment of AIH involves the corticosteroids prednisone (PD) or prednisolone, either as monotherapy or in combination with azathioprine (AZA). Corticosteroids readily interfere with the immune system by affecting cytokine production and inhibiting T lymphocyte activation. AZA exerts its immunosuppressive effects by blocking the maturation of lymphocyte precursors, which may take at least 3 mo for the full effect to be accomplished[4].

In 2015, the European Association for the Study of Liver Diseases (EASL) published their practice guidelines on AIH diagnosis and management[5], followed by the American Association for the Study of Liver Diseases (AASLD) in 2019[6] and the Asian Pacific Association for the Study of the Liver in 2021[7]. Both the AASLD and EASL recommend delaying the introduction of AZA for the first 2 wk. This approach, though untested in clinical trials, may aid in distinguishing rare instances of AZA-induced hepatotoxicity from non-response. AZA is primarily employed for its steroid-sparing effect and for maintenance therapy since it is less effective in inducing a response. Once steroid responsiveness is confirmed and thiopurine methyltransferase deficiency is ruled out, AZA can be prescribed, according to these guidelines. Genetic mutations affecting thiopurine methyltransferase occur in up to 0.6% of the population, but the consequences of using AZA in these individuals can be severe[8].

For over 40 years, this was the standard first-line treatment. Randomized trials involving ursodeoxycholic acid and cyclosporine, as well as intermittent or pulse corticosteroid treatment, yielded negative results[9-13]. In 2010, a clinical trial suggested that budesonide (BD) might be more effective than PD in inducing a response, with the added advantage of causing fewer cosmetic side effects[14]. BD is a glucocorticosteroid with a potent topical effect and a high (> 90%) first-pass uptake[15]. However, this study faced criticism due to the rapid reduction in PD doses, irrespective of biochemical relapse or non-response, reaching 10 mg per day within 8 wk. Importantly, the effectiveness in the control group was much smaller than expected: Only 39% in 6 mo. Despite the criticism, the AASLD guidelines recommend BD as a first-line option for patients without cirrhosis, particularly those at risk of adverse corticosteroid-related side effects. Other medical societies take a more cautious approach, suggesting BD as an alternative pending further study.

A recent multicenter real-life study[16] that included treatment-naïve, non-severe AIH patients without cirrhosis revealed that clinicians prescribed BD in only 5% of cases. Notably, BD was more commonly used in patients with significantly lower liver test results (median alanine aminotransferase 198 IU/L *vs* 753 IU/L), with a relative risk of response of 0.20 compared to PD. However, effectiveness was similar in patients with alanine aminotransferase or aspartate aminotransferase levels < 2 times the normal limit. Complete biochemical response was 87% with PD and 51% with BD when equivalent initial doses were used (50 mg PD *vs* 9 mg BD), which was in contrast with the clinical trial favoring budesonide.

Current guidelines recommend mycophenolate mofetil (MMF) only for AZA-intolerant patients. MMF is the prodrug of mycophenolic acid. It exerts an antiproliferative action on lymphocytes by inhibiting inosine monophosphate dehydrogenase, the rate-limiting enzyme in de novo purine synthesis[15]. However, MMF has been found to be superior to AZA in renal transplant protocols, reducing acute rejection and graft loss[17,18]. Accordingly, two recent studies compared MMF to AZA on a head-to-head basis. The first was a Greek multicenter study published in 2022[19]. It was designed in a way that patients could choose whether to receive AZA (1-2 mg/kg/d up to 150 mg) or MMF (1.5-2.0 mg/d) in addition to a starting PD dose of 40 mg. Notably, the MMF group achieved greater rates of complete biochemical response (96% *vs* 87%) and smaller rates of therapeutic failures (8% *vs* 19%) or treatment modification due to incomplete response or intolerance (11% *vs* 44%) after 4.8 years of follow-up.

Last year in the EASL Liver Meeting, a randomized control trial (the CAMARO trial) was presented[20]. Patients in the AZA arm received a maximum daily dose of 100 mg, while patients in the MMF arm were treated with up to 2000 mg daily, per protocol. In treatment-naïve patients, MMF was superior to AZA for induction of remission (55.3% *vs* 25.8%) with less cessation due to adverse effects (5.1% *vs* 25.8%). Some may argue that the AZA maximum dose was not equivalent to that of MMF, but the magnitude of the difference between the treatment arms was relevant, and an increase of the AZA dose would lead to even greater side effect rates.

After 50 years, an important modification in the first-line treatment of AIH is anticipated, as MMF could be considered an alternative to AZA. Second-line or third-line drugs such as tacrolimus, rituximab, and infliximab need controlled trials for further evaluation, requiring a multicentric effort due to the large sample sizes needed.

Regarding long-term therapy, the prolonged use of corticosteroids is associated with well-established side effects, while maintaining monotherapy with AZA carries risks such as skin cancer and lymphoma[21]. It is recommended to consider suspending treatment upon achieving a complete response. However, the suspension of treatment remains a topic of controversy in the management of AIH. Historical data has shown that relapses can lead to the progression of AIH to cirrhosis, liver failure, and even death. Nevertheless, recent publications with closer follow-up have not found these serious complications. A consolidation period of at least 18 mo is recommended, considering that histological remission typically lags behind biochemical remission[6,22].

Plasma cell infiltrates and interface hepatitis have been associated with relapse after treatment suspension[23,24], but those publications defined response as a reduction to less than twice the upper limit of liver tests. It was later demonstrated that complete biochemical normalization, including aminotransferases and gamma globulin levels, correlated with more favorable clinical outcomes. Unfortunately, even using these criteria, relapses still occurred in 46%-81% of patients after 3 years of follow-up[25,26]. However, these publications evaluating relapse risk are potentially biased because of their retrospective nature. Indeed, there is currently no controlled trial to support any treatment withdrawal strategy. A prospective trial in this regard would be invaluable, but it would need to be multicentric to include a sufficient sample size. There is a need to evaluate prospectively whether a liver biopsy is needed before treatment suspension, or if strict biochemical criteria alone are sufficient.

Encouraging results have emerged from single-center studies involving the use of chloroquine diphosphate, demonstrating a significant reduction in relapse rates compared to a placebo. Chloroquine plays an established role in the treatment of autoimmune rheumatic diseases, potentially by interfering with lysosomal phagocytic function, antigen presentation, cytokine production, and other immunoregulatory effects. However, it is important to note that these intriguing findings have yet to be independently replicated by other research groups[27-29].

Promising therapeutic agents, such as those acting on cytokine, chemokine, and signaling pathways, cell-based therapy, microbiome modulation, or nanomedicine, are still in the early stages of research[30].

CONCLUSION

The clinical management of autoimmune hepatitis is primarily rooted in landmark clinical trials conducted over 50 years ago. While certain aspects of this management have evolved, recent research has provided data that hold the potential to refine our current guidelines. Nonetheless, achieving optimal strategies for response induction, treatment maintenance, and suspension will require ongoing research and efforts.

FOOTNOTES

Author contributions: Mucenic M wrote and revised the manuscript.

Conflict-of-interest statement: The author declares having no conflicts of interest.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 140-145

DOI: 10.4254/wjh.v16.i2.140

ISSN 1948-5182 (online)

EDITORIAL

Interleukins in liver disease treatment

Ming Yang, Chun-Ye Zhang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chatterjee B, India

Received: December 4, 2023
Peer-review started: December 4, 2023
First decision: December 17, 2023
Revised: December 22, 2023
Accepted: January 8, 2024
Article in press: January 8, 2024
Published online: February 27, 2024

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Abstract

Cytokines play pleiotropic roles in human health and disease by regulating both innate and adaptive immune responses. Interleukins (ILs), a large group of cytokines, can be divided into seven families, including IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, and IL-17 families. Here, we review the functions of ILs in the pathogenesis and resolution of liver diseases, such as liver inflammation (*e.g.*, IL-35), alcohol-related liver disease (*e.g.*, IL-11), non-alcoholic steatohepatitis (*e.g.*, IL-22), liver fibrosis (*e.g.*, Il-17a), and liver cancer (*e.g.*, IL-8). Overall, IL-1 family members are implicated in liver inflammation induced by different etiologies, such as alcohol consumption, high-fat diet, and hepatitis viruses. IL-2 family members mainly regulate T lymphocyte and NK cell proliferation and activation, and the differentiation of T cells. IL-6 family cytokines play important roles in acute phase response in liver infection, liver regeneration, and metabolic regulation, as well as lymphocyte activation. IL-8, also known as CXCL8, is activated in chronic liver diseases, which is associated with the accumulation of neutrophils and macrophages. IL-10 family members contribute key roles to liver immune tolerance and immunosuppression in liver disease. IL-12 family cytokines influence T-cell differentiation and play an essential role in autoimmune liver disease. IL-17 subfamilies contribute to infection defense, liver inflammation, and Th17 cell differentiation. ILs interact with different type I and type II cytokine receptors to regulate intracellular signaling pathways that mediate their functions. However, most clinical studies are only performed to evaluate IL-mediated therapies on alcohol and hepatitis virus infection-induced hepatitis. More pre-clinical and clinical studies are required to evaluate IL-mediated monotherapy and synergistic therapies.

Key Words: Interleukins; Family members; Liver disease; Treatment; Clinical trials

Core Tip: Interleukins as a large group of cytokines play pleiotropic roles in liver homeostasis and disease by regulating both innate and adaptive immune responses. They can be divided into seven families, and all of them are involved in the pathogenesis and resolution of chronic liver diseases. Currently, interleukin-mediated therapies are applied in patients with hepatitis induced by alcohol or hepatitis virus infection.

Citation: Yang M, Zhang CY. Interleukins in liver disease treatment. *World J Hepatol* 2024; 16(2): 140-145
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/140.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.140

INTRODUCTION

Cytokines coordinate both innate and adaptive immune responses, and they display pleiotropic roles in healthy and disease conditions[1]. Interleukins (ILs), a large group of cytokines, play important roles in immune cell growth, differentiation, and activation, as well as other tissue-resident cells by interacting with their receptors[2]. Acute and chronic liver diseases are characterized by liver inflammation and cell death[3,4], which are commonly associated with infiltration of different immune cells and activation of hepatic parenchymal cells to secrete ILs[5,6]. ILs as a major type of cytokines are involved in the pathogenesis and resolution of liver diseases, such as liver inflammation (*e.g.*, IL-35)[7], alcohol-related liver disease (*e.g.*, IL-11)[8], non-alcoholic steatohepatitis (*e.g.*, IL-22)[9], liver fibrosis (*e.g.*, Il-17a)[10], and liver cancer (*e.g.*, IL-8)[11].

Herein, we review the members of IL families and their functions in liver disease. Especially, we summarize the current findings for liver disease treatment by targeting different ILs in clinical trials.

INTERLEUKIN FAMILIES

Interleukins can be divided into seven families (Table 1), including IL-1 family[12,13], IL-2 family[14,15], IL-6 family[16,17], IL-8 family[18,19], IL-10 family[20,21], IL-12 family[22,23], and IL-17 family[24,25]. All the families of interleukins are involved in the liver disease. For example, IL-1 family cytokines are implicated in liver inflammation induced by different etiologies[26,27], such as alcohol consumption, high-fat diet, and hepatitis viruses. IL-2 family members mainly regulate T lymphocyte and NK cell proliferation and activation, and the differentiation of T cells[28-30]. IL-6 family cytokines play important roles in acute phase response in liver infection, liver regeneration, and metabolic regulation, as well as lymphocyte activation[31,32]. IL-8, also known as CXCL8, is activated in chronic liver diseases, which is associated with the accumulation of neutrophils and macrophages[33,34]. IL-10 family members contribute key roles to liver immune tolerance and immunosuppression in liver disease[35,36]. IL-12 family cytokines influence T-cell differentiation and play an essential role in autoimmune liver disease[37,38]. IL-17 subfamilies contribute to infection defense, liver inflammation, and Th17 cell differentiation[39,40]. Commonly, several IL families function together in each liver disease, contributing to liver disease progression and resolution. Therefore, targeting interleukins provides therapeutic strategies for liver disease.

INTERLEUKIN RECEPTORS

Cytokines such as interleukin family members can bind their receptors to activate intracellular signaling pathways (*e.g.*, Janus kinase/signal transduction and transcription activation or JAK/STAT signaling pathway) to regulate cell biological functions. Cytokine receptors are mainly classified into two classes, type 1 and type 2 receptors. Most receptors of IL family members belong to type 1 receptors (Table 2), such as IL-2 and IL-6, and IL-10 and IL-10 family cytokine (*e.g.*, IL-19) receptors belong to type 2 receptors[41,42], while IL-1 family member receptors have both type 1 and type 2 receptors [12]. Type 1 cytokine receptors have a conserved Trp-Ser-X-Trp-Ser (WSXWS) motif at their C-terminals and four conserved cysteine residues at their N-terminals, and they can interact with cytokines with four-helical bundle motifs [43]. Most type 2 cytokine receptors are heterodimers (Table 2), and their intracellular domains are linked by a Janus kinase which can activate the STAT signaling pathway[44].

IL-MEDIATED THERAPIES IN CLINICAL TRIALS

Given the important roles of ILs in liver diseases, many clinical trials are undergoing to evaluate their direct and synergistic functions in liver disease treatment. The cases (Table 3) were reviewed from the website https://www.clinicaltrials.gov/ (accessed on December 3, 2023). To date, most studies have been performed to evaluate IL-mediated therapies on alcohol and hepatitis virus infection-induced hepatitis.

Table 1 Interleukin families in liver diseases

IL family	Members	Functions
IL-1	IL-1α, IL-1β, IL-18, IL-33, IL-36, IL-37, and IL-38	Mediate inflammatory responses to a wide range of stimuli in both innate and adaptive immune systems, with pro- and anti-inflammatory functions[12,13]
IL-2	IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21	Regulate T cell proliferation and activation, NK cytolytic activity, and the differentiation of regulatory T cells[14,15]
IL-6	IL-6, IL-11, IL-27, IL-31, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor, cardiotrophin 1, and cardiotrophin-like cytokine factor 1s	Play important roles in B-cell stimulation, the balance between regulatory and effector T cells, metabolic regulation, hepatic acute phase reaction, and many neural functions [16,17]
IL-8	IL-8, also known as CXCL8	It is a member of the chemokines, which has biological functions on cells expressing CXCR1 and CXCR2 receptors, such as polymorphonuclear leukocytes (neutrophils), epithelial cells, endothelial cells, fibroblasts, and neurons[18,19]
IL-10	IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26	Display immunosuppressive functions, elicit innate defense mechanisms against viral, bacterial, and fungal infections, promote tissue repair and regeneration, and provide therapeutic targets for autoimmune diseases and cancers[20,21]
IL-12	IL-12, IL-23, IL-27 and IL-35	Regulate immune responses and influence naïve T cell differentiation in many inflammatory diseases, autoimmune diseases, and various cardiovascular diseases[22,23]
IL-17	IL-17A to IL-17F (IL-17E also known as IL-25)	Defense against microbial (bacteria, fungi, and helminth) infection, recruit neutrophils, and modify T-helper cell differentiation[24,25]

IL: Interleukins.

Table 2 Interleukins and their receptors

Interleukin	Type 1 receptors	Interleukin	Type 2 receptors	IL-1 family member	Receptor
IL-2	IL-2Rα, IL-2Rβ, IL-2Rγ	IL-10	IL-10Rα, IL-10Rβ	IL-1α, IL-1β	IL-1R1, IL-1R3
IL-3	IL-3Rα, CSF2Rβ	IL-19, IL-20, IL-24	IL-20Rα, IL-20Rβ	IL-1β	IL-1R2, IL-1R3
IL-4	IL-4R, IL-2Rγ/IL-13Rα1	IL-22	IL-22Rα1, IL-10Rβ	IL-1Rα	IL-1R
IL-5	IL-5Rα, CSF2Rβ	IL-20, IL-24	IL-22Rα1, IL-20Rβ	IL-18	IL-1R5, IL-1R7
IL-6	IL-6Rα, gp130	IL-26	IL-10Rβ, IL-20Rα	IL-33	IL-1R4, IL-1R3
IL-7	IL-7Rα, IL-2Rγ	IL-28, IL-29	IL-28Rα, IL-10Rβ	IL-36	IL-1R6, IL-1R3
IL-9	IL-9R, IL-2Rγ			IL-37	IL-1R5, IL-1R8
IL-11	IL-11Rα, gp130			IL-38	IL-1R6, IL-1R9
IL-12	IL-12Rβ1, IL-12Rβ2				
IL-13	IL-13Rα1, IL-13Rα2, IL-4R				
IL-15	IL-15Rα, IL-2Rβ, IL-2Rγ				
IL-16	CD4, CD9				
IL-21	IL-21R, IL-2Rγ				
IL-23	IL-12Rβ1, IL-23R				
IL-27	IL-27Rα, gp130				
IL-31	IL-31Rα, OSMR				
IL-34	CSF-1R				
IL-35	IL-12Rβ2, gp130				

IL: Interleukins.

Table 3 Interleukin-mediated therapies in liver disease

Clinical trials	Phase	Liver disease	Interleukin therapy
NCT00565539	1	Chronic hepatitis C virus (HCV) infection	PEGylated recombinant interleukin 29 (PEG-rIL-29) or in combination with daily oral ribavirin (an antiviral drug)
NCT03882307	1	Hepatitis C virus (HCV) infection	Test the association of serum levels of IL-6 and TGF-β in response to antiviral therapy (sofosbuvir and daclatasvir) for chronic hepatitis C patients
NCT02431312	1	Chronic hepatitis B	Evaluate the safety, tolerability, and immunogenicity of dose combinations of INO-1800 (DNA plasmids encoding hepatitis B surface antigen and hepatitis B core antigen) and INO-9112 (DNA plasmid encoding human interleukin 12) delivered by electroporation
NCT02655510	1/2	Alcoholic hepatitis	To test the efficacy of F-652, a recombinant fusion protein containing human IL-22 and human immunoglobulin G2 (IgG2)-Fc produced in CHO cells in serum-free culture
NCT03775109	2	Alcoholic hepatitis	To evaluate the potential benefits of the IL-1β antibody Canakinumab in the treatment of alcoholic hepatitis
NCT01988506	2	Autoimmune hepatitis, and other autoimmune and auto-inflammatory diseases	Low-dose IL-2 to induce regulatory T cells
NCT00196586	2	Chronic hepatitis C	Evaluate the efficacy and safety of the addition of IL-2 to pegylated interferon α-2a and ribavirin in patients with HCV/HIV coinfection
NCT01697501	3	Chronic hepatitis B	Evaluating the IL-28B polymorphism in patients with HBeAg-negative chronic hepatitis B treated with pegylated interferon α-2a
NCT03090035	3	Chronic hepatitis C	Test IL-28B (rs12979860) genotypes in patients with chronic hepatitis C infection treated with pegylated interferon α2 plus ribavirin
NCT02360592	4	Chronic hepatitis B	Evaluate the efficacy and safety of interferon α-2b therapy plus IL-2 and hepatitis B therapeutic vaccine compared to interferon α-2b alone
NCT03734783	Observational	Chronic hepatitis B	Investigate the levels of IL-35-secreting B regulatory cells in peripheral blood cells in patients with chronic hepatitis B and their functions on Th1 and Th2 cell levels

IL: Interleukins.

CONCLUSION

In summary, all seven families of ILs play pivotal roles in liver homeostasis and pathogenesis by regulating both innate and adaptive immune responses. However, current studies mainly focus on evaluating the roles of ILs in alcohol and hepatitis virus infection-induced hepatitis. Pre-clinical and clinical evaluations of IL effects in different chronic liver diseases should be further studied by testing the efficacy of interleukin monotherapy or synergistic effects with other therapies.

FOOTNOTES

Author contributions: Yang M and Zhang CY designed, collected data, wrote, revised, and finalized the manuscript, contributed equally, and shared the first authorship.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 146-151

DOI: 10.4254/wjh.v16.i2.146

ISSN 1948-5182 (online)

EDITORIAL

Changes in the etiology of liver cirrhosis and the corresponding management strategies

Jin-Jin Dai, Yue-Ying Liu, Zhen-Hua Zhang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Sintusek P, Thailand

Received: December 10, 2023
Peer-review started: December 10, 2023
First decision: January 15, 2024
Revised: January 16, 2024
Accepted: January 30, 2024
Article in press: January 30, 2024
Published online: February 27, 2024

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Abstract

We read with interest the article by Xing Wang, which was published in the recent issue of the *World Journal of Hepatology* 2023; 15: 1294-1306. This article focuses particularly on the prevalence and trends in the etiology of liver cirrhosis (LC), prognosis for patients suffering from cirrhosis-related complications and hepatocellular carcinoma (HCC), and management strategies. The etiology of cirrhosis varies according to geographical, economic, and population factors. Viral hepatitis is the dominant cause in China. Vaccination and effective treatment have reduced the number of people with viral hepatitis, but the overall number is still large. Patients with viral hepatitis who progress over time to LC and HCC remain an important population to manage. The increased incidence of metabolic syndrome and alcohol consumption is likely to lead to a potential exponential increase in metabolic dysfunction-associated steatotic liver disease (MASLD)-associated LC and alcoholic liver disease in the future. Investigating the evolution of the etiology of LC is important for guiding the direction of future research and policy development. These changing trends indicate a need for greater emphasis on tackling obesity and diabetes, and implementing more effective measures to regulate alcohol consumption in order to reduce the occurrence of MASLD. In an effort to help cope with these changing trends, the authors further proposed countermeasures for healthcare authorities doctors, and patients.

Key Words: Liver cirrhosis; Etiology; Viral hepatitis; Alcoholic liver disease; Hepatocellular carcinoma; Metabolic dysfunction-associated steatotic liver disease

Core Tip: China is aiming to eradicate viral hepatitis as a public health threat by 2030. It is expected that the prevalence of viral hepatitis will decrease in the coming years. The increasing prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) may emerge as a leading cause of liver cirrhosis. Additionally, excessive alcohol consumption is a significant risk factor. These shifting trends necessitate innovative management strategies. There is a need for sustained implementation of measures to eliminate viral hepatitis, as well as greater efforts to control obesity, diabetes and alcohol consumption to reduce the incidence of MASLD and Alcoholic liver disease.

Citation: Dai JJ, Liu YY, Zhang ZH. Changes in the etiology of liver cirrhosis and the corresponding management strategies. *World J Hepatol* 2024; 16(2): 146-151
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/146.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.146

INTRODUCTION

Liver cirrhosis (LC) is the final stage of progressive liver fibrosis attributed to various etiologies. The etiology of LC varies according to geographical region, economy, lifestyle, and population. Globally, hepatitis B virus (HBV) and hepatitis C virus (HCV) are the leading causes of LC and liver-related mortalities. With the development of control of HBV and cure of HCV, the prevalence of viral hepatitis has decreased. Unfortunately, LC morbidity and mortality are increasing rather than declining. The prevalence of metabolic risk factor-associated liver disease has increased substantially due to the rising prevalence of metabolic syndrome (Mets) and obesity, coupled with increased alcohol consumption and aging. It is expected that metabolic dysfunction-associated steatotic liver disease (MASLD) will soon become the leading cause of LC worldwide[1].

LC and its complications are a major public health challenge worldwide, with a significant economic and health burden. China is ''leader in liver diseases''[2]. In 2017, there were 10.6 million decompensated and 112 million compensated LC cases worldwide[3]. Approximately 2 million people worldwide die from liver disease each year[4]. According to the Global Burden of Disease project, LC caused about 1 million deaths in 2010 and 1.3 million in 2016[5]. Latin America and North Africa recorded the highest LC mortality rates, while the West Coast of the Pacific and Southeast Asia had the highest number of absolute deaths[6]. LC-related complications and hepatocellular carcinoma (HCC) are the main causes of death[7]. While HBV and HCV infections are significant acquired risk factors for HCC, excessive alcohol consumption and associated conditions such as Mets, type 2 diabetes mellitus, obesity, and MASLD have also emerged as important risk factors. Liver cancer morbidity and mortality are estimated to increase by more than 20% in the coming 50 years if the rate of incidence does not decrease by at least 3% per year[8]. In the recent issue of the *World Journal of Hepatology* 2023; 15: 1294-1306[9], that study addresses an important issue: The etiology of LC in China is also changing. Viral hepatitis cirrhosis is gradually decreasing, while non-viral hepatitis cirrhosis is gradually increasing, especially alcoholic liver disease (ALD) and MALSD.

The etiology of LC differs among regions and countries. From 1990 to 2016, China's provinces had very different epidemiological patterns[10]. China is a large and rapidly growing country with a vast population. Regional disparities in the burden of LC may be attributed not only to differences in economic development and healthcare, but also to variations in the distribution of risk factors. The burden of LC also changes over time, making it essential to conduct regionalization studies to analyze the evolution of disease burden and trends in different regions. Access to localized high-quality data is crucial for developing and refining cost-effective strategies for the prevention and treatment of LC, which is necessary to address the increasing burden of chronic liver disease. Wang found that HCC and acute-on-chronic liver failure (ACLF) were identified as the strongest risk factors for in-hospital mortality[9].

ETIOLOGIES DIFFERENCES AND TEMPORAL TRENDS IN BURDEN OF LIVER CIRRHOSIS

Viral hepatitis

Viral hepatitis continues to be the leading cause of LC. Recent studies have shown that approximately 56% of HCC cases are attributed to HBV, while 20% are due to HCV[11]. The lifetime risk of HCC for HBV carriers is estimated to be 10%-25%[12], with the incidence depending on active HBV infection and/or LC. In 2016, China had approximately 12 million LC patients, with 48.9% of cases caused by HBV infection[10]. China also has the largest population of HCV-infected individuals, estimated at 9.8 million[13]. On a global scale, it is projected that chronic HCV infection will not significantly change from 2020 to 2030, but long-term outcomes such as liver-related deaths, HCC, and decompensated LC are expected to increase by 14%-17% in adults[14].

China has implemented a comprehensive strategy to prevent HBV transmission, which includes immunization, interruption of mother-to-child transmission, safe injection practices, and ensuring the safety of blood donations. Hepatitis B vaccination is recognized as the most effective tool for preventing and eliminating HBV infection. China's Hepatitis B Virus prevention policy, launched in 1985, aims to increase neonatal immunization coverage. The use of the recombinant vaccine was approved nationwide in 1992, and the HBV-free vaccination program for children under 14

years was expanded in 2002[15]. Additionally, a catch-up HBV vaccination program for children aged 8-15 was implemented during 2009-2011[16]. The success of these programs in China has led to significant population-wide health benefits, with an estimated 120 million HBV infections and 28 million chronic infections averted.

The incidence of HCV has increased dramatically in China, almost ten folds from 2003 to 2017, due to improved testing technology and government focus[2]. Direct-acting antivirals (DAA) were approved in the United States in 2013, Europe in 2014, and in China in 2017, and have proven to be effective in treating HCV infection. However, of the 15.2 million people diagnosed with HCV worldwide from 2015-2019, only 9.4 million were receiving DAA medication[7]. It is important to establish and improve appropriate surveillance mechanisms to work towards eliminating HCV.

Non-viral hepatitis

43% of the world's population currently consumes alcohol, and the global prevalence of alcohol use disorders (AUD) is 5.1% (283 million individuals)[1]. Alcohol is the primary cause of LC worldwide, with nearly 60% of cirrhosis cases in Europe, North America, and Latin America attributable to alcohol[17]. AUD tends to be more common in high-income countries, while low-income countries are likely to underreport and underdiagnose. The highest prevalence of AUD is in European countries, but the absolute burden may be higher in Asia[1]. ALD has gradually become the second leading cause of advanced liver disease in the country due to increased alcohol consumption[13]. There is a clear tendency for the rate of ALD to increase among young people and women[7]. Most of the burden of ALD falls on the 15-44 age group, representing the young and vigorous years of life[18]. Women tolerate alcohol less well than men, tend to develop ALD after lower alcohol exposure, and are more likely to have progression of ALD[19]. The proportion of female drinkers is expected to rise as the proportion of working and single women in China continues to increase. Individuals with ALD are more likely to progress to cirrhosis than those with other causes of liver diseases, including non-alcoholic fatty liver disease (NAFLD). Obesity and Mets may also act synergistically to increase the severity of all stages of ALD. Alcohol abstinence reduces deaths[20]. Therefore, reducing alcohol consumption should be prioritized in public health efforts. The World Health Organization urges countries to develop preventive policies and actions to reduce alcohol consumption and harm. China has a long history of alcohol culture, and hazardous drinking behaviors are prevalent. Alcohol has become a major contributor to the overall burden of disease in China[21]. Despite the implementation of alcohol control strategies in China since 1990, including reforms to alcohol taxation policies, restrictions on alcohol advertising, bans on drink driving, alcohol restrictions for civil servants, and monitoring underage drinking, per capita alcohol consumption has increased dramatically over the past 30 years[22]. Based on available evidence, no level of alcohol consumption can be considered safe, and in order to minimize health effects, consumption should be zero. Challenges remain for China's alcohol control public health strategy.

NAFLD, now known as MASLD, affects a quarter of adults worldwide[23]. The prevalence of NAFLD is 24%-48% in North America[24], 23%-33% in Europe[24], and 28%-32.4% in Asia[25]. NAFLD is the second leading cause of liver transplants in the United States and Europe, and the primary cause of liver disease in females[26]. Approximately 20%-30% of individuals with NAFLD will develop non-alcoholic steatohepatitis (NASH), and 10%-20% of those with NASH will develop HCC[27]. However, NAFLD-associated cirrhosis is often under-recognized or referred to as 'cryptogenic cirrhosis'. Being overweight in late adolescence has been shown to be significantly associated with an increased risk of end-stage liver disease and liver-related mortality in adulthood[28]. Metabolic risk factors emerge as the greatest threat to the health of children and adolescents. As the population ages, MASLD-associated LC is expected to grow exponentially in the coming decades[29]. Despite MASLD being an urgent public health problem, no country has yet developed a national or local public health response[30].

The prevalence of autoimmune hepatitis (AIH)[31] and primary biliary cholangitis (PBC)[32] is increasing worldwide. Reports of PBC are increasing in eastern countries[33]. There is a high prevalence in females, while males appear to have a more aggressive disease and a poorer prognosis[34]. AIH is often detected in the later stages of the disease and is associated with higher mortality. Among individuals with AIH, those with LC were more likely to develop cancer, with a 29.18-fold increased risk of HCC, particularly with prolonged immunosuppressive treatment[35]. Therefore, early diagnosis and treatment could improve the outcome of AIH-related LC.

MORTALITY AND RISK FACTORS OF LIVER CIRRHOSIS

Compensated cirrhosis is typically asymptomatic and often overlooked, but once decompensation occurs, mortality and morbidity significantly increase. The incidence of decompensation is 11% per year, but varies based on the underlying etiology[36]. ACLF is linked to organ failure and high short-term mortality in LC[37]. In the US, hospitalizations and costs related to ACLF have increased over the last decade[38]. The lowest incidence of ACLF but highest short-term mortality is observed in patients with HCV or MASLD[39]. HBV reactivation is a major predisposing factor for ACLF in China[40].

According to China Cancer Registry data, China's crude liver cancer death rate was 23.7 per 1 million in 2015[41]. Between 2020 and 2040, the number of new cases of HCC is expected to increase by 55.0%, with 1.3 million people estimated to die from HCC in 2040[8]. Effective treatment of HBV and HCV has an impact on the incidence of viral hepatitis-associated HCC. The HBV vaccination program is a key strategy to prevent HCC. A study in Taiwan reports more than 80% reduction in HCC incidence in adults vaccinated in infancy compared with the unvaccinated[42]. The annual incidence of HCC in patients with HCV-associated LC is 0.5%-10%[43]. A 70% reduction in the incidence of HCC following a sustained virological response was observed in a prospective study of French patients with HCV cirrhosis. This study suggests that DAA will play an important role in significantly reducing HCC rates in the future[44]. Increasing evidence suggests that HCC risk is increased by excessive alcohol consumption, Mets, atherosclerotic dyslipidemia, and

consumption of aflatoxin-contaminated foods, all of which can be prevented[45]. The government can reduce the incidence of HCC by focusing on risk factor prevention and comprehensive HCC surveillance.

We generally agree with the views and conclusions presented in the text, which to some extent may also reflect the trend of etiological changes in hospitalized LC patients in southern China. Additionally, we find some interesting results in the text. Looking at the temporal trends in LC etiology, the overall incidence of hepatitis B-associated LC showed a decreasing trend but peaked significantly in 2011. The possible reason is that the author's hospital is one of the leading liver disease treatment centers in southern China, and over the years it has been actively developing new technologies and treatments for hepatitis B liver failure, attracting more hepatitis B patients to come to the clinic. This phenomenon peaked in 2011-2012. The total number of hepatitis B cases began to decline in 2013, in line with the overall downward trend. When considering the characteristics of the study population over 20 years, we found that the severity of the patients' conditions lessened, in addition to being associated with an increase in quality of care, was, in our opinion, due to the following reasons: (1) With economic development, improved health insurance policies, and more convenient transportation, the awareness and attention of patients to diseases has increased, leading to more hospital admissions for mild diseases; and (2) The authors' hospitals have evolved in their specialties, expanded their wards, and relaxed their indications for hospitalization, allowing them to provide medical care to a greater number of patients and have the capacity to admit and treat more patients with relatively minor illnesses.

CLINICAL IMPLICATIONS

LC and its complications continue to pose a significant public health burden, despite some improvements in HBV and HCV. The impact of targeting the elimination of viral hepatitis is just emerging, but an increase in other risk factors may add to the overall burden of LC. National health planning should be adapted to take these changes into account, including sustainable implementation of programs to eliminate viral hepatitis, expanding screening and treatment options for HBV/HCV, primary prevention of diabetes and obesity, as well as stronger measures for controlling alcohol. It is recommended that patients with chronic liver disease should have serum aminotransferase and alpha-fetoprotein tests, liver ultrasound and elasticity tests every six months, and those with LC or HCC every three months.

Investment in the prevention, detection, and treatment of liver disease has the potential to decrease the number of deaths caused by associated liver disease, lower the incidence of complications from advanced liver disease, and reduce the associated management costs. Tracking trends of LC is essential to identify effective strategies appropriate to the local disease burden and to implement cost-effective interventions.

CONCLUSION

We are at a crucial turning point in the recognition, prevention, and treatment of liver disease. Management strategies are needed not only at the national level, but also localized policies for various regions. Early detection and treatment of cirrhosis, with a focus on ALD and NASH, and continued implementation of strategies to eliminate viral hepatitis, must be given particular attention. Establishing well-functioning and comprehensive national health systems to achieve universal coverage is crucial. For hepatologists, it is critical to increase screening of high-risk groups, identify and eliminate disease-causing factors early, and improve monitoring and follow-up with LC and HCC. For patients, maintaining good lifestyle habits, making behavioral changes, and taking necessary precautions can reduce the risks.

ACKNOWLEDGEMENTS

We would like to express special thanks to Jun Zhang for their assistance with language polishing.

FOOTNOTES

Author contributions: Dai JJ and Liu YY contributed to this paper; Zhang ZH designed the overall concept and outline of the manuscript; Liu YY contributed to the discussion and design of the manuscript; Dai JJ contributed to the writing, and editing the manuscript, illustrations, and review of literature.

Supported by Anhui Provincial Natural Science Foundation, No. 2108085MH298; University Scientific Research Project of Anhui Provincial Education Department, No. KJ2021A0323; Fund of Anhui Medical University, No. 2021xkj196; and Clinical Medicine Project of Anhui Medical University, No. 2021LCXK027.

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

Country/Territory of origin: China

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S-Editor: Gong ZM
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 152-163

DOI: 10.4254/wjh.v16.i2.152

ISSN 1948-5182 (online)

REVIEW

Metabolic-associated fatty liver disease and sarcopenia: A double whammy

Aditya Viswanath, Sherouk Fouda, Cornelius James Fernandez, Joseph M Pappachan

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Cossiga V, Italy

Received: December 6, 2023
Peer-review started: December 6, 2023
First decision: December 18, 2023
Revised: December 26, 2023
Accepted: January 17, 2024
Article in press: January 17, 2024
Published online: February 27, 2024

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Abstract

The prevalence of metabolic-associated fatty liver disease (MAFLD) has increased substantially in recent years because of the global obesity pandemic. MAFLD, now recognized as the number one cause of chronic liver disease in the world, not only increases liver-related morbidity and mortality among sufferers but also worsens the complications associated with other comorbid conditions such as cardiovascular disease, type 2 diabetes mellitus, obstructive sleep apnoea, lipid disorders and sarcopenia. Understanding the interplay between MAFLD and these comorbidities is important to design optimal therapeutic strategies. Sarcopenia can be either part of the disease process that results in MAFLD (*e.g.*, obesity or adiposity) or a consequence of MAFLD, especially in the advanced stages such as fibrosis and cirrhosis. Sarcopenia can also worsen MAFLD by reducing exercise capacity and by the production of various muscle-related chemical factors. Therefore, it is crucial to thoroughly understand how we deal with these diseases, especially when they coexist. We explore the pathobiological interlinks between MAFLD and sarcopenia in this comprehensive clinical update

review article and propose evidence-based therapeutic strategies to enhance patient care.

Key Words: Metabolic-associated fatty liver disease; Sarcopenia; Sarcopenic obesity; Lean metabolic-associated fatty liver disease; Cardiovascular disease; Liver-muscle axis

Core Tip: Metabolic-associated fatty liver disease (MAFLD) is associated with sarcopenia in a significant proportion of individuals. Sarcopenia can be a consequence of the comorbidities associated with MAFLD (such as obesity or adiposity) or a direct result of advanced stages of MAFLD, such as fibrosis and cirrhosis. On the other hand, sarcopenia can worsen MAFLD due to reduced exercise capacity and the release of various myokines. Understanding the strong interlink between MAFLD and sarcopenia is important to plan appropriate therapeutic strategies. We discuss the pathobiological aspects of this interlink and the potential clinical and metabolic complications of the coexistence of MAFLD and sarcopenia in this comprehensive clinical update review.

Citation: Viswanath A, Fouda S, Fernandez CJ, Pappachan JM. Metabolic-associated fatty liver disease and sarcopenia: A double whammy. *World J Hepatol* 2024; 16(2): 152-163
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/152.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.152

INTRODUCTION

Metabolic-associated fatty liver disease (MAFLD) and sarcopenia are two chronic health conditions with profound adverse implications in modern society. MAFLD encompasses a spectrum of liver conditions, ranging from simple steatosis (fatty liver) to non-alcoholic steatohepatitis (NASH) which involves inflammation and liver cell damage, advanced liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). On the other hand, sarcopenia represents the progressive loss of muscle mass, strength, and function associated with aging, sedentarism, obesity, and conditions that cause reduced mobility. These two conditions have gained considerable attention due to their parallel rise in prevalence and potential interconnections. MAFLD is currently the most common liver disorder worldwide, affecting nearly one-third of the global population[1]. The definition and diagnostic criteria for sarcopenia have evolved in recent years. The European Working Group on Sarcopenia in Older People and the Asian Working Group for Sarcopenia have proposed consensus definitions that consider muscle mass, muscle strength, and physical performance measures[2,3]. Recent studies have highlighted the association between sarcopenia and various chronic diseases, including cardiovascular disease, diabetes mellitus, and liver disease, emphasizing the importance of early detection and intervention[4,5]. MAFLD and sarcopenia are two interrelated conditions that pose significant challenges in clinical practice.

The intricate link between MAFLD and sarcopenia involves shared mechanisms, such as chronic low-grade inflammation, oxidative stress, insulin resistance (IR), and alterations in adipokines and myokines[6,7]. MAFLD contributes to sarcopenia through negative impacts on muscle protein synthesis and metabolism, leading to wasting. Conversely, sarcopenia exacerbates MAFLD by influencing IR, dyslipidaemias, and systemic inflammation, and promoting liver fat accumulation through physical inactivity, weight gain, and central obesity[8,9]. The cumulative effect creates a cycle that worsens health outcomes and highlights the complex interplay between metabolic, inflammatory, and hormonal factors.

The clinical association between MAFLD and sarcopenia has important implications for patient management and outcomes. Understanding this association is crucial for identifying patients at high risk and implementing appropriate interventions to mitigate the disease progression. Recognizing and addressing these conditions early in clinical practice can help to improve patient prognosis and overall well-being. This review aims to explore the relationship between MAFLD and sarcopenia, shedding light on underlying mechanisms, common risk factors, potential consequences, and possible interventions.

THE BIDIRECTIONAL RELATIONSHIP BETWEEN MAFLD AND SARCOPENIA

Central to understanding mechanisms between MAFLD and sarcopenia is the concept of myosteatosis, which represents the infiltration of fat into skeletal muscle, contributing to functional decline as well as the loss of skeletal muscle mass seen in sarcopenia. Myosteatosis is seen in both NASH as well as MAFLD. It may be a superior indicator and predictor of hepatocellular deterioration compared to sarcopenia, especially in the early stages of NASH, since it precedes the onset of sarcopenia[10,11]. Additionally, myosteatosis has a strong association with liver stiffness in obese patients with MAFLD [12], highlighting its role in the dynamic relationship between sarcopenia and MAFLD.

Numerous shared risk factors augment the bidirectional relationship between MAFLD and sarcopenia, acting as a precipitator of metabolic dysregulation. Factors include the male gender, physical inactivity, metabolic syndrome, older

age, and raised total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol. Additionally, factors such as alcohol consumption, diabetes mellitus leading to elevated glycated haemoglobin (HbA1c) levels, and other co-morbidities contribute to the bidirectional risk[13-16]. Table 1 and Table 2 provide an overview of studies illustrating both sarcopenia as a risk factor for MAFLD and vice versa.

PATHOGENIC PATHWAYS IN THE LIVER-MUSCLE AXIS

The intricate relationship between MAFLD and sarcopenia is characterized by shared pathogenic mechanisms, complicating efforts to identify a primary instigator. Central to this is IR, a pivotal factor in influencing both metabolic and growth processes. Physiologically, insulin plays a key role in lowering blood glucose and fatty acid levels through the suppression of adipose tissue lipolysis and hepatic glucose output[29]. It also increases glucose uptake into adipose and muscle tissue through phosphorylation of insulin receptor substrate and subsequent exocytosis *via* glucose transporter type 4[30]. Additionally, insulin plays a role in muscle protein synthesis, mediated *via* key signalling pathways such as Akt/protein kinase B and mammalian target of rapamycin[31]. IR in adipose and muscle tissue therefore contributes significantly to the clinical progression of both MAFLD and sarcopenia. IR has strong associations with obesity and metabolic syndrome as well as its association with low muscle mass due to age-related degeneration, as seen in sarcopenia. Adipose tissue IR leads to heightened release of free fatty acids (FFAs), prominently observed in MAFLD patients[32], contributing to liver triglyceride accumulation, and fostering a pro-inflammatory environment[33,34].

MAFLD is associated with low levels of growth hormone (GH) and insulin-like growth factor (IGF-1), contributing to increased hepatic IR and body adiposity. GH is crucial in increasing beta-oxidation of FFAs, potentially ameliorating hepatic lipid content, whilst IGF-1 exerts anti-inflammatory and anti-fibrotic effects in the liver[35]. Hormonal changes, particularly in post-menopausal women and individuals with altered testosterone levels, further complicate this relationship. The prevalence of sarcopenia is high amongst post-menopausal women[36], with menopausal hormonal therapy demonstrating a possible protective effect[37]. Testosterone is essential for muscle regeneration in males, and its deficiency contributes to reduced lean muscle mass in sarcopenia. Additionally, this deficiency may coincide with obesity and culminate in a pro-inflammatory state through the release of mediators such as IL-6 and TNF-α[38-40], thereby worsening sarcopenia and steatohepatitis.

In MAFLD, inflammation is further intensified by lipotoxicity, leading to increased reactive oxygen species and oxidative stress. This cascade results in intrahepatic cellular damage and higher circulating levels of FFAs within the cytosol[41,42]. Myokines, signalling molecules produced by skeletal muscle, play a role in energy metabolism. Sarcopenic MAFLD individuals show reduced levels of myokines, attributed to muscle loss and lack of physical activity[43]. Irisin, an exercise-induced myokine, demonstrates a protective effect against fatty liver[44] and shows a positive relationship with fibroblast growth factor 21 in animal studies, suggesting its potential therapeutic effects of reversing hepatic steatosis[45,46] (Figure 1).

CLINICAL EVIDENCE LINKING MAFLD AND SARCOPENIA

The relationship between fatty liver disease (formerly non-alcoholic fatty liver disease (NAFLD), now MAFLD) and sarcopenic muscle degeneration has been extensively explored in numerous studies, though variations in selection criteria exist due to the recent redefinition of the disease. The shift to MAFLD has broadened the identification of individuals with liver disease.

Between 2018-19, four meta-analyses investigated the link between NAFLD and sarcopenia, with one study exploring the progression of fatty liver disease. One meta-analysis reported a significantly increased risk of NAFLD in patients with sarcopenia (pooled odds ratio of 1.54), emphasizing a substantial association despite there being statistical heterogeneity [47]. Another meta-analysis confirmed this increased risk of both NAFLD and a heightened risk of significant fibrosis [48]. In concordance with these results, a strong association was found between sarcopenia and advanced liver disease, with an odds ratio of 2.41[49]. Cai *et al*'s meta-analysis involving 19 studies, further reported higher risks of NAFLD, NASH, and significant fibrosis in individuals with reduced skeletal mass[50].

Diagnostic variation between studies is evident as there are no standardized diagnostic criteria to measure skeletal muscle mass and fat in the liver. Diagnostic computed tomography (CT)/magnetic resonance imaging (MRI) are superior modalities for skeletal mass measurement however, they pose a challenge in larger study settings. Dual-energy X-ray absorptiometry (DXA) is a preferred tool for its ease of use, but there are limitations in estimating lean mass and quantifying intramuscular adiposity, especially in cases of myosteatosis[51].

Recent studies, focusing on MAFLD as opposed to NAFLD, provide a pragmatic clinical evaluation accounting for metabolically deranged individuals. A large study involving 8371 patients revealed increased risks of significant liver fibrosis and atherosclerotic cardiovascular disease in those with both MALFD and sarcopenia. Sarcopenic individuals with MAFLD exhibited higher odds ratios for significant fibrosis (Fibrosis 4 Index: odds ratio - 4.51, NAFLD fibrosis score: odds ratio - 5.72) and cardiovascular disease (odds ratio 4.08) compared to non-sarcopenic counterparts[52]. Another investigation involving 6424 subjects, using Fibro scan and bioimpedance analysis (BIA), found an association between MAFLD and increased risk of low muscle mass adjusted for weight and BMI, with the diabetic MAFLD subgroup showing the highest risk[53]. Notably a reverse relationship was found between appendicular skeletal muscle mass and the risk of MAFLD across both sexes, with appendicular skeletal mass of the highest quartile being associated with the least risk of MAFLD[54].

Table 1 Sarcopenia as a risk factor for metabolic-associated fatty liver disease

Ref.	Study design	Study population	Size	Sarcopenic assessment	MAFLD assessment	Conclusions
Seo *et al*[17], 2022	Longitudinal	Korean	115568	BIA	Non-invasive models	Increases in relative skeletal muscle mass over time may lead to benefits in prevention of development of NAFLD or the resolution of existing NAFLD
Zhai *et al*[18], 2018	Cross-sectional	Chinese	494	DXA	US	NAFLD is not independently associated with sarcopenia
Wijarnpreecha *et al*[19], 2019	Cross-sectional	American	11325	BIA	US	Sarcopenia was independently associated with increased odds of NAFLD and NAFLD-associated advanced fibrosis independent of well-defined risk factors
Hsieh *et al*[20], 2021	Cross-sectional	Korean	521	CT	Liver biopsy	Patients with significant fibrosis had lower Skeletal muscle index and muscle attenuation than those without
Zhao *et al*[21], 2023	Cross-sectional	American	2065	DXA	LUTE	Higher appendicular skeletal muscle mass was associated with a lower risk of MAFLD, while the risk of significant fibrosis in females was increased with the trunk skeletal muscle mass
Hsieh *et al*[22], 2023	Longitudinal	Korean	338	CT	Liver biopsy	Severe myosteatosis is significantly associated with early NASH and fibrosis progression in early-stage MAFLD
Tanaka *et al*[23], 2020	Cross-sectional	Japanese	632	CT	Non-invasive models	Both skeletal muscle index and skeletal muscle density are independently associated with the prevalence of MAFLD
Choe *et al*[24], 2023	Cohort	Korean	4038	BIA	Non-invasive models	Both lower muscle mass index and genetic risk variants are important contributors to the development of MAFLD

BIA: Bioimpedance analysis; DXA: Dual Xray absorptiometry; LUTE: Liver ultrasound transient elastography; MAFLD: Metabolic-associated fatty liver disease; NAFLD: Non-alcoholic fatty liver disease; US: Ultrasonography.

Table 2 Metabolic-associated fatty liver disease as a risk factor for sarcopenia[28]

Ref.	Study design	Study population	Size	Sarcopenic assessment	MAFLD assessment	Conclusions
Roh *et al*[25], 2022	Longitudinal	Korean	1595	DXA	Non-invasive models	The presence of NAFLD may predict future risk of low muscle mass and low muscle strength, with a greater impact on LMS than on LMM
Sinn *et al*[26], 2022	Cross-sectional	Korean	52815	BIA	US	Participants with NAFLD were at increased risk of sarcopenia, indicated by faster loss of skeletal muscle mass
Altajar *et al*[27], 2023	Cross-sectional	Korean	6414	BIA	CAP	The presence of MAFLD is significantly associated with an increased risk of low muscle mass with varying risks according to the MAFLD subgroups

BIA: Bioimpedance analysis; DXA: Dual Xray absorptiometry; LUTE: Liver ultrasound transient elastography; MAFLD: Metabolic-associated fatty liver disease; NAFLD: Non-alcoholic fatty liver disease; US: Ultrasonography; CAP: Controlled attenuation parameter; LMM: Low muscle mass; LMS: Low muscle strength.

LEAN MAFLD AND SARCOPENIA

Lean MAFLD poses a challenge to clinicians as fatty liver disease presents in individuals with a lower BMI and adipose tissue and contradicts the criteria of MAFLD, which is usually associated with metabolic syndrome and obesity[55]. A United States-based population study found the prevalence of NAFLD to be 4 per 100000 and lean NAFLD to be 0.6 per 100000, where patients with lean NAFLD tended to be older, females, smokers, and of Asian race[56]. Global estimation of lean NAFLD was around 4.1%, with Asian populations having the highest prevalence (4.8%)[57]. Ha *et al*'s investigation revealed that lean MAFLD individuals faced a relative risk of 1.12 for cardiovascular mortality and 1.88 for liver-related mortality compared to non-lean individuals[58]. However, varied findings from a Chinese cohort study indicate that, while obese NAFLD individuals have a higher cardiovascular disease risk, lean NAFLD individuals still face elevated risks of all-cause death, digestive system cancers, and obesity-related cancers[59].

Despite having milder features of metabolic syndrome, lean individuals have been shown to have a higher prevalence of metabolic abnormalities such as dyslipidaemia, hypertension, IR, and diabetes mellitus[60]. Sarcopenic patients have

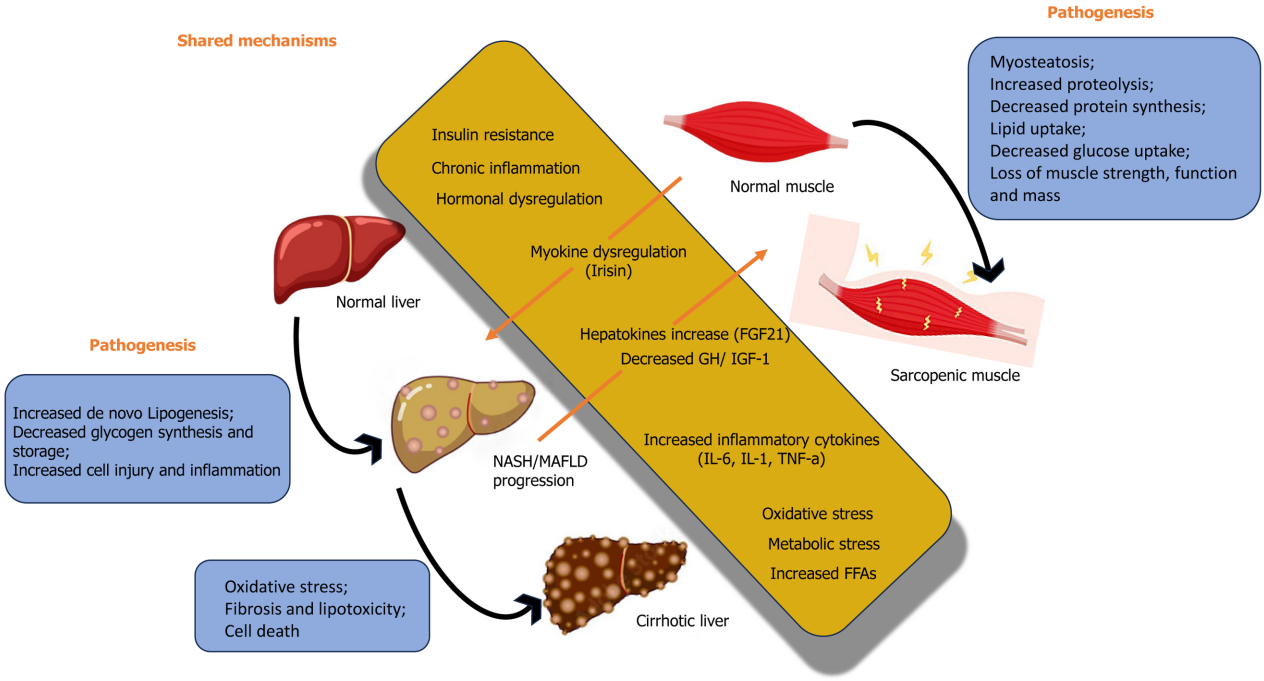

Figure 1 The pathways contributing to the progression of both liver and muscle dysfunction in metabolic-associated fatty liver disease and sarcopenia. MAFLD: Metabolic-associated fatty liver disease; FFAs: Free fatty acids; IL: Interleukin; TNF-α: Tumor necrosis factor alpha; NASH: Non-alcoholic steatohepatitis; FGF21: Fibroblast growth factor 21; GH: Growth hormone; IGF-1: Insulin-like growth factor 1.

shown upregulated serum levels of N-acetylneuraminyl-glycoproteins, lactic acid, LDL triglycerides, and VLDL5 Levels, along with reduced HDL4 levels[61]. A study by Nabi *et al*[62] confirmed patients with lean NAFLD were associated with advanced liver fibrosis (odds ratio = 1.26) compared to non-lean individuals, highlighting the necessity to re-evaluate assumptions about the health of lean individuals. The optimization in skeletal muscle mass, as opposed to the sole reduction in visceral adiposity, has been suggested for optimal management of lean MAFLD[63].

SARCOPENIC-OBESITY AND MAFLD

Sarcopenic obesity, being the co-existence of loss of muscle mass alongside increased body adiposity/fat mass, is a prevalent condition affecting 11% of older adults globally[64]. In males, identified risk factors include increased body mass index (BMI), waist circumference, and triglyceride levels, along with a decreased skeletal muscle mass index. For females, height, weight, BMI, waist circumference, and systolic blood pressure, as well as smoking status and fasting glucose are identified as significant risk factors, according to a large nationwide Korean study[65].

The pathophysiology of sarcopenic obesity can be attributed to a multitude of mechanisms such as aging, IR, low-grade inflammation, and hormonal changes[66]. Aging induces a progressive loss in muscle mass and an increase in adiposity due to a decline in basal metabolic rate, inactivity, and hormonal fluctuation. The increased visceral fat and net loss of muscle mass, coupled with aging can encourage a reduction in GH production[67], negatively impact protein synthesis, and exacerbate sarcopenia.

Diagnosis, as detailed by the European Society for Clinical Nutrition and Metabolism[68], involves screening for high BMI and waist circumference, using surrogate indicators for sarcopenia with diagnostic cut-offs based on ethnicity, age, and gender. Functional assessments, including muscle strength evaluation and body composition analysis based on DXA, BIA, and CT, are used to finalize the diagnosis. Advanced liver disease warrants the use of CT/MRI imaging for accuracy, due to its ability to effectively eliminate the confounding effects of fluid retention, particularly ascites, and oedema, associated with portal hypertension[69].

Sarcopenic obesity has been shown to increase the risk of all-cause mortality, and fragility fractures in elderly patients with type 2 diabetes mellitus[70,71]. Low muscle-to-fat ratio in older adults has been linked to impaired health outcomes and increased cardiometabolic and cardiovascular risk[72,73]. Sarcopenic obesity is also associated with a significant increase in the risk of coronary artery calcification[74]. In children, sarcopenic obesity is linked to the development of metabolic syndrome and worsened outcomes in type 2 diabetes mellitus[75].

Moreover, sarcopenic obesity also increases the risk of MAFLD development and fibrosis progression[76]. Increased visceral adiposity and IR, resulting in hepatic fat accumulation[77], contribute to worsened hepatic fibrosis, as demonstrated by Kim *et al*[78]. Understanding the relationship between sarcopenic obesity and these health outcomes is crucial for holistic patient care.

CLINICAL COMPLICATIONS AND THEIR SIGNIFICANCE

In MAFLD, the presence of sarcopenia worsens disease progression and outcomes. Reduced muscle mass leads to physical inactivity, which can exacerbate metabolic dysfunction, IR, and the accumulation of visceral adiposity[79]. Inflammation in the liver is further aggravated by the sarcopenia-associated cytokine and adipokine profiles. Conversely, MAFLD can contribute to the development of sarcopenia through chronic inflammation, leading to muscle breakdown.

IR, resulting from MAFLD, impairs glucose uptake into the muscle, and metabolic alterations in MAFLD can lead to increased oxidant stress in muscle tissue. IR, a common feature in MAFLD, sarcopenia, and diabetes mellitus, worsens glycemic control, inflammation, and metabolic dysfunction, thereby escalating the risk of cardiovascular disease. Notably, the presence of sarcopenia in diabetes mellitus increases the risk of all-cause mortality and cardiovascular mortality[80]. Studies, such as the post-hoc analysis of the ATTICA study, detailed that a lower skeletal muscle perc-entage is associated with an increased risk of MAFLD, with a substantial rise in cardiovascular risk when sarcopenia exists, irrespective of waist circumference[81,82]. These disease outcomes were also reaffirmed in a study of 11,065 Sarcopenic MAFLD patients, who faced a 28% higher likelihood of all-cause mortality, all with elevated risk of cancer and diabetes-related mortality[83].

A further study of 852 diabetic participants demonstrated a higher risk of carotid atherosclerotic progression over a 6-8-yr span in those with both sarcopenia and MAFLD (odds ratio 2.2)[84]. Albuminuria, a marker of renal dysfunction and cardiovascular risk, was also shown to have significantly higher rates in patients with both conditions[85].

Moreover, the progression of fibrosis in the presence of sarcopenia has been extensively studied. In a recent study of 2422, sarcopenic NAFLD demonstrated higher liver fibrosis rates than NAFLD alone. Rates of significant fibrosis were elevated (18.3% *vs* 3.2%) and a similar marked increase was seen in advanced fibrosis[86]. The interplay between the two conditions has been shown to worsen surgical outcomes and long-term post-operative survival of HCC patients with MAFLD[87]. Sarcopenia was identified as an independent risk factor for both recurrence-free survival and overall survival in sarcopenic MAFLD patients with HCC. Additionally, sarcopenia in MAFLD is associated with higher rates of depression and fatigue, along with a reduced quality of life[88].

The synergy of both conditions leads to increased metabolic dysregulation, contributing to a more adverse clinical course of MAFLD, emphasizing the need for a comprehensive assessment to address the multiple implications of having both sarcopenia and MAFLD. Figure 2 illustrates the clinical implications of sarcopenia and MAFLD, as discussed prior.

CLINICAL MANAGEMENT OF MAFLD WITH SARCOPENIA

A multifaceted approach must be used to address the combination of MAFLD and sarcopenia. Conservative management of MAFLD involves gradual weight loss and regular physical activity, both of which improve hepatic steatosis and quality of life[89,90], as well as improving liver stiffness[91]. Aerobic and resistance training, especially moderate resistance training, was found to be beneficial in ameliorating IR[92]. Bariatric surgery may be offered to individuals with BMI > 40 kg/m^2 and obesity-related comorbidities, with the exclusion of those with decompensated cirrhosis and concomitant portal hypertension[93].

When managing sarcopenia, a meta-analysis highlights the effectiveness of nutritional supplementation and physical activity for outcomes such as muscle mass, strength, and physical performance[94]. Strength training induces muscle hypertrophy and mitigates the decline in lean muscle tissue[95]. Although physical activity has been shown protective effects against both conditions[96], its utility is limited due to the heightened frailty seen in the advanced stages of these diseases[97]. Evaluation of sarcopenia through imaging and muscle strength assessment is crucial, before commencing treatment. Whilst protein supplementation alone may not be useful for sarcopenia[98], branched-chain amino acids show promise, especially in sarcopenic patients with cirrhosis[99].

The implication of diet in sarcopenia and MAFLD remains an area for exploration, although it is a smaller prognostic factor in patients with sarcopenic MAFLD compared to physical activity[100]. Pharmacological treatment often targets pre-existing co-morbidities such as cardiovascular disease, diabetes mellitus, and lipid abnormality, focusing on regulating the patients' metabolic status. For example, Vitamin E supplementation demonstrates significant improvements in alanine aminotransferase/aspartate aminotransferase, fibrosis, and steatosis for patients with MAFLD, with a notable reduction in fibrosis score[101]. A meta-analysis has shown the use of GLP-1 analogue leads to notable enhancements in liver enzymes, liver fat content, HbA1c, and weight in individuals with both type 2 diabetes mellitus and MAFLD[102]. Whilst thiazolidinediones have shown efficacy in MAFLD treatment, GLP-1 analogues appear to be superior[103]. SGLT2 inhibitors also demonstrate similar efficacy to thiazolidines, with the added benefit of weight reduction[104]. Statins have anti-inflammatory properties, and their use has further been associated with a lower prevalence of NASH and fibrosis, highlighting their protective role against the progression of MAFLD[105]. As seen, a comprehensive approach to managing MAFLD and sarcopenia involves lifestyle interventions, targeted pharmacotherapy, and ongoing research for optimal care.

AREAS OF UNCERTAINTY/EMERGING RESEARCH QUESTIONS

Emerging pharmacological interventions aim to target inflammatory and fibrotic pathways in fatty liver disease. A review by Rojas *et al*[106] outlines therapies in phase II/III clinical trials, which focus on reducing fatty acid accumulation and regressing fibrosis.

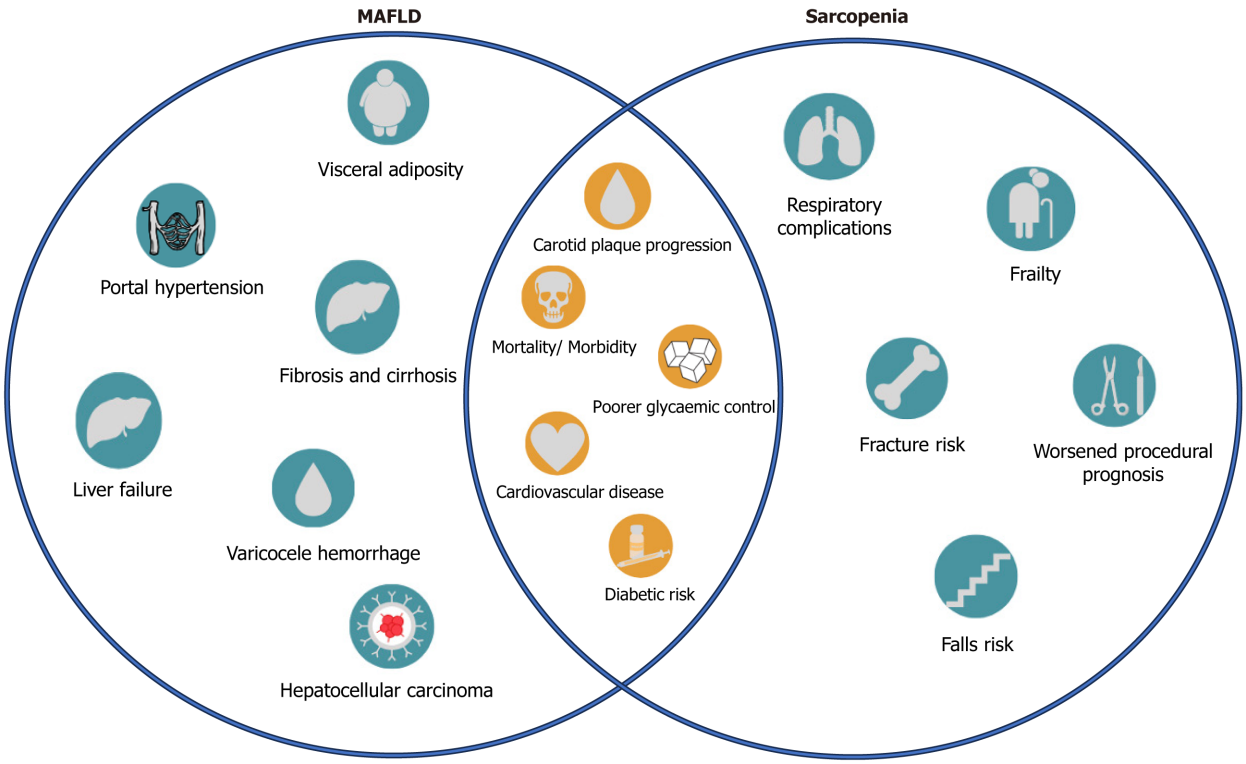

Figure 2 Clinical complications associated with metabolic-associated fatty liver disease and sarcopenia. MAFLD: Metabolic-associated fatty liver disease.

Novel mechanisms have been researched to explain the pathogenesis of both conditions, as well as to unravel their bidirectional nature. A recent study was done on the involvement of the phosphoenolpyruvate carboxykinase 1 (PCK1) enzyme in MAFLD and NASH progression[107]. This study aimed to investigate the role of PCK1, a gluconeogenic enzyme, in the promotion of MAFLD through the study of rodents. It was found that PCK1 was downregulated in NASH patients and rodents with MAFLD. A further study by Xu *et al*[108] investigated transcription pattern mapping to identify the core genes and possible therapeutic targets that regulate MAFLD and sarcopenia, revealing 8 shared genes with common pathways.

Another area of interest is the impact of aging and associated low-grade inflammation on metabolic-associated diseases. As opposed to metaflammation, which is the inflammation present under overnutrition and metabolic disease, Inflammaging is a relatively new term that describes a low-grade inflammation that arises through aging[109]. Its mechanism is not fully understood; however, it is a common factor in the development of both sarcopenia and MAFLD[110]. Future research may be directed at not only understanding these mechanisms but also developing targeted therapeutics to reverse such pathological outcomes.

CONCLUSION

Understanding the clinical association between MAFLD and sarcopenia is crucial for a comprehensive approach to patient care. By recognizing the bidirectional relationship, shared risk factors, and impact on various outcomes, healthcare providers can implement targeted interventions, promote early detection, and optimize treatment strategies for individuals affected by these conditions. Further research is needed to unravel the complex mechanisms, explore targeted interventions, and develop personalized treatment strategies for individuals with this complex clinical association.

FOOTNOTES

Author contributions: Viswanath A performed initial literature search, interpretation of relevant literature, article drafting, revision and figure preparation and is the first author of the work; Fouda S substantially contributed to the conception of the work with additional literature review and revision of the article critically for important intellectual content; Fernandez CJ and Pappachan JM contributed to the conceptual design of the paper and critically supervised the whole drafting, revision and modifications of the paper including figure construction and share final authorship; all authors have read and approved the final version of the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Gao CC
L-Editor: A
P-Editor: Zhang YL

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 164-176

DOI: 10.4254/wjh.v16.i2.164

ISSN 1948-5182 (online)

REVIEW

Precision targeting in hepatocellular carcinoma: Exploring ligand-receptor mediated nanotherapy

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Palomino ZJ, Brazil

Received: December 20, 2023
Peer-review started: December 20, 2023
First decision: January 5, 2024
Revised: January 10, 2024
Accepted: January 18, 2024
Article in press: January 18, 2024
Published online: February 27, 2024

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and poses a major challenge to global health due to its high morbidity and mortality. Conventional chemotherapy is usually targeted to patients with intermediate to advanced stages, but it is often ineffective and suffers from problems such as multidrug resistance, rapid drug clearance, nonspecific targeting, high side effects, and low drug accumulation in tumor cells. In response to these limitations, recent advances in nanoparticle-mediated targeted drug delivery technologies have emerged as breakthrough approaches for the treatment of HCC. This review focuses on recent advances in nanoparticle-based targeted drug delivery systems, with special attention to various receptors overexpressed on HCC cells. These receptors are key to enhancing the specificity and efficacy of nanoparticle delivery and represent a new paradigm for actively targeting and combating HCC. We comprehensively summarize the current understanding of these receptors, their role in nanoparticle targeting, and the impact of such targeted therapies on HCC. By gaining a deeper understanding of the receptor-mediated mechanisms of these innovative therapies, more effective and precise treatment of HCC can be achieved.

Key Words: Targeting; Hepatocellular carcinoma; Receptor; Nanomedicine; Chemotherapy

Core Tip: This review explores the innovative field of nanoparticle-mediated targeted drug delivery in hepatocellular carcinoma (HCC), focusing on the critical role of various overexpressed cellular receptors in improving the therapeutic specificity and efficacy of nanomedicines. It comprehensively analyzes recent advances in the development of receptor-targeted nanoparticles, revealing the complex mechanisms behind receptor-mediated drug delivery at the nanoscale. This exploration not only emphasizes the potential of nano-therapies to transform the treatment of HCC, but also provides valuable insights for future research and clinical applications.

Citation: Zhou XQ, Li YP, Dang SS. Precision targeting in hepatocellular carcinoma: Exploring ligand-receptor mediated nanotherapy. *World J Hepatol* 2024; 16(2): 164-176
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/164.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.164

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer worldwide and the second leading cause of cancer-related deaths, claiming approximately 700000 lives each year[1]. The 5-year survival rate of HCC in the North American area is 15%-19%, while in China it is only around 12.1%. This high mortality rate is partly due to the aggressive nature of the disease and the fact that most patients are diagnosed at a late stage[2,3]. These bring heavy mental pressure and economic burden to the patient's family and society. The incidence of HCC is closely related to chronic viral hepatitis, of which hepatitis B virus (HBV) and hepatitis C virus (HCV) are the main causative factors[4,5]. Globally, HBV accounts for 54.4% of liver cancer cases and up to 50% of HCC occurrences. This high rate is attributed to the virus's ability to integrate into the host DNA, causing direct genetic alterations[6]. In contrast, HCV-induced HCC usually results from a different mechanism, primarily through chronic inflammation, cirrhosis, and subsequent cellular changes leading to malignancy [7]. Patients with HCV-associated cirrhosis are at higher risk of developing HCC compared to HBV[8]. Other risk factors for HCC include chronic alcohol abuse, non-alcoholic fatty liver disease (NAFLD), and exposure to aflatoxins[9,10]. Aflatoxin B1, produced by Aspergillus fungi and present in contaminated staple foods, is particularly prevalent in certain regions of Africa and Asia, significantly increasing the incidence of HCC in these areas[11]. In addition, the rising incidence of obesity and type 2 diabetes has led to an increase in the number of NAFLD-associated HCC cases[12]. As a result, innovative therapeutic approaches are urgently needed for the treatment of HCC.

Currently, the therapeutic strategies for HCC are diverse, including options such as liver transplantation, surgical resection, embolization, stereotactic body radiation therapy, ablative procedures, and systemic therapy[13-17]. Treatment of HCC is highly dependent on the stage of the disease at diagnosis. Liver transplantation offers the best long-term survival rate for patients with early-stage HCC, with a 5-year survival rate exceeding 70% for suitable patients[18]. Surgical resection is another treatment option, and patients with early-stage HCC without cirrhosis have a 5-year survival rate of 50%-70%[19]. However, only about 15%-20% of HCC patients are candidates for liver transplantation or surgical resection at the time of diagnosis. Transarterial chemoembolization (TACE) is a widely used treatment for patients with intermediate (stage B) HCC. This approach takes advantage of the unique feature that HCC tumors predominantly receive their blood supply from the hepatic artery[20]. By delivering chemotherapeutic agents such as doxorubicin (DOX), or mitomycin C directly to the tumor through the hepatic artery, TACE concentrates the drugs on the tumor while minimizing the impact on surrounding healthy liver tissue[21]. Approximately only 10%-15% HCC patients are candidates for TACE, making it an important option for patients with unresectable mid-stage HCC. However, the onset of HCC is insidious and the disease progresses slowly, and most patients are often diagnosed in the late stages, when treatment becomes more challenging and the efficacy of existing therapies is greatly reduced. In the advanced stages of HCC, systemic therapy becomes the primary treatment modality. This includes molecular targeted therapy that specifically targets the molecular pathways which contribute to HCC growth, and immunotherapy that stimulates the body's immune system to attack cancer cells[22]. Systemic therapy is preferred for advanced HCC because it is relatively less painful and more cost-effective than other advanced treatments.

In recent years, systemic therapy for HCC has undergone significant evolution with the development of several chemotherapeutic agents. Sorafenib, approved by the United States Food and Drug Administration (FDA) in 2007 as the first systemic treatment for advanced HCC, marked a critical milestone in this journey. As a multikinase inhibitor, Sorafenib disrupts angiogenesis by inhibiting vascular endothelial growth factor receptors (VEGFR) and platelet-derived growth factor receptors (PDGFR) and prevents tumor from obtaining the nutrients and oxygen they need to grow[23]. Additionally, Sorafenib blocks the Raf-MEK-ERK signaling pathway by targeting Raf kinases, effectively slowing down cancer cell proliferation. This dual-action mechanism extended the overall survival of HCC patients to about 10.7 months, compared to 7.9 months in the placebo group[24]. Following this, Lenvatinib was approved by the FDA in 2018 as a first-line treatment for unresectable HCC, marking another major advancement in HCC treatment. Lenvatinib, inhibiting multiple kinases including VEGFR1-3, fibroblast growth factor receptor1-4, PDGFR, RET, and KIT, demonstrates effective control over tumor proliferation and angiogenesis[25]. Clinical trials have shown that Lenvatinib can extend the survival of patients to about 13.6 months. A significant leap was made in 2020 with the FDA approval of the combination therapy of Atezolizumab and Bevacizumab, setting a new standard for first-line treatment in terms of efficacy[26]. Atezolizumab, an immunoglobulin G 1 monoclonal antibody, specifically targets and binds to programmed death-ligand 1 (PD-L1),

blocking its interaction with the PD-1 receptor and thereby enhancing T-cell activity against cancer cells. Concurrently, Bevacizumab, a humanized monoclonal antibody targeting VEGF, inhibits tumor angiogenesis, further impeding the progression of HCC[27]. The progression in second-line treatments for HCC began with the approval of Regorafenib in 2017 and Cabozantinib in 2020. Regorafenib, like Sorafenib but with a broader spectrum of kinase inhibition, notably enhances the antiangiogenic effects through the simultaneous blockade of VEGFR2 and epidermal growth factor homology domain2 pathways[28]. Cabozantinib demonstrates significant antitumor activity in HCC, primarily through its dual inhibition of MET and VEGFR2[29]. Furthermore, FDA approved Ramucirumab in 2019, an antibody targeted against VEGFR2, providing a specialized option for patients with elevated alpha-fetoprotein levels[30]. In addition, nucleic acid-based drugs, including small interfering RNA (siRNA), microRNA, and antisense oligonucleotides, represent another promising area in HCC treatment, particularly in terms of their specificity and p potential to target the molecular basis of the disease[31]. For example, In HCC, high levels of Polo-like kinase 1 (PLK1) are associated with aggressive tumor growth and poor prognosis. Researchers have developed siRNA molecules that specifically target and silence the *PLK1* gene to inhibit the proliferation of HCC cells[32]. All of the above advancements provided more targeted and effective options for HCC treatment.

However, despite these advancements, systemic therapy for HCC continues to face significant challenges, including the management of side effects. Sorafenib often causes hand and foot skin reactions in up to 30% of patients. Lenvatinib can lead to hypertension in about 23% of patients, as well as proteinuria and cardiac dysfunction in some patients[33]. Immunotherapy also presents unique side effects, including autoimmune reactions such as colitis, hepatitis, dermatitis, and endocrinopathies[34]. These side effects result from the nonspecific effects of the chemical drugs that may inadvertently harm healthy cells while killing cancer cells. The uneven drug distribution at the tumor site and the emergence of multi-drug resistance (MDR) further challenge the effectiveness of chemotherapy[35]. Consequently, the search for more targeted and effective systemic therapeutical agents remains a key issue that needs to be addressed.

NANOTECHNOLOGY APPLIED IN HCC TREATMENT

Nanomedicine or nanoparticle drug delivery system (NDDS) with particle sizes of 1-1000 nm, offers a revolutionary way to circumvent the side effects associated with traditional systemic therapy. The history of nanomedicine dates back to the late 20th century, with the advent of liposomal formulations being one of the earliest applications[36-38]. These nanoscale carriers improve the solubility and stability of chemotherapeutic drugs, which often presents a challenge in conventional formulations[39]. Moreover, these nanoparticles can deliver therapeutic drugs directly to the tumor site. This targeted approach not only enhances the efficacy by increasing the concentration of drug within the tumor, but also minimizes the impact on surrounding healthy tissues, thereby significantly reducing the adverse side effects typically associated with systemic therapy. In addition, one key mechanism of drug resistance is through the overexpression of efflux pumps, such as P-glycoprotein[40]. These pumps are capable of actively transporting chemotherapy drugs out of the cancer cells, significantly reducing the intracellular concentration of these drugs, thereby diminishing their efficacy. However, nanomedicine can bypass these efflux pumps because drugs are encapsulated within nanoparticles that are less likely to be recognized and expelled by these pumps. This property is particularly important for cancers that have become resistant to chemotherapy regimens. Nanomedicine's ability to deliver drugs in a more controlled and precise manner opens new avenues in cancer treatment, offering the potential to significantly improve the efficacy of systemic therapies while simultaneously reducing their side effects[41]. This innovative field continues to evolve, with ongoing research and development aimed at further refining and personalizing cancer treatment through advanced nanotechnology.

NDDS has revolutionized the management of HCC at various stages, including surveillance, diagnosis, and treatment [42]. Their application in surveillance has notably improved the detection of early-stage HCC, offering higher sensitivity and specificity, which is crucial for timely intervention. On the diagnostic side, contrast-enhanced nanoparticles can improve the clarity and accuracy of imaging modalities such as magnetic resonance imagings and computed tomography scans, resulting in more precise visualization of HCC tumors[43]. When it comes to treatment, the unique physiological and biochemical properties of the liver, particularly its dual blood supply from the hepatic artery and the portal vein, are crucial for nanomedicine delivery[44]. Specifically, liver tumors typically have abnormal and leaky vasculature, which may enhance the permeability and retention effect, allowing nanoparticles to accumulate more efficiently in tumor tissues than in normal liver tissues[45]. In addition, nanomedicines can be designed for active targeting by modifying the surface of nanoparticles with ligands that have a high affinity for receptors overexpressed in liver cancer cells, thereby reducing the impact on healthy liver cells. Another critical aspect of NDDS in HCC treatment is their role in overcoming drug resistance, a common challenge in cancer therapy[46]. The liver's complex enzyme system often contributes to this resistance, but nanoparticles can be engineered to circumvent these mechanisms, enhancing the efficacy of drug delivery and reducing the likelihood of resistance development.

The applications of NDDS in HCC systemic treatment are diverse and can be categorized based on their therapeutic function and type. These include targeted therapy, stimuli-responsive therapy, immune-modulating therapy, TACE therapy, nucleic acid-based therapy, and so on. Each category plays a distinct and pivotal role in improving the efficacy of HCC systemic treatment[47,48]. Thus, this review paper will primarily focus on targeted therapy, particularly emphasizing ligand-receptor mediated delivery. This approach underscores the crucial role of NDDS in advancing HCC management strategies, highlighting how targeted therapy, through specific ligand-receptor interactions, represents a significant advancement in the precision and effectiveness of HCC treatment.

SURFACE RECEPTOR FOR SPECIFIC TARGETING IN HCC THERAPY

In targeted therapy, the uptake of nanoparticles by HCC cells is facilitated through the interaction between targeting agents on the nanoparticle surface and receptors that are abundantly expressed on the membrane of HCC cells[49]. Therefore, a thorough understanding of these surface receptors on HCC cells is essential for the effective design and surface modification of nanoparticles to ensure that they are accurately localized on target cells. Next, we will delve into the key receptors that are characteristically overexpressed on liver cancer cells. We will also discuss their corresponding ligands, which play a key role in targeted therapies for HCC, thus providing a clearer perspective on the strategies employed for receptor-mediated nanotherapies for this complex disease. Figure 1 illustrates a summary of receptors that are overexpressed on hepatoma cells.

Glypican-3

In the area of nano-targeted therapies for HCC, Glypican-3 (GPC3) stands out as a pivotal molecular target. As a heparan sulfate proteoglycan, GPC3 is significantly overexpressed in the cell membrane and cytoplasm of HCC cells, whereas it is conspicuously absent in normal hepatocytes[50]. This unique expression pattern makes GPC3 a prime candidate for therapeutic targeting, and a series of *in vitro* and *in vivo* studies have validated this potential. In addition, the presence of GPC3 is strongly associated with advanced HCC stage, higher tumor grade, vascular invasion and poorer patient prognosis[51]. Hsu *et al*[52] discovered that GPC3 mRNA was present in 74.8% of both primary and recurrent HCC cases, in contrast to its mere 3.2% occurrence in normal liver tissues[52]. This significant difference highlights GPC3's utility as a biomarker for tumor staging and assessing the aggressiveness of HCC. This is due to the role of GPC3 in promoting HCC growth through the wnt/β-catenin signaling pathway and its potential as a therapeutic target. The pathological process driven by GPC3 in HCC can be demonstrated by the fact that the gene silencing inhibits HCC cell proliferation and induces apoptosis.

Various therapeutic strategies targeting GPC3 have been explored in the treatment of HCC, with a particular focus on anti-GPC3 monoclonal antibodies (mAbs). Among these, GC33 was the first therapeutic mAb developed against GPC3 [53]. As a humanized mouse antibody, GC33 is known for its high-affinity binding to the C-terminal region of GPC3 and has shown substantial cytotoxic activity against GPC3-positive hepatoma cells. In preclinical studies using xenograft models, GC33 demonstrated a significant ability to reduce tumor size, highlighting its potential as an effective treatment for HCC. In a noteworthy study by Shen *et al*[54] sorafenib-loaded polymer nanoparticles were modified with the hGC33 antibody[54]. These nanoparticles specifically targeted GPC3-positive HepG2 cells, binding to GPC3 on their surface. The treatment was shown to inhibit wnt-induced signal transduction and down-regulate cyclin D1 expression, thereby halting the cell cycle in the G0/1 phase. This led to a reduction in HCC cell migration by inhibiting the epithelial–mesenchymal transition, offering a promising approach to HCC therapy. In addition to GC33, several other mAbs targeting GPC3 are currently being evaluated in various stages of research. These include the human antibodies MDX-1414 and HN3, as well as the humanized mouse antibody YP7. Each of these antibodies offers a unique approach to targeting GPC3, expanding the potential treatment options for HCC. For instance, Hanaoka *et al*[55] developed YP7-modified albumin-bound paclitaxel nanoparticles[55]. This innovative formulation not only induced targeted necrotic cell death, but also enhanced the concentration of paclitaxel within tumors, demonstrating its efficacy in HCC treatment. Table 1 presents various studies that have employed nanotechnology to target GPC-3 in the treatment of HCC[54-58].

Asialoglycoprotein receptor

The Asialoglycoprotein receptor (ASGPR), commonly known as the Ashwell-Morell receptor, is predominantly found on the sinusoidal surfaces of hepatocytes and is less common in non-liver cells[59,60]. This C-type lectin receptor is chiefly involved in the endocytosis and clearance of glycoproteins from the bloodstream. It binds specifically to glycoproteins that have exposed terminal galactose (GAL) or N-acetylgalactosamine (GalNAc) residues. In HCC, there is an observed increase in ASGPR expression across both early and advanced stages of the disease[61]. Utilizing this characteristic, drugs or therapeutic nanoparticles can be effectively conjugated with ligands that precisely target ASGPR. This targeted approach is designed to enhance drug delivery directly to the liver, thereby increasing the concentration of therapeutic agents in the target area while significantly reducing the potential for off-target effects on non-hepatic tissues.

In a recent study, Faris *et al*[62] developed chitosan nanoparticles, with a size of less than 100 nm, were loaded with simvastatin and modified with Chondroitin sulfate (ChS)[62]. ChS, containing GalNAc, has a specific affinity for ASGPR found on hepatocyte membranes. This modification enhanced the cytotoxicity of simvastatin against HepG2 cells, due to its targeted delivery and increased cellular uptake. However, targeting HCC cells presents a unique challenge since both cancerous cells and healthy hepatocytes express ASGPR. Wang's group tackled this problem by synthesizing nanoparticles conjugated with eight different types of GAL derivatives[63]. Their findings revealed that nanoparticles decorated with phenyl β-D-galactoside were particularly effective in delivering drugs to HCC cells, achieving greater specificity compared to normal hepatocytes. To provide a comprehensive overview, Table 2 includes several examples of HCC-targeting ligands that have been modified on nanoparticles for ASGPR-targeted delivery[62,64-69].

Transferrin receptor

The Transferrin receptor (TfR), a membrane glycoprotein, plays a crucial role in cellular iron regulation. When transferrin binds to TfR on the cell surface, the complex is internalized into the cell where the acidic environment of the endosome causes transferrin to release its iron ions[70]. There are two primary types of TfR: TfR1 and TfR2, both responsible for mediating cellular iron uptake. TfR1 is ubiquitously expressed and exhibits a significantly higher affinity for transferrin compared to TfR2. In recent years, TfR has gained attention for its notable overexpression in various tumor cells,

Table 1 Summary of nanoformulations utilizing Glypican-3 as a targeting receptor in hepatocellular carcinoma treatment

Targeting ligand	Particle size	Nanocarrier	Payload	*In vitro* or/and *in vivo* results
GC33[54]	100-150 nm	PEG PLGA	Sorafenib	GC33 modified nanoparticles *in vitro*: Specifically target GPC3-positive HepG2 cells, resulting in cell cycle arrest at G0/1 phase; *in vivo*: Inhibit the growth of liver cancer and improve the survival rate of tumor-bearing mice
YP7[55]	N/A	Albumin	Paclitaxel	YP-7 bounded-nanoparticles induce rapid target-specific necrotic cell death and increase the concentration of paclitaxel within HCC tumors
Clone 9C2 [56]	85-99 nm	TPGS PCL	Sorafenib	9C2 antibody conjugated nanoparticles *in vitro*: Have a higher cellular uptake and a 7.5-fold increase in IC50 value compared to free sorafenib; *in vivo*: Can greatly inhibit tumor growth with no significant side effects
Peptide G12 [57]	Approximately 100 nm	Liposome	Sorafenib	G12-modified liposomes *in vitro*: Have enhanced specific-targeting and internalization into GPC3-positive cancer cells; *in vivo*: Show a superior precise antitumor effect with marked tumor suppression
Peptide[58]	105-117 nm	PEG PLGA	Sorafenib	Peptide-labeled nanoparticles *in vitro*: Significantly increase cytotoxicity against Hep3B cells; *in vivo*: Show good uptake and inhibited tumor growth

HCC: Hepatocellular carcinoma; GPC3: Glypican-3.

Table 2 List of different nanoformulations for Asialoglycoprotein Receptor targeted therapy in hepatocellular carcinoma

Targeting ligand	Particle size	Nanocarrier	Payload	*In vitro* or/and *in vivo* results
Lactose[64]	Approximately 115 nm	PCL-PEG-CHO	Sorafenib Curcumin	Lactose modified nanoparticles *in vitro*: Improve the efficiency of loaded drugs and exhibit better cytotoxicity; *in vivo*: The inhibition rate is 77.4%
Galactose [65]	92-136 nm	PEG PCL; Micelles	Paclitaxel	IC50 values of Gal decorated nanoparticles decreased from 11.7 to 1.1 μg/mL with increasing Gal concentration from 10% to 30%, supporting receptor-mediated endocytosis mechanism
ASP[66]	Approximately 228 nm	Deoxycholic acid	Doxorubicin	ASP modified nanoformulations *in vitro*: Internalize into HepG2 cells *via* ASGPR-mediated recognition and inhibit cell proliferation; *in vivo*: Suppress the tumor growth and reduce the side effects of free DOX
CS[62]	Approximately 80 nm	Chitosan	Simvastatin	CS decorated nanoparticles enhance the cytotoxicity of the loading drug against HepG2 cells owing to its enhanced cellular uptake
LA[67]	Approximately 310 nm	Cholesterol Liposome	Oxaliplatin	LA presents as a promising ligand for targeted drug delivery in the treatment of BEL7402 cancer cells
Pullulan[68]	140-170 nm	PLGA; PBAE	Paclitaxel; Combretastatin A4	Pullulan labeled nanoparticles enhance targeting capability and efficacy in HCC treatment both *in vivo* and *in vitro*
Pectin[69]	Approximately 300 nm	$Ca(OH)_2$; $NaHCO_3$	5-Fu	Pectin-based nanoparticles reduced the IC50 value to 0.17 mol/L in HepG2 cells, a significant decrease compared to the 0.45 mol/L IC50 value for free 5-Fu

ASP: Angelica sinensis polysaccharide; LA: Lactobionic acid; CS: Chondroitin sulfate; 5-Fu: 5-fluorouracil; HCC: Hepatocellular carcinoma.

including HCC. It is particularly pronounced on the surface of several HCC cell lines such as HepG2, J5, Bel-7402, Huh7, and SK-Hep-1. This marked overexpression establishes TfR as a significant target for effective drug delivery strategies in HCC therapy[71]. Specifically, research indicates that in human HCC, the mRNA level of TfR1 is upregulated, whereas that of TfR2 is downregulated. This differential expression pattern further highlights the potential of targeting TfR1 in HCC therapy.

Exploiting this trait, Xiao *et al*[72] developed innovative transferrin nanovesicles, incorporating Fe3+ ions and encapsulating the chemotherapeutic drug sorafenib[72]. In both *in vivo* and *in vitro* studies, SOR@TF-Fe3+ NVs demonstrated a preferential accumulation in the liver, specifically targeting HCC cells that overexpress the TfR. This targeted approach not only enhances the therapeutic effectiveness of sorafenib by directing it to the tumor site but also potentially reduces the systemic distribution and associated side effects, highlighting the potential of TfR-targeted therapies in the treatment of HCC. In addition, Malarvizhi *et al*[73] developed nanoparticles conjugated with human serum transferrin, innovatively incorporating DOX within a poly(vinyl alcohol) nano-core and sorafenib in an albumin nano-shell[73]. This design utilized transferrin ligands for targeted delivery, resulting in notably enhanced cellular uptake. The study demonstrated that these transferrin-conjugated nanoparticles achieved synergistic cytotoxicity, effectively inducing cell death in approximately 92% of the targeted cells. This outcome was significantly more efficient compared to the 75% cell death rate observed with nanoparticles that were not modified with transferrin, highlighting the efficacy of transferrin-

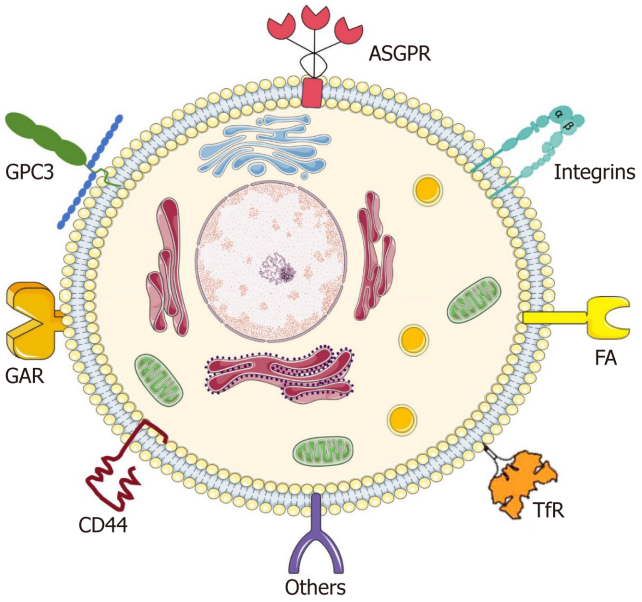

Figure 1 Schematic representation of different types of targeting receptors expressed on hepatocellular carcinoma. ASGPR: Asialoglycoprotein receptor; FA: Folic acid; TfR: Transferrin receptor; GAR: Glycyrrhetinic acid receptors; GPC3: Glypican-3.

mediated targeting in enhancing the therapeutic impact in HCC treatment.

Folate receptor

In HCC, the rapid proliferation of tumor cells creates an increased demand for essential nutrients and organic compounds, including vital vitamins such as folic acid (FA), biotin, retinoic acid (RA), and dehydroascorbic acid. FA, also known as vitamin B9, vitamin M, and vitamin Bc, is a water-soluble vitamin crucial in eukaryotic cell metabolism[74]. It is integral to the biosynthesis of methionine, purine, and pyrimidine, as well as in the interconversion of serine and glycine and histidine catabolism. Animal cells, unable to synthesize FA due to the absence of key enzymes, rely on the uptake of exogenous FA for these vital biosynthetic pathways[75]. The FA receptor (FAR), a glycosylphosphatidylinositol-anchored membrane protein, is significantly overexpressed in HCC cells and offers a strategic target for anticancer therapies. FAR mediates the cellular uptake of FA through receptor-mediated endocytosis, a process that efficiently internalizes this essential nutrient. This overexpression of FAR in HCC cells, coupled with the critical role of FA in cellular metabolism, makes FAR a prime target for delivering therapeutic agents. This approach aims to capitalize on the unique metabolic requirements of rapidly proliferating cancer cells, potentially leading to more effective and targeted therapeutic strategies in the treatment of HCC.

3,4-seco-lupane triterpenes show a potent cytotoxic activity against HepG2 cells, however, the poor solubility of the drug has limited its further application. Wang *et al*[76] formulated FA-conjugated polyethylene glycol albumin nanoparticles which encapsulated lupane triterpenes inside[76]. With the help of FA ligand, these nanoparticles showed enhanced toxicity and specific uptake in FAR-positive HepG2 cells, demonstrating their targeted anticancer efficacy. While FA-functionalized drug delivery systems can induce apoptosis in tumor cells, HCC cells often possess various anti-apoptotic mechanisms that can hinder the effectiveness of such therapies. To overcome this challenge, down-regulation of anti-apoptotic genes through RNA interference has emerged as a viable strategy to induce cell death in HCC cells. In this context, Xia *et al*[77] made a significant contribution by developing selenium nanoparticles loaded with siRNA and linked with FA[77]. These nanoparticles, approximately 115 nm in size, demonstrated enhanced cellular uptake and were notably effective in inhibiting the proliferation of HepG2 cells. Furthermore, they were successful in inducing cell cycle arrest at the G0/G1 phase in HepG2 cells. Highlighting the potential of FAR-targeted therapies in the treatment of HCC. Based on the multifunctionality of FA in targeted therapies that can provide dual drug treatment options. Cao *et al*[78] extended this approach by developing polymeric nanoparticles loaded with both BCL-2 siRNA and DOX and functionalized with FA for targeted delivery[78]. This FA-mediated targeting greatly improved the therapeutic efficacy; delivery of BCL-2 siRNA *via* these FA-modified nanoparticles produced more pronounced gene silencing, as evidenced by a dramatic reduction in BCL-2 mRNA and protein expression levels. This targeted delivery mechanism not only induces apoptosis of cancer cells more effectively, but also extends the therapeutic effect of co-delivered DOX. The study demonstrates the potential of using FA as a ligand in multidrug nanoparticle systems, providing a more targeted and effective approach to the treatment of cancer, especially in terms of enhanced gene suppression and drug synergy.

Integrins

Integrins, a class of heterodimeric transmembrane glycoproteins, play an important role in regulating various cellular functions, including adhesion, migration, invasion, proliferation, and apoptosis[79]. In contrast to normal cells, certain integrins are often overexpressed or aberrantly activated in HCC cells, which is associated with aggressive behavior of cancer cells, including proliferation, invasion, and metastasis. The Arginine-Glycine-Asparagine (RGD) tripeptide is

crucial in these cellular interactions, particularly in its specific targeting of integrins[80]. RGD peptides have a high affinity for integrin receptors, a feature that is strategically utilized in the design of targeted nanoparticles for HCC treatment. This targeted approach ensures that the nanoparticles, often carrying therapeutic agents, are more likely to adhere to and be absorbed by HCC cells rather than normal cells, thereby enhancing the efficacy and specificity of the treatment directed against these cancer cells. Recently, Wu *et al*[81] prepared a RGD-modified polydopamine-paclitaxel-loaded nanoparticles[81]. These nanoparticles are uniquely designed to target HCC cells by specifically recognizing and binding to αvβ3/αvβ5 integrins, which are often overexpressed in HCC cells. Li *et al*[82] engineered another novel therapeutic approach by developing gold nanoparticles coated with polydopamine and conjugated with RGD peptides [82]. The conjugation with RGD peptides enabled the nanoparticles to target integrin αvβ3-overexpressing HepG2 cells specifically. These receptor-mediated targeting led to an enhanced uptake of the nanoparticles by the cancer cells, resulting in increased cytotoxicity compared to non-targeted treatment approaches.

Cancer stem cell biomarker

Recent studies have underscored the critical role of cancer stem cells (CSCs) in HCC, particularly their capacity to initiate tumors and drive recurrence and metastasis[83]. These CSCs, a distinct subpopulation within the tumor, are notably resistant to conventional chemotherapies, highlighting the need for targeted therapeutic strategies. Among the notable CSC biomarkers in HCC, Cluster of Differentiation 44 (CD44) has been identified as a key player. This transmembrane glycoprotein, primarily a receptor for hyaluronic acid (HA), also interacts with osteopontin, collagens, and matrix metalloproteinases[84]. Nanoparticles can be specifically designed to target CD44, utilizing ligands that bind to this receptor. Cannito *et al*'s group has prepared HA and PEGylated liposomes as promising approaches for the treatment of HCC[85]. In cell culture experiments, HA-liposomes demonstrated enhanced internalization in Huh7 cells that over-express CD44 compared to HepG2 cells with lower receptor expression, indicating CD44's potential as a target for nanoparticle-based therapies.

Besides CD44, several other markers have been identified for liver CSC, including CD133, CD90, OV6, and epithelial cell adhesion molecule (EpCAM). CD133, in particular, stands out as one of the most important surface markers for liver CSCs. Jin *et al*[86] have contributed to this field by developing paclitaxel-loaded PLGA nanoparticles decorated with anti-CD133 antibodies[86]. These targeted nanoparticles showed a substantial improvement in therapeutic response by selectively eliminating the CD133 positive subpopulation in both *in vitro* and *in vivo* experiments. Another breakthrough came from Yamashita *et al*[87] who identified EpCAM-positive cancer cell subpopulations in HCC. These cells have the ability to self-renew, initiate tumors, and form distant metastases, *etc*. EpCAM overexpression in HCC is associated with a poor prognosis, and thus it has been positioned as a potential risk stratification biomarker. Utilizing EpCAM-specific antibodies, researchers have developed modified nanoparticles for effectively treating malignant tumors in HCC patients with EpCAM positive carcinomas. For example, Zhang *et al*[88] prepared magnetic nanoliposomes targeting EpCAM capable of encapsulating Lenvatinib[88]. This nanoparticle showed significant efficacy in inhibiting HCC cell proliferation and promoting apoptosis, as well as specific targeting and magnetic resonance imaging tracking of HCC cells.

Glycyrrhetinic acid receptor

Glycyrrhizic acid (GA), a GA derivative extracted from licorice root, has attracted great interest in the field of HCC therapy. GA has been reported to inhibit cancer cell proliferation, invasion, and metastasis, and induce cell cycle arrest, autophagy, and apoptosis[89,90]. Recent advances in nanoparticle technology have witnessed the development of GA-modified drug delivery systems. These nano-delivery systems have shown good hepatocyte and liver targeting efficiency both *in vitro* and *in vivo*. The efficacy of GA in targeting HCC cells is primarily due to its ability to bind to Glycyrrhetinic acid receptors (GAR) present on the surface of these cells. Furthermore, the prevalence of GAR is reportedly higher in tumor tissues compared to normal tissues[91]. This differential expression makes GA an optimal ligand for targeted drug delivery in HCC.

For instance, Lv *et al*'s group developed GA-modified mesoporous silica nanoparticles (MSN) containing Curcumin [92]. These nanoparticles not only exhibited satisfactory loading capacity but also increased drug uptake by GA receptor-positive cells. In vitro experiments revealed a significant increase in apoptotic cells treated with MSN/Curcumin/GA, indicating the efficacy of GA-functionalized nanoparticles in inducing apoptosis in HepG2 cells. Similarly, Tian *et al*[93] prepared liver-targeted DOX delivery using GA-modified chitosan/PEG nanoparticles. These nanoparticles exhibited significant liver-targeting and retention, and the accumulation in the liver was 2.6 times higher than that of non-GA-modified nanoparticles. Furthermore, the DOX-loaded chitosan/PEG–GA nanoparticles effectively inhibited tumor growth in H22 cell-bearing mice, showcasing the potential of GA in enhancing the therapeutic efficacy of nanoparticle-based drug delivery systems in HCC treatment. In conclusion, the incorporation of GA into nanoparticle formulations represents a major advancement in targeted therapy for HCC, which leverages the unique properties of GA to improve drug delivery and therapeutic efficacy.

Other receptors in HCC nanotherapy

In the evolving development of nanotherapies for HCC, several receptors beyond the previously discussed ones are being targeted for more effective treatments. Notably, the epidermal growth factor receptor (EGFR) plays a crucial role in HCC. Often overexpressed in HCC, EGFR is linked to accelerated tumor growth and poor prognosis[94]. Targeting EGFR with nanoparticles, such as the adriamycin-loaded polymer-lipid hybrid nanoparticles developed by Gao *et al*[95] conjugated with EGFR-specific antibodies, demonstrates enhanced targeting and cytotoxicity against EGFR-expressing HCC cells [95].

Another important target is the low-density lipoprotein receptor (LDLR), which shows increased expression in HCC compared to adjacent liver tissue[96]. Utilizing the natural affinity of the major cholesterol transporter, LDL, for the LDLR, nanoparticles can be designed to mimic or conjugate with LDL particles. This approach is employed to deliver therapeutic agents directly to HCC cells, capitalizing on their increased demand for cholesterol. The Wang *et al*'s group utilized Apolipoprotein B-100, recognized by LDLR, to modify lipid nanoparticles[97]. These nanoparticles exhibited higher cellular internalization and tumor targeting in LDLR-overexpressing liver cancers.

c-Met, the receptor for hepatocyte growth factor, is another significant target in HCC. It contributes to cell proliferation, survival, migration, and invasion[98]. Nanoparticles carrying c-Met inhibitors, such as crizotinib or cabozantinib, have been developed for targeting HCC cells. These nanoparticles can be functionalized to bind specifically to c-Met, allowing for targeted delivery and disruption of c-Met signaling pathways.

Furthermore, C-X-C chemokine receptor type 4 (CXCR4) plays a multifaceted role in HCC progression, including promoting angiogenesis and tumor cell evasion of immune surveillance. The Chen group developed nanoparticles where the CXCR4 antagonist AMD3100 serves a dual function; it is encapsulated within the nanoparticles and also modifies their surface[99]. This innovative design allows AMD3100 to act both as an intracellular delivery agent for siRNA targeting malignant HCC cells and as a CXCR4 blocker, enhancing its anti-cancer efficacy.

In summary, these advancements in targeting EGFR, LDLR, c-Met, and CXCR4 through nanoparticle technology represent significant strides in the personalized treatment of HCC. By exploiting the unique molecular characteristics of HCC cells, these targeted therapies offer the potential for more effective and less toxic treatments.

Multiple receptors: Dual-targeting

Dual-ligand nanoparticle modification is an advanced strategy for the treatment of HCC that enhances targeting and specificity by simultaneously binding to multiple receptors or pathways on cancer cells. This approach enables more precise targeting of HCC cells and ensures better cellular uptake and internalization of the drug, thereby improving drug efficacy and specificity. In addition to this, dual-ligand nanoparticles can provide synergistic therapeutic agents that produce enhanced effects and offer diverse therapeutic strategies by combining different therapeutic modalities. Table 3 lists some successful examples of dual-ligand modified nanoparticles targeting multiple HCC receptors[100-105].

LIMITATIONS OF NANOTECHNOLOGY IN THE TREATMENT OF HCC

While numerous studies have demonstrated the effectiveness of targeted ligand-modified nanoparticles in enhancing the anticancer properties of drugs for HCC treatment, the advancement of nanotechnology in this field encounters several complex challenges[106-108]. Achieving precise targeted drug delivery is a major challenge, which includes not only the precise localization of nanoparticles within a specific body region, but also the control of their release and dosage. This precision is essential to maximize efficacy and minimize adverse effects. Another major challenge is the body's immune response and potential rejection of these nanocarriers. The immune system usually recognizes these nanoparticles as foreign entities, leading to reduced efficacy or adverse immune reactions. Successful application of nanomedicines for the treatment of HCC requires various strategies to evade immune detection and minimize immunogenicity. There are technical difficulties in synthesizing nanoparticles with uniform and predictable properties in a controlled, rapid and reproducible manner. This challenge also includes ensuring precise manufacturing processes for the systematic screening and characterization of nanoparticles, which is critical for maintaining consistency of efficacy. Scaling up production for mass market availability while ensuring quality, performance and biocompatibility is another hurdle. Ensuring cost-effectiveness is key to making these advanced treatments economically viable and widely available for clinical use. Regulatory and ethical considerations are also central to the development and application of nanomedicines. These include stringent regulation of safety and efficacy, ethical considerations such as patient privacy, and understanding the long-term impact of nanomaterials on human health and the environment. Addressing these multifaceted challenges requires a multidisciplinary approach that encompasses the fields of materials science, medicine, pharmacology, engineering, and ethics. Collaboration between these disciplines is critical to refining nanoparticle design, improving their therapeutic applications in HCC, and transitioning these advanced technologies from the laboratory to the clinical setting.

CONCLUSION

HCC is one of the most challenging malignant tumors, characterized by its complex nature and increasing morbidity and mortality. Utilizing the unique advantages of nanotechnology to improve the efficacy of HCC heralds a new era of precision medicine. In this review, we delved into the application of nanomedicine in HCC, with special emphasis on the role of ligand-receptor interactions in improving treatment specificity and efficacy. We investigated a series of receptors that are critical to the pathophysiology of HCC, including GPC3, ASGPR, FAR, TfR, Integrins, GAR and several CSC receptors. Targeted therapies developed to interact with these receptors demonstrate how nanomedicines can be tailored to address the various complexities of HCC. These therapies are expected to not only improve efficacy but also reduce side effects compared to conventional therapies. Of particular note are dual-ligand modified nanoparticles. By targeting multiple receptors or pathways simultaneously, these nanoparticles provide a multifaceted approach to combating HCC, a strategy that is critical to addressing challenges such as MDR and enhanced targeting. However, the process of moving

Table 3 Summary of dual-targeted nanoformulations in hepatocellular carcinoma therapy

Ligand 1	Ligand 2	Nanocarrier	Payload	Particle size
Folic acid[100]	Lactobionic acid	Chitosan	5-Fu	163 ± 10 nm
Folic acid[101]	Lactobionic acid	Berberine; Diosmin	Casein micelles	Approximately 200 nm
Glycyrrhetinic acid[102]	Hyaluronic acid	Carbodiimide	Paclitaxe	200-320 nm
Lactobionic acid[103]	Glycyrrhetinic acid	Chitosan; Acrylic acid	DOX	Approximately 274 nm
Lactoferrin[104]	Lactobionic acid/Glycyrrhetinic acid	Phospholipid complex	Sorafenib; quercetin	169 ± 1.5; 230 ± 1.7
Biotin[105]	Lactobionic acid	PEG; PLGA	Curcumin 5-Fu	110-187 nm

5-Fu: 5-fluorouracil; DOX: Doxorubicin.

from laboratory research to clinical application remains fraught with challenges, including ensuring the precision of targeted delivery in the human body, mitigating immune responses, enabling controlled and reproducible nanoparticle synthesis, scaling up production, and addressing cost-effectiveness issues. In addition, regulatory pathways and addressing ethical issues are critical steps in bringing these innovations to patients. As we make progress in developing and refining these targeted therapeutic strategies, the future looks bright for dramatically improving HCC treatment outcomes. However, this will require continued collaboration across multiple scientific and medical disciplines to realize the full potential of nanotechnology in the fight against HCC.

FOOTNOTES

Author contributions: Zhou XQ and Li YP collected and analyzed the information and wrote the manuscript; Dang SS reviewed and edited the manuscript; All authors have given approval to the final version of the manuscript.

Supported by Xi'an Jiaotong University Medical "Basic-Clinical" Integration Innovation Project, No. YXJLRH2022067; and Shaanxi Postdoctoral Research Program "Orlistat-loaded Nanoparticles as A Targeted Therapeutical Strategy for The Enhanced Treatment of Liver Cancer", No. 2023BSHYDZZ09.

Conflict-of-interest statement: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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S-Editor: Fan JR
L-Editor: A
P-Editor: Zhang YL

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 177-185

DOI: 10.4254/wjh.v16.i2.177

ISSN 1948-5182 (online)

MINIREVIEWS

Dynamic changes and clinical value of lipocalin 2 in liver diseases caused by microbial infections

Feng Chen, Shan-Shan Wu, Chao Chen, Cheng Zhou

Specialty type: Infectious diseases

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: El-Arabey AA, Egypt; Perazzo JC, Argentina

Received: August 28, 2023
Peer-review started: August 28, 2023
First decision: November 20, 2023
Revised: December 4, 2023
Accepted: January 9, 2024
Article in press: January 9, 2024
Published online: February 27, 2024

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Abstract

Lipocalin 2 (LCN2) plays a pivotal role in iron metabolism, particularly in the context of microbial infection resistance (*e.g.*, viruses, bacteria, parasites, *etc.*). LCN2 combats microbial infection by directly assisting the body in competing with microorganisms for iron, inducing immune cells to secrete various cytokines to enhance systemic immune responses, or recruiting neutrophils to infectious sites. The liver serves as the primary organ for LCN2 secretion during microbial infections. This review encapsulates recent advances in dynamic changes, clinical values, and the effects of LCN2 in infectious liver diseases caused by various microbial microorganisms.

Key Words: Lipocalin 2; Microbial infection; Immunity; Liver diseases

Core Tip: Lipocalin 2 (LCN2) is a sensitive marker for infections because its change can be detected at the very early stage of various pathogenic microorganism infections. Infection with a variety of pathogens can cause liver damage, and it is well established that LCN2 is expressed differently in different clinical conditions. By observing the level of LCN2, doctors can evaluate the progression of the disease and the treatment efficacy. LCN2 is also a predictor marker in some end-stage liver diseases and is promising as a new diagnostic marker. Due to its strong binding with iron, targeting LCN2 also shows great potential in the treatment of infectious diseases.

Citation: Chen F, Wu SS, Chen C, Zhou C. Dynamic changes and clinical value of lipocalin 2 in liver diseases caused by microbial infections. *World J Hepatol* 2024; 16(2): 177-185
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/177.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.177

INTRODUCTION

Lipocalin 2 (LCN2) is a secreted protein from the Lipocalin family with a molecular weight of 25 kDa, consisting of 178 amino acid residues[1]. It is widely expressed across various cell types, including neutrophils, macrophages, activated leukocytes, adipocytes, hepatocytes, tumor cells, stromal cells, and osteoblasts. The LCN2 mRNA was initially isolated from a kidney cell cDNA library infected with SV40 in 1989 by Hraba-Renevey *et al*[2], and it was named 24p3. Subsequently, in 1993, Kjeldsen *et al*[3] successfully purified LCN2 from human neutrophils. LCN2's diverse functions were discovered in different cell types, resulting in several names inspired by its various roles. In addition to the original designation as 24p3[2], it was referred to as 25 kDa protein for its molecular weight[3], neutrophil gelatinase-associated lipocalin (NGAL or HNL) for its aggravating inflammatory response *via* recruitment of neutrophils[4], oncogenic lipocalin for its promotion of tumor growth[5], superinducible protein (SIP24)[6] for its heightened expression in response to fibroblast growth factor in BALB/c 3T3 cells, uterocalin for its elevated level around parturition in the uterus[7], and siderocalin for its function in sequestering iron[8]. In 2004, Flo TH published a paper in Nature officially named it LCN2, following the nomenclature of the lipocalin family, a gene group evolutionarily conserved and found in all kingdoms of life. In the Flo TH paper, LCN2 was demonstrated to play an important role in innate immunity against iron-dependent bacterial infections. Over time, the name LCN2 gained widespread acceptance and standardization[9,10].

Similar to other members of the Lipocalins family, LCN2 has a highly conserved core structure, characterized by an eight-stranded, antiparallel β-barrel with the calyx, or ligand-binding site. Compared with other members, LCN2 exhibits a calyx that is unusually shallower and broader, featuring a lining of positively charged residues. Consequently, LCN2 does not directly bind positively charged ions but rather sequesters irons by binding the negatively charged ferric siderophore with a sub-nanomolar dissociation constant[11]. The ligands housed in the calyx suggests that LCN2's primary function is intricately linked to iron metabolism.

LCN2 has been reported to be expressed in many organs, including the liver[12-15], kidney[16-18], lungs[19,20], brain [21,22], heart[23,24], bone marrow[25], and spleen[26], bronchus[27], stomach[28], small intestine[29], pancreas[30], prostate gland[31], thymus[32]. Despite its presence in numerous organs, the liver emerges as the major site of LCN2 generation. During the body's response to harmful microorganisms, 90% of the LCN2 upregulation originates from the liver. It has been reported that in mice, upon bacterial infection, the mRNA level of LCN2 increased by 30-40-fold in the liver, in contrast to an approximately 1.5-fold elevation in the spleen and lungs[33]. Physiologically, hepatocytes take up iron majorly by transferrin-mediated pathway, and LCN2 was demonstrated to be not essential in hereditary hemochromatosis (HH)[34]. However, when the body with HH was infected by microorganisms, such as Salmonella Typhimurium, iron-capturing LCN2 was inducted to confer the host resistance to systemic infection with Salmonella and improve control of bacterial replication[35]. Therefore, LCN2 is proposed to play an important role in microbial infection-induced hepatitis.

IN VIRAL INFECTIOUS LIVER DISEASES

As a key organ of detoxification, the liver assumes a crucial role in the body's defense mechanisms. Viral infections, including hepatitis B virus (HBV) and hepatitis C virus (HCV), can induce a series of inflammatory pathological changes in the liver, leading to varying degrees of hepatitis. This progression may result in fibrosis, cirrhosis, liver failure, and ultimately, liver cancer. Individuals at different stages of HBV-related liver diseases manifest varying degrees of iron metabolic disorders[36]. Following viral infection, LCN2 levels begin to rise and exhibit variation during different phases of liver disease, underscoring the potential of LCN2 in the diagnosis and treatment of viral hepatitis. Due to the lack of suitable mouse models for viral hepatitis, studies on LCN2 in viral liver diseases mainly focus on disease diagnosis.

LCN2 is mainly related to the degree of inflammation in the body during viral liver disease and can serve as an indicator of various complications, such as cirrhosis, ascites, peritonitis, hepatorenal syndrome, nephritis, *etc*. Our previous study showed that the serum LCN2 levels in patients with chronic hepatitis B were significantly higher than those in normal controls[37]. Lu *et al*[38] examined the serum of patients with HBV-associated acute-on-chronic liver failure (ACLF) and found that LCN2 levels were significantly higher than that of patients without ACLF. Moreover, the

serum LCN2 levels significantly correlated with the total bilirubin, international normalized ratio, and model for end-stage liver disease model (MELD). The MELD score was independently associated with the overall survival in patients with HBV-ACLF, and serum LCN2 is also an independent risk factor for hepatorenal syndrome. Thus, the above data showed significant value in predicting the prognosis of HBV-ACLF.

In patients with liver cirrhosis, Gungor *et al*[39] reported that in patients with stable cirrhosis, serum LCN2 levels were not significantly different when compared with controls, while LCN2 levels in plasma and urine were significantly higher in cirrhosis patients with hepatorenal syndrome. Cox regression analysis revealed that plasma LCN2 and MELD-Na scores independent predicted of mortality. Nevertheless, conflicting perspectives exist, Borkham-Kamphorst *et al*[40] found that there was no difference in LCN2 between cirrhosis and non-cirrhosis patients, with compensatory cirrhosis patients exhibiting similar LCN2 levels to end-stage liver disease patients.

Concerning the relationship between LCN2 and viral load, studies in the patients infected with HCV showed no correlation between LCN2 levels and HCV viral load[41]. The diagnostic value of LCN2 in HCV infection-associated renal glomerular injury remains unclear. Strazzulla *et al*[42] observed significant increase in urine LCN2 levels in HCV-infected patients after one year of treatment with direct antiviral drugs, whereas Nada *et al*[43] reported a decrease in urine LCN2. Both studies, however, showed unchanging glomerular filtration rate. The disparate results may stem from individual differences in patient samples, systemic inflammation, and drug toxicity, necessitating further investigation (Table 1).

Because of its small molecular weight, LCN2 may directly leak from the kidney into the urine, allowing it to be clinically detected through noninvasive methods. Urinary LCN2 levels can be used to assess kidney injury in chronic liver disease complications or renal toxicity after antiviral drug administration[16,17,44]. To evaluate the efficacy of urinary LCN2 as a diagnostic biomarker for different etiology of acute kidney injury (AKI) in cirrhosis and its role as a prognostic marker, Hamdy *et al*[44] studied 83 patients with liver cirrhotic AKI due to HCV or combined with HBV infections, and they revealed that different urine LCN2 levels matched different types of kidney dysfunction in cirrhotic patients, thus providing suggestions for management decisions in the diagnostic process. It needs to be pointed out that, in addition to a systemic inflammatory reaction, the kidneys themselves are also damaged in the virus-induced hepatorenal syndrome [45], resulting in a significant increase of LCN2.

IN BACTERIAL INFECTIOUS LIVER DISEASES

Bacterial infections pose an escalating global health challenge, and the LCN2 has been repetitively found to play an important role in the body's defense against bacterial infections (Figure 1).

LCN2 inhibits bacterial growth primarily by sequestering iron, an essential nutrient for life. Bacterial invasion, growth, and reproduction rely on iron, creating a natural competition with the body's iron-dependent activities. Mechanistically, bacteria acquire iron by synthesizing siderophores and forming siderophore-Fe complexes. The host employs LCN2 to directly bind the siderophores-Fe complex, thereby controlling bacterial growth. Therefore, LCN2 is an important component of the innate immune system in defense against bacterial infection[46]. In LCN2 deficient mice, a challenge with a sublethal dose of Escherichia coli intraperitoneally resulted in a substantially higher amount of bacteremia and bacterial burden in the liver compared to the control mice. However, no significant differences in other components of the immune response, including leukocyte numbers, neutrophil infiltration, and various cytokines, such as toll-like receptor (TLR)-induced tumor-necrosis factor, interleukin (IL)-12, IL-6 and macrophage inflammatory protein (MIP)-2 were found between wild-type and lipocalin-2-deficient mice[9].

In vivo, LCN2 may also act as an antioxidant by regulating iron homeostasis to combat bacteria. Studies in LCN2 deficient mice revealed decreased levels of tissue redox state indicators cysteine and glutathione in the liver and plasma, elevated indices of liver damage such as transaminasemia, lactate dehydrogenase, and increased mortality. Moreover, the application of an iron chelator, Desferoxamine, was able to protect LCN2-deficient mice from LPS-induced toxicity and reduce mortality[47].

As a constituent of innate immunity, the antibacterial effects of LCN2 are intricately regulated by the immune system. During the early phase of infection, the innate immunocytes, such as neutrophils and macrophages, are activated, leading to the secretion of substantial quantities of LCN2. LCN2 then stimulates these immunocytes to produce a variety of cytokines and chemokines,such as IL-6, IL-10, tumor necrosis factor (TNF)-α, monocyte chemoattractant protein(MCP)-1, *etc.*, thereby enhancing the migration and phagocytosis of macrophages to bolster antibacterial function. Deletion of the LCN2 gene results in impaired functions of immune cells, including compromised homeostasis and morphological development of neutrophils, decreased migration ability and exudation, as well as reduced secretions of cytokines and chemokines, such as TNF-α, IL-6, IL-1β, MCP-1, and MIP-2[48]. LCN2 knockout mice exhibit higher expression of Th17 cell polarization markers, with transcription factor RORγt and cytokines IL-17A and IL-21 significantly up-regulated[49].

LCN2 itself also interacts with other immune factors in various types of cells, such as TLR ligands (*e.g.*, TLR4 ligands), cytokines (*e.g.*, IL-6, IL-1, IL-22, TNF-α, interferon (IFN)-γ), and growth factors (*e.g.*, insulin-like growth factor)[33,50,51]. It was reported that IL-6 treatment stimulated hepatocytes to produce more LCN2 *in vitro* and in vivo, and the elevation of LCN2 was abrogated in the IL-6R Hep$^{-/-}$, IL-6 $^{-/-}$mice, and signal transducers and activators of the transcriptions 3 (STAT3) Hep$^{-/-}$ mice. Hepatocyte-specific LCN2 knockout mice showed increased susceptibility to infection with Klebsiella pneumoniae or Escherichia coli, leading to increased bacterial translocation from the gut to mesenteric lymph nodes and reduced liver regeneration after partial hepatectomy. These findings suggest that the production of hepatocyte-specific LCN2 depends on IL-6 activation of the STAT3 signaling pathway. Hepatocyte-derived LCN2 protects against bacterial infection and promotes liver regeneration[33]. Another study demonstrated that rIL-22-induced antimicrobial activity mediated by IL-22 receptor alpha 1 (IL-22Rα1) and STAT3 signaling is partially dependent on

Table 1 Expression of lipocalin 2 in different viral hepatitis

Sample	Cases	LCN2 level	Ref.
Blood	675 mild chronic hepatitis B, 178 moderate chronic hepatitis B, 199 severe chronic hepatitis B and 317 fulminant hepatitis B, 246 healthy volunteers	Chronic hepatitis, fulminant hepatitis significantly higher than control	[37]
Blood	54 patients with HBV-ACLF and 49 patients with CHB	HBV-ACLF higher than CHB	[38]
Blood and urine	64 patients with cirrhosis and 23 control subjects	Cirrhosis with hepatorenal syndrome patients is significantly higher than controls, no difference between stable cirrhosis and control	[39]
Blood	192 patients with chronic liver diseases of variable etiology and clinical severity in comparison to 91 healthy controls	Chronic liver diseases were higher than healthy controls No significant differences between cirrhotic and non-cirrhotic patients	[40]
Blood	48 patients with chronic HCV	Not correlated with HCV viral load	[41]
Blood	87 Egyptian patients with chronic hepatitis C infection	Significantly decrease after HCV treatment with DAA	[42]
Blood	102 chronic hepatitis C virus infection	Significantly increase after HCV treatment with DAA	[43]

LCN2: Lipocalin 2; HBV-ACLF: Hepatitis B virus –related acute-on-chronic liver failure; CHB: Chronic hepatitis B; HCV: Hepatitis C virus; DAA: Direct-acting antivirals.

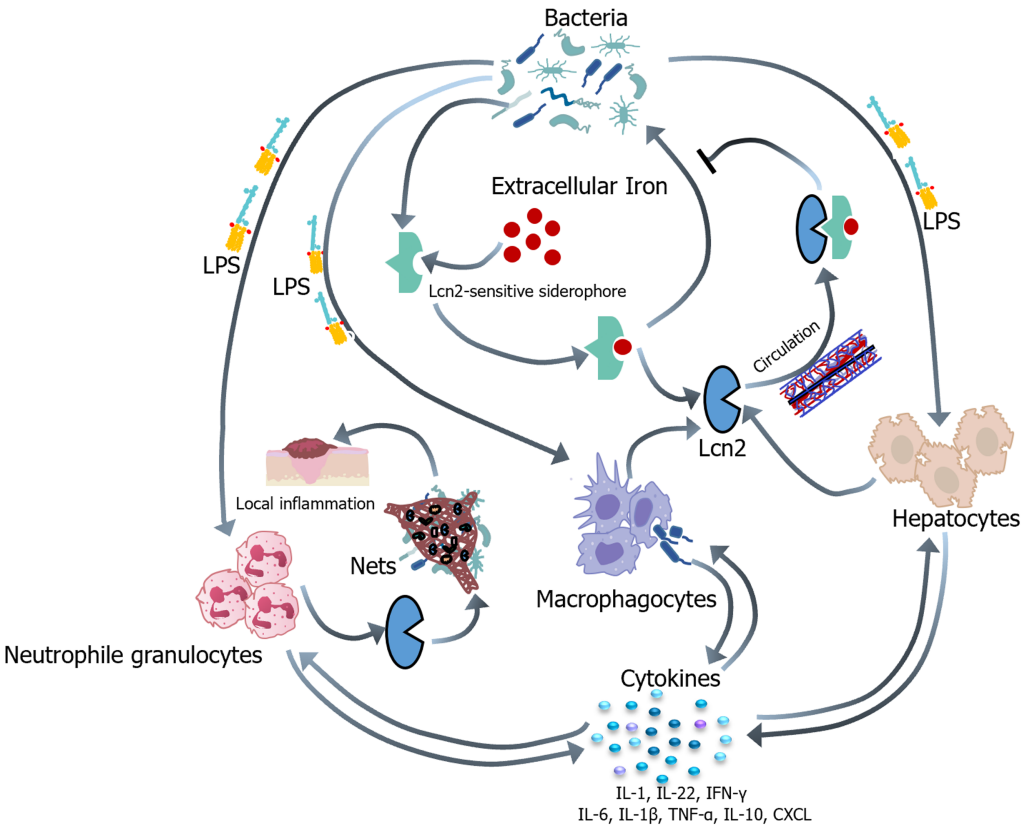

Figure 1 Schematic outline of lipocalin 2 antibacterial effects. During bacterial infections, most lipocalin 2 (LCN2) is secreted by liver cells, while some are derived from immune cells such as macrophages and neutrophils. LCN2 exerts an antibacterial effect mainly in two ways. One, it chelates LCN2-sensitive siderophores to form the LCN2-siderophores-Fe complex, which prevents the bacteria from absorbing iron and inhibits bacterial growth. Two, it stimulates the antibacterial immune response through stimulating immunocytes, which secrete various inflammatory cytokines and chemokines, promoting the migration and phagocytosis of macrophages. The cytokines and chemokines from immunocytes then further stimulate immunocytes and hepatocytes to produce more LCN2, forming a positive feedback loop to enhance the antibacterial immune response. LCN2 derived from hepatocytes and macrophages enters the systemic circulation to fight bacteria, and LCN2 is a component of NETs from neutrophils, which plays an important role in local inflammation. LCN2: Lipocalin 2; LPS: Lipopolysaccharide; IL: Interleukin; CXCL: Chemokines; IFN: Interferon; TNF: Tumor necrosis factor.

LCN2[51].

During bacterial infection, elevated levels of LCN2 expression are detected in both hepatocytes and neutrophils. The LCN2 from these distinct sources coordinates to combat bacteria, albeit with differing roles. While extracellular LCN2, secreted by liver cells, serves to restrict systemic bacterial infection, neutrophils transported LCN2 are carried in specific granules of neutrophils to local sites, contributing to the resistance against local bacterial infection through a network of neutrophil extracellular traps (NETs). The recently discovered mechanism, NETs, describes the process by which neutrophils kill bacteria through both cell-death-dependent and cell-death-independent pathways. Among the more than 80 proteins identified as components of NETs, LCN2 emerges as one of the crucial proteins. Notably, LCN2 in NETs is exclusively derived from neutrophils and not from hepatocytes or other cells. Complete or specific genetic deletion of the LCN2 gene in neutrophils does not impact NETs formation but does reduce the bactericidal effect of NETs *in vitro*[52].

LCN2 also resists bacterial invasion by maintaining intestinal microecological stability. It plays an important role in maintaining the species and abundance of intestinal flora and contributes to the gastrointestinal antibacterial barrier. Studies have shown that in the gut of LCN2 knockout mice, there is a significant and dramatic alteration in the species and abundance of intestinal flora change. Notably, iron-dependent strains expand significantly, leading to dysbiosis and continuous colonization by segmented filamentous bacteria (SFB). SFB is a special symbiotic intestinal bacterium within the firmicutes, fundamental for the production of innate and acquired immunity in the gastrointestinal and respiratory tracts and necessary for the maturation of the host intestinal immune barrier[53]. Deletion of LCN2 may provide a favorable environment for SFB colonization by inhibiting the antibacterial response of epithelial cells, altering the mucous composition, or establishing the antibacterial barrier. The increased SFB, in turn, can upregulate the level of LCN2. A study reported a significant increase in LCN2 levels in the liver and serum of SFB-colonized mice, accompanied by upregulation of the pro-inflammatory TH17 and TH1 cells in the liver-draining lymph nodes[54].

The dynamic changes in LCN2 levels during bacterial infection present potential applications for the early diagnosis of complications in liver diseases. Behairy *et al*[55] demonstrated that the level of LCN2 was significantly elevated in the patients with chronic liver diseases complicated with bacterial infection compared with those without bacterial infection. This finding suggests that LCN2 is an early diagnostic indicator for chronic liver disease with bacterial infection. Similarly, Liu *et al*[56] found that the level of LCN2 in ascites from decompensated liver cirrhosis with spontaneous bacterial peritonitis (SBP) was significantly higher than that in the non-SBP group, and the LCN2 level was positively correlated with ascitic polymorphonuclear leukocyte and negatively correlated with ascitic albumin. Furthermore, the dynamic changes of ascitic LCN2 were able to predict the clinical prognosis of SBP patients. Another study by Cullaro *et al*[57] showed a close relationship between ascitic LCN2 level and patient mortality, indicating that LCN2 serves as a molecular diagnostic biomarker of peritonitis in liver cirrhotic patients and an independent predictor of short-term hospitalization mortality. In addition, a study in the sepsis animal model of cecal ligation and puncture showed the LCN2 Levels in mouse liver and lung increased significantly during the early hyper-inflammatory phase, despite the dysfunction of innate immunity characterized by a severely decreased expression of most inflammatory mediators. This highlights the potential importance of LCN2 in the diagnosis of sepsis[58]. Thus, LCN2 is suggested to be an important clinical biomarker for early diagnosis of sepsis.

As a siderophore-binding protein, LCN2 provides wide application in the treatment of bacterial infections. Due to its multidrug resistance and the lack of effective antimicrobial drugs, carbapenem-resistant Acinetobacter baumannii (A. baumannii) has been designated by the World Health Organization as a priority critical pathogen for the development of novel therapeutics. Sheldon *et al*[59] observed the transcriptional profile in the A. baumannii-infected mice and revealed that the expression of LCN2 gene was the most highly upregulated during A. baumannii bacteremia. *In vitro* studies have also shown that LCN2 inhibits iron-dependent growth of A. baumannii and induces iron-regulated gene expression. In an LCN2 knockout mouse model, although LCN2 gene deletion did not alter the number of microflora in A. baumannii-infected tissues, it significantly aggravated the severity of infection and increased mortality.

Similarly, in a sepsis animal model induced by the infection of A. baumannii, the injection of recombinant mouse LCN2 prolonged the survival time of mice by decreasing the number of bacteria in macrophages and multiple organs, including the liver, spleen, and lungs[60]. LCN2 has been regarded as novel therapeutics to combat A. baumannii infection.

IN PARASITIC INFECTIOUS LIVER DISEASES

Infection caused by a variety of parasites, such as Schistosoma, Leishmania, and Plasmodium, can lead to liver damage. Iron, being an essential trace element for parasite survival, is acquired by intracellular pathogens from multiple sources within host cells, such as heme, ferlactoferrin or ferrictransferin. This ability may contribute to parasites' survival in different environmental conditions within the host[61]. As a metabolic enzyme and a cofactor in oxidative transport, iron plays a crucial role in immune surveillance. Therefore, controlling iron homeostasis is one of the central battlegrounds in combating pathogen infection.

LCN2's robust iron-binding ability assumes a crucial role in the body's resistance to parasitic infections[62]. Dighal *et al*[63] studied the intramonocytic labile iron pool (LIP) in Indian post Kala-azar dermal Leishmaniasis and found enhanced gene expressions of the iron influx gateways, including LCN2, possibly contributing to the heightened LIP. Therefore, restricting the availability of iron for parasites is regarded as a potential therapeutic strategy against parasitic infections.

Emerging evidence has shown that macrophage polarization plays a critical role in the initiation and progression of liver diseases. The underlying molecular mechanisms are intricate and involve various signaling pathways, including TLR4/nuclear factor kappa-B (NF-κB), janus kinase/STATs, transforming growth factor-β/smads, peroxisome proliferators-activated receptor, Notch, and miRNA signaling pathways[64]. Various factors, such as microorganisms, hypoxia,

metabolism, *etc*., can influence the macrophage polarization. As an iron-related protein involved in innate immune response, LCN2 is modulated by the host's immune system and interacts with macrophages. Studies have reported that LCN2 promotes M1 polarization of macrophages under *S. japonicum* soluble worm antigens (SWA) treatment. In addition, during the early infection stage in mice treated with *schistoma japonicum*, the expression of LCN2 significantly increased in the liver, mainly located in macrophages *via* the upregulation of NF-κB signaling. This study highlighted the importance of NF-κB/LCN2 in migration and phagocytosis of M1 macrophages stimulated by SWA, emphasizing the essential role of NF-κB/LCN2 in early innate immune responses to infection[65]. Recently, it has been reported that GATA3 is a master regulator for macrophage polarization and infiltration[66,67]. Therefore, investigating the relationship between LCN2 and GATA3 in macrophage polarization of various liver diseases may provide valuable insights.

Besides, oxidative stress can also lead to an increase in LCN2. In *in vitro* experiments, the up-regulation of LCN2 expression after H_2O_2 treatment was found to be offset by the addition of antioxidants, dimethyl sulfoxide or cysteamine [68]. Al-Shaebi *et al*[69] found that in the mouse model of malaria induced by Plasmodium chabaudi infection, the expression of the LCN2 gene reduced significantly after treatment with a plant antioxidant, Indigofera oblongifolia leaf extracts (ILE). ILE demonstrated a protective effect on mouse liver injury infected with Plasmodium chabaudi by enhancing the antioxidant capacity of the liver and significantly reducing the red blood cell count and hemoglobin content in mice caused by infection.

CONCLUSION

In summary, LCN2 emerges as a sensitive marker for infections, as its changes can be detected at the very early stage of various pathogenic microorganism infections, even preceding the detection of the commonly used clinical acute phase protein α2 macroglobulin. Infection with a variety of pathogens can lead to liver damage. Monitoring the level of LCN2 allows doctors to evaluate disease progression and treatment efficacy. LCN2 also serves as a predictive marker in certain end-stage liver diseases, holding promise as a novel diagnostic marker. Additionally, due to its robust iron-binding capacity, targeting LCN2 presents great potential in the treatment of infectious diseases.

Indeed, the application of LCN2 in clinical setting is not without challenges. First, given that LCN2 is expressed at different levels in different organs, plasma, and urine, it is crucial to determine the appropriate situations to test specific samples and develop standardized sample-handling protocols. Second, the variability in LCN2 data across studies is attributed to different methods of detection. Addressing this issue would entail standardizing the detection methods and establishing specific thresholds for diagnosis, necessitating future work with large sample verification. Third, LCN2, being a very sensitive indicator of inflammation, is also sensitive to various other factors. Therefore, evaluating and mitigating the impact of confounding factors on LCN2 reading is essential. Finally, despite promising results in drug studies targeting LCN2, extensive experiments are required to confirm whether regulating iron metabolism *via* LCN2 may lead to unexpected side effects, as iron involves many aspects of the body's functions.

FOOTNOTES

Co-first authors: Feng Chen and Shan-Shan Wu.

Author contributions: Chen F and Zhou C contributed to the concept and design of the whole study; Chen F prepared the draft; Chen F and Zhou C wrote and revised the manuscript; Chen C contributed to drawing the figure; All authors contributed to preparing, reading, and approving the final manuscript; Chen F and Wu SS have been working together on the research of LCN2; Wu SS participated in the conception of the paper and was instrumental and responsible for the comprehensive literature search, figure plotting, preparation and submission of the current version of the manuscript; Chen F and Wu SS have made crucial and indispensable contributions to the publication of this manuscripts as the co-first authors.

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

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S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 186-192

DOI: 10.4254/wjh.v16.i2.186

ISSN 1948-5182 (online)

MINIREVIEWS

Recent advances in the diagnosis of drug-induced liver injury

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: He YH, China

Received: September 29, 2023
Peer-review started: October 1, 2023
First decision: November 7, 2023
Revised: January 3, 2024
Accepted: February 3, 2024
Article in press: February 3, 2024
Published online: February 27, 2024

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Abstract

Drug-induced liver injury (DILI) is a major problem in the United States, commonly leading to hospital admission. Diagnosing DILI is difficult as it is a diagnosis of exclusion requiring a temporal relationship between drug exposure and liver injury and a thorough work up for other causes. In addition, DILI has a very variable clinical and histologic presentation that can mimic many different etiologies of liver disease. Objective scoring systems can assess the probability that a drug caused the liver injury but liver biopsy findings are not part of the criteria used in these systems. This review will address some of the recent updates to the scoring systems and the role of liver biopsy in the diagnosis of DILI.

Key Words: Drug induced liver injury; Liver biopsy; Diagnosis; RUCAM; RECAM

Core Tip: Diagnosing drug induced liver injury (DILI) remains a challenge in the absence of a reliable biomarker. This review highlights some of the recent advances in causality assessment in DILI that will allow clinicians to be more certain in making a diagnosis.

Citation: Ahmed T, Ahmad J. Recent advances in the diagnosis of drug-induced liver injury. *World J Hepatol* 2024; 16(2): 186-192
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/186.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.186

INTRODUCTION

Drug-induced liver injury (DILI) is a major problem in the United States, commonly leading to hospital admission. Diagnosing DILI is difficult as it is a diagnosis of exclusion requiring a temporal relationship between drug exposure and liver injury and a thorough work up for other causes. In addition, DILI has a very variable clinical and histologic presentation that can mimic many different etiologies of liver disease. Objective scoring systems can assess the probability that a drug caused the liver injury but liver biopsy findings are not part of the criteria used in these systems. This review will address some of the recent updates to the scoring systems and the role of liver biopsy in the diagnosis of DILI.

DIAGNOSING DILI

The diagnosis of DILI is challenging due to the lack of specific biomarkers and the variable presentation. DILI is a diagnosis of exclusion but there should be a temporal relationship to exposure to a drug, herbal product, or dietary supplement (HDS)[1-3]. DILI can essentially present as any type of liver disease so viral disease,autoimmune hepatitis, vascular liver disease, biliary tract disease and malignancy, all need to be excluded when assessing causality. Several society guidelines on DILI agree that to make a diagnosis of DILI, there should be thorough testing for other etiologies of liver disease[4-6]. The type of testing will vary depending on the clinical situation. In patients with hepatocellular injury, viral serology with hepatitis C viral RNA is indicated, even without an obvious risk factor[4,7]. Hepatitis E testing is important in older patients and in parts of the world where hepatitis E is endemic[8]. For cholestatic injury, imaging of the biliary tree is mandatory to exclude obstruction from gallstones or malignancy and to look for evidence of biliary disease such as primary sclerosing cholangitis (PSC)[4-6]. A recent study has suggested that DILI can present with a sclerosing cholangitis type picture[9], making differentiation with PSC difficult. Vascular imaging with computed tomography or Doppler ultrasound is indicated if Budd-Chiari or vascular compromise is suspected[4-6]. Autoimmune hepatitis (AIH) is a special situation as some drugs can lead to a drug-induced AIH and differentiating de novo AIH from drug-induced AIH can be difficult, even with autoimmune serology and liver biopsy[4]. Drugs associated with drug induced AIH include older agents such as nitrofurantoin, minocycline, hydralazine and methyldopa, but also several newer drugs, particularly immune checkpoint inhibitors[10]. In addition, a history of concomitant medications, latency (time from drug start/end to injury onset), dechallenge (improvement in liver tests after cessation of medication) and rechallenge are important factors[3,4,11].

National and international registries of DILI exist and typically employ expert consensus opinion to assess whether a drug caused a liver injury (*causality)*, such as in the Drug-Induced Liver Injury Network (DILIN). DILIN is funded by the National Institutes of Health in the United States and is an observational cohort study that enrolls patients with suspected DILI in multiple centers across the country. Causality is assessed based on a 5 point categorical scale but is undertaken 6 months after the onset of liver injury which allows interpretation of all the data. Experts review a clinical narrative summarizing the initial presentation and clinical and laboratory outcomes along with a summary of all available laboratory, radiological, and histological data[3]. Critically, this review is undertaken 6 months after the injury occurred and means there is plenty of time to review the course of the injury and various tests including serology and liver biopsy. When available, the liver biopsy is reviewed by an expert liver pathologist. This does not mirror the typical clinical situation where the diagnosis has to be made in real-time.

Practicing clinicians trying to make a diagnosis of DILI must rely on the history and local laboratory testing and abdominal imaging. If liver biopsy is performed it is unlikely to be reviewed by expert liver pathologists and expert hepatology opinion is rarely available. Fortunately, over the last 10 years, an online resource- livertox.nih.gov- has become a very valuable tool that details the typical liver injury from most prescription medications and many HDS[12]. The *livertox* database is regularly updated and provides an approximation to expert opinion.

DILI SCORING SYSTEMS

As well as *livertox*, a readily available online or digital tool for assessing DILI causality would be very useful. The Roussel Uclaf Causality Assessment Method (RUCAM) score (developed by the Council of International Organizations of Medical Sciences, an entity established by the World Health Organization and the United Nations Educational, Scientific and Cultural Organization was designed to be such a tool[13]. First introduced 30 years ago, the RUCAM score uses 8 factors in 7 domains (age over 55 years; presence of alcohol or pregnancy; latency (time from drug start to liver injury); time for dechallenge; exclusion of other causes; hepatoxicity (published or mentioned in the package insert; concomitant medications and positive rechallenge) and assigns points in each to produce an overall score. It has 5 categories based on the numerical score- highly probable (> 8), probable (6-8), possible (3-5), unlikely (1-2) and excluded (0 or less). Liver histology is not one of the domains included in the RUCAM score[13].

There are several criticisms of the RUCAM domains that suggest it may not be very accurate. RUCAM favors DILI if the temporal association to onset of injury is within 5-90 d from drug administration with initial treatment and within 15 d with subsequent exposure. The rate of decline of biochemical tests (the dechallenge) after discontinuation of the drug is also an important criteria with a decrease in alanine aminotransferase (ALT) > 50% from the upper limit of normal within 8 dwithout a subsequent uptrend in one month highly suggestive of DILI. Although uncommon, readministration or rechallenge of the drug with a two-fold increase in ALT favors DILI. However, evaluation of the latency period and the

dechallenge has changed with the recognition that certain drugs have a typically very short or long latency. The domain regarding the published literature of hepatotoxcity of the potential offending agent will inevitably change with time.

The points assigned in the age and alcohol domains were based on older literature and are not considered important today. Concomitant medications often present a problem as most drugs can cause DILI. In the case of polypharmacy it is often difficult to identify the offending agent, particularly if there are over the counter medications that are taken intermittently and the use of HDS is notorious for not being disclosed[14].

In addition, excluding other causes of liver disease involves more testing than was previously available, particularly with regards to viral hepatitis and the presence of underlying chronic liver disease is not taken into account in the RUCAM[4-6].

Comparing the RUCAM score with the structured expert opinion process which DILIN utilizes, demonstrated that the DILIN expert process yielded a higher overall causality consensus of DILI probability. Additionally, RUCAM assessment did not perform as well as expert opinion in cases restricted to a single implicated agent. This highlights the subjectivity of certain RUCAM criteria when even experts have difficulty agreeing, particularly as expert opinion is not available in routine practice[3].

An updated RUCAM version has been proposed with pre-scoring consideration for hepatocellular, cholestatic or mixed liver injury patterns[15]. This is determined with calculation of the R factor [ALT/alkaline phosphatase (ALP) on initial presentation and suspicion of DILI]. Hepatocellular injury is defined by $R \geq 5$, cholestatic injury with $R \leq 2$, and a mixed hepatic and cholestatic pattern with an $R > 2$ but < 5. The corresponding scoring method is then used based on the pattern of liver injury to aid causality assessment in DILI. Primary differences between the hepatocellular injury and cholestatic/mixed injury updated RUCAM is the percentage of improvement of ALT *vs* ALP, respectively, as discussed in criteria 2 of the original 1993 RUCAM. Similarly, criteria 7 measures any interval increase in ALT *vs* ALP in the hepatocellular *vs* cholestatic/mixed injury assessments, respectively, with re-exposure to the drug. Given the shortcomings of the RUCAM, the DILIN and the Spanish DILI Registry developed and validated a revised tool into an easily accessible electronic version termed the revised electronic causality assessment method (RECAM)[15]. Major changes included a much more detailed point-system regarding latency including time to injury after the drug was first taken and when stopped (domains 1a & 1b), dechallenge period standardized irrespective of R-value (domain 2), omission of risk factors (RUCAM criteria 3) and concomitant drugs (RUCAM criteria 4) since they are assessed separately. The major revision was the addition of domain 3 (literature supporting liver injury) using the National Institute of Diabetes and Digestive and Kidney Diseases LiverTox category for each drug[12]. Excluding other causes of liver disease such as viral hepatitis (including hepatitis A, B, C, and E), auto-immune disease, alcohol, biliary tract disease, infection/sepsis and ischemic liver injury encompasses domain 4 (former RUCAM criteria 5). Domain 5 was termed additional data with points awarded for several situations including rechallenge and liver biopsy findings (see below). Additional information, if available, is also included in domain 5 such as the presence of severe skin reactions, or atypical viral testing. Moreover, a warning is issued if a firm alternate diagnosis is suspected, or injury timing is inconsistent with DILI to the user making the diagnosis of DILI highly unlikely. Table 1 delineates the key differences between the original RUCAM and RECAM. Although more complex, incorporation of additional criteria make the RECAM a more accurate tool to assess causality in DILI.

The RECAM has the advantage of being adaptable to new findings, particularly in domain 3 as more reports of liver injury from certain drugs increases the likelihood category in LiverTox. This raises the possibility of newer domains being added. A good example of this is genetic risk factors that may affect the risk of DILI, especially variants in genes involved in drug metabolism or immune response. Multiple HLA and non-HLA polymorphisms have been described that can increase the risk of liver injury considerably but these are of limited clinical use currently as they are drug specific[10]. Recent examples include HLA-B35:01 for green tea extract associated DILI (7-fold increased risk)[16], HLA-B*53:01 for phenytoin associated DILI in African Americans (9-fold increase)[17], and HLA-DRB1*11:04 for nitrofurantoin DILI (4-fold increase)[18]. Newer genetic polymorphisms have been described, such as the PTPN22 gene, a gene associated with many immune-mediated diseases, that increase the risk of DILI for many drugs but only at a low level with an odds ratio of 2[19]. Since DILI is a rare event, even a 10 or 100 fold increased risk does not make the event common enough to warrant genetic testing prior to prescription.

LIVER BIOPSY

Half of all patients enrolled into DILIN undergo a liver biopsy. The liver biopsy is usually undertaken in a situation where the diagnosis is uncertain or in more severe cases[20]. For management decisions, the liver biopsy is performed early during the course of the injury as it may can determine if steroids are required or if the disease is very severe and there is a need to consider liver transplantation. Few diseases have a diagnostic pathologic finding but liver biopsy can support a diagnosis and can eliminate other causes of liver injury (such as autoimmune hepatitis and hemochromatosis) [21-23]. In DILI registries, hepatocellular injury is the most common presentation and pathologic changes can range from mild injury to confluent hepatocellular necrosis and in severe cases results in acute liver failure with massive necrosis. In 5-10% of cases, acute hepatocellular injury can progress to chronic injury mimicking alcoholic cirrhosis, autoimmune hepatitis, or chronic viral hepatitis. Several drugs are associated with this type of injury including TNF-alpha inhibitors [24] and antibiotics such as minocycline[25] and nitrofurantoin[18]. Similarly, certain drugs classically cause a cholestatic injury with characteristic findings on liver biopsy such as bland cholestasis with bile plugging and minimal hepatocellular injury mainly seen with oral contraceptives and anabolic steroids[26]. Mixed liver injury or cholestatic hepatitis has histologic findings of cholestasis with surrounding hepatocyte injury and portal inflammation that can be seen with

Table 1 Differences between The Roussel Uclaf Causality Assessment Method and revised electronic causality assessment method scores

RUCAM	RECAM
Criteria 1: Latency	Domain 1a: Latency from drug start
	Domain 1b: Latency from drug stop
Criteria 2: Dechallenge	Domain 2: Dechallenge
Different time cut-offs based on *R* value	Same time cut-offs regardless of *R* value
Criteria 3: Risk factors	Eliminated
Criteria 4: Concomitant drugs	Domain 3: Literature supporting drug toxicity (LiverTox)
Criteria 5: Exclusion of non-drug etiologies	Domain 4: Exclusion of non-drug etiologies
Criteria 6: Known hepato-toxicity of drug	Became domain 3
Criteria 7: Rechallenge response	Domain 5: Rechallenge response - both prospectively documented with lab testing and retrospective based on patient history. Includes additional data: Liver biopsy results, atypical viral testing, and presence of severe skin reactions

RUCAM: The Roussel Uclaf Causality Assessment Method; RECAM: Revised electronic causality assessment method.

use of amoxicillin-clavulanate, erythromycin, and herbal supplements[21-23].

It is not feasible to conduct a randomized clinical trial to determine the role liver biopsy plays in causality assessment in DILI. Selection bias is a problem when examining patients suspected of DILI that have already undergone a liver biopsy, as the result of the biopsy may have influenced subsequent management. To try and answer the question of how liver biopsy findings impact causality assessment, DILIN investigators in the United States assessed causality in a cohort of patients with suspected DILI, prior to obtaining a liver biopsy, and then repeated causality assessment after reviewing the liver biopsy[20]. All subjects in this study had been enrolled in the DILIN database and had had a liver biopsy performed within 60 d of DILI onset. Investigators reviewed data obtained before the liver biopsy was performed and assigned a causality score (the pre-score) and then reviewed the biopsy with an expert liver pathologist an assigned a post-biopsy causality score. The liver biopsy altered causality assessments in 68% of cases with an increase in DILI likelihood in 48% and made exclusion of DILI more certain in 20% of cases with a cumulative clinically meaningful change in 16% of cases. However, situations exist where the injury from DILI can be virtually indistinguishable such as differentiating AIH from drug-induced AIH[27].

A few caveats should be considered when considering the role of liver biopsy in support of a DILI diagnosis. While there is a suggested timing for DILI with temporal association between drug use and onset of symptoms, there is no suggested timing for obtaining liver biopsies with suspected DILI. The liver injury in DILI can evolve from initially hepatocellular to cholestatic later in the course and the degree of jaundice can worsen. Additionally, when a patient is biopsied, the zone of hepatocellular or cholestatic injury may not be found in the particular lobe or segment from which the biopsy is obtained as seen with acute zonal hepatocellular DILI[22,23]. Biopsies are often inadequate without enough portal tracts or poorly stained. Similar to the updated RUCAM score which is reliant on expert opinion, a biopsy read is pathologist-dependent, often read by community pathologists without much liver pathology experience. Kleiner *et al*[23] described an approach for evaluating hepatic histological findings in patients with suspected DILI correlating pathology with causality and clinical outcome. Up to 10 sections of liver biopsies were obtained from each patient for various staining and reviewed by the same blinded hepatic pathologist. Causality assessment as definite, very likely, probable, possible, or unlikely was assigned to each case. Biopsies in the definite to probable criteria had statistically significant increase in eosinophils ($P = 0.04$), decreased ductal reaction ($P = 0.04$), and decreased hepatocellular iron accumulation ($P = 0.0008$). Nearly 70% of the reviewed samples were implicated with a single agent, while the remaining samples were involved 2 or more hepatotoxic agents. Most common associated drugs included antibiotics such amoxillicin-clavulanate, nitrofurantoin, and sulfamethaxazole-trimethoprim. However, this again highlights some of the limitations of biopsy as these samples met strict criteria in terms of the size and number of portal tracts and were all reviewed by a very experienced liver pathologist. In addition, the drugs that were associated with liver injury in this study are the most common prescription drugs associated with DILI in the United States, suggesting the pre-biopsy likelihood of DILI was already quite high.

Liver biopsy may be helpful in prognosticating the severity and possible course or recovery of DILI. Composite data of patients enrolled in DILIN revealed favorable clinical outcomes in patients with hepatic eosinophil infiltration and eosinophilia[23]. However, hepatocyte drop-out or necrosis, microvesicular steatosis, and fibrosis were seen in severe or fatal cases. It is worth noting that although eosinophilia is associated with hypersensitivity features of fever and rash as seen in drug reaction with eosinophilia and systemic symptoms syndrome, these symptoms were seldom seen in the included patients with high suspicion for DILI. Although fibrosis can be seen with amiodarone or nitrofurantoin use, it may indicate undiagnosed underlying chronic hepatic disease and limit response to injury. Micro and macrovesicular

steatosis reflect mitochondrial injury secondary to fatty acid oxidation and is usually associated with higher clinical severity as seen in acute fatty liver of pregnancy[23,28].

Society guidelines on DILI have recommendations on when to perform liver biopsy in DILI but are hampered by low quality evidence so are not very definitive. The American College of Gastroenterology guideline suggests liver biopsy in situations where AIH is on the differential diagnosis and immunosuppression is being contemplated; if the injury is not improving; and if the injury persists for more than 180 d[4]. The Asia Pacific Association of Study of Liver guideline is more general and states to consider liver biopsy if an alternative diagnosis needs to be ruled out or if patients fail to respond after the suspected offending agent is stopped[6]. The European Association for the Study of the Liver guideline are similar, suggesting liver biopsy in selected patients; if AIH is suspected; and if the liver injury persists or worsens[5].

The conclusion from these guidelines are that liver biopsy can be considered in patients where the diagnosis is not certain, particularly if the injury is not improving and if AIH is on the differential diagnosis.

The diagnosis of DILI continues to be a clinical challenge due to several confounding variables that albeit known, undisclosed, or undiagnosed at the time of initial clinical evaluation, affect attributing a correct diagnosis of DILI. A recent review of patients enrolled in DILIN reported 1.5% of cases from 2004-2016 were found to have acute hepatitis C in the 6 month follow up period from enrollment[7]. At enrollment, serologic assessment was done to exclude other causes of hepatic injury including testing for viral hepatitis. Routinely, anti-hepatitis C antibodies were collected, however, hepatitis C virus (HCV) RNA was sent at the discretion of the investigator at that time. At the 6-month follow up period, stored serum samples were retrospectively analyzed for HCV RNA if this had not been initially tested, revealing 23 cases of acute hepatitis C with varying initial degrees of suspicion for DILI from highly probable to unlikely. As with any diagnosis of exclusion, cumulative and complete data with a possible temporal advantage helps illuminate missed underlying diagnoses. However, it is important to note that uncovering acute hepatitis C in these patients was possible due to stored sera from which is not routinely possible in every clinical encounter. Other retrospective studies evaluated the presence of acute hepatitis E in patients enrolled in DILIN[8,29]. Stored sera were tested for anti-hepatitis E antibody (anti-HEV), HEV IgM, HEV IgG, and HEV RNA levels. The results revealed 1.5% of patients with active hepatitis E at the time of suspected DILI. These were predominantly older men almost all of whom presented with typical acute viral hepatitis-like symptoms including fatigue, nausea, abdominal pain, and jaundice. Similarly, this study reiterates the difficulty and importance to differentiate whether an acute hepatic injury is attribute to another etiology rather than DILI.

CONCLUSION

In conclusion, while the diagnosis of DILI remains challenging for clinicians due to the absence of a standardized diagnostic criteria or a specific biomarker, recent literature has reiterated the importance of complete exclusion of other etiologies for hepatic injury as seen in uncovered cases of acute hepatitis C and E with repeat serologies or liver biopsy results that significantly changed expert opinion regarding DILI likelihood. Additionally, the revision and digitalization of RUCAM into RECAM facilitates the diagnostic evaluation and probability of a DILI diagnosis. While a liver biopsy is not necessary for establishing a DILI diagnosis, histologic findings can augment or exclude DILI in certain patients and help to differentiate AIH from DILI and the need for immunosuppression.

FOOTNOTES

Author contributions: Ahmed T and Ahmad J contributed equally to this work; All authors have read and approve the final manuscript.

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

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zheng XM

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 193-210

DOI: 10.4254/wjh.v16.i2.193

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Predicting major adverse cardiovascular events after orthotopic liver transplantation using a supervised machine learning model: A cohort study

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Ferrarese A, Italy

Received: November 2, 2023
Peer-review started: November 2, 2023
First decision: December 1, 2023
Revised: December 27, 2023
Accepted: February 4, 2024
Article in press: February 4, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

Liver transplant (LT) patients have become older and sicker. The rate of post-LT major adverse cardiovascular events (MACE) has increased, and this in turn raises 30-d post-LT mortality. Noninvasive cardiac stress testing loses accuracy when applied to pre-LT cirrhotic patients.

AIM

To assess the feasibility and accuracy of a machine learning model used to predict post-LT MACE in a regional cohort.

METHODS

This retrospective cohort study involved 575 LT patients from a Southern Brazilian academic center. We developed a predictive model for post-LT MACE

(defined as a composite outcome of stroke, new-onset heart failure, severe arrhythmia, and myocardial infarction) using the extreme gradient boosting (XGBoost) machine learning model. We addressed missing data (below 20%) for relevant variables using the k-nearest neighbor imputation method, calculating the mean from the ten nearest neighbors for each case. The modeling dataset included 83 features, encompassing patient and laboratory data, cirrhosis complications, and pre-LT cardiac assessments. Model performance was assessed using the area under the receiver operating characteristic curve (AUROC). We also employed Shapley additive explanations (SHAP) to interpret feature impacts. The dataset was split into training (75%) and testing (25%) sets. Calibration was evaluated using the Brier score. We followed Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis guidelines for reporting. Scikit-learn and SHAP in Python 3 were used for all analyses. The supplementary material includes code for model development and a user-friendly online MACE prediction calculator.

RESULTS

Of the 537 included patients, 23 (4.46%) developed in-hospital MACE, with a mean age at transplantation of 52.9 years. The majority, 66.1%, were male. The XGBoost model achieved an impressive AUROC of 0.89 during the training stage. This model exhibited accuracy, precision, recall, and F1-score values of 0.84, 0.85, 0.80, and 0.79, respectively. Calibration, as assessed by the Brier score, indicated excellent model calibration with a score of 0.07. Furthermore, SHAP values highlighted the significance of certain variables in predicting postoperative MACE, with negative noninvasive cardiac stress testing, use of nonselective beta-blockers, direct bilirubin levels, blood type O, and dynamic alterations on myocardial perfusion scintigraphy being the most influential factors at the cohort-wide level. These results highlight the predictive capability of our XGBoost model in assessing the risk of post-LT MACE, making it a valuable tool for clinical practice.

CONCLUSION

Our study successfully assessed the feasibility and accuracy of the XGBoost machine learning model in predicting post-LT MACE, using both cardiovascular and hepatic variables. The model demonstrated impressive performance, aligning with literature findings, and exhibited excellent calibration. Notably, our cautious approach to prevent overfitting and data leakage suggests the stability of results when applied to prospective data, reinforcing the model's value as a reliable tool for predicting post-LT MACE in clinical practice.

Key Words: Liver transplantation; Major adverse cardiac events; Machine learning; Myocardial perfusion imaging; Stress test

Core Tip: This study presents a robust machine learning model, utilizing the XGBoost algorithm, to predict major adverse cardiovascular events (MACE) following liver transplantation. The model demonstrated high accuracy and calibration, with key factors such as noninvasive cardiac stress test outcomes, use of nonselective beta-blockers, direct bilirubin levels, blood type O, and dynamic alterations on myocardial perfusion scintigraphy identified as significant predictors. This tool offers valuable insights into the risk assessment of post-liver transplant MACE, particularly in an aging and comorbid patient population.

Citation: Soldera J, Corso LL, Rech MM, Ballotin VR, Bigarella LG, Tomé F, Moraes N, Balbinot RS, Rodriguez S, Brandão ABM, Hochhegger B. Predicting major adverse cardiovascular events after orthotopic liver transplantation using a supervised machine learning model: A cohort study. *World J Hepatol* 2024; 16(2): 193-210
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/193.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.193

INTRODUCTION

The population of liver transplant (LT) candidates has become older and sicker, experiencing higher morbidity[1]. This might be due to the increasing prevalence of metabolic-associated fatty liver disease (MAFLD) as a cause of cirrhosis and end-stage liver disease (ESLD)[2-5]. As a result, there is an expected rise in the incidence of major adverse cardiovascular events (MACE) following LT, a well-documented complication of LT that negatively impacts prognosis[6-10].

The occurrence of MACE in the post-LT period is a significant concern, since these events contribute to increased mortality and jeopardize the success of LT[11]. Previous literature suggests that the incidence of post-LT MACE can be as high as 41% within the first 6 months following LT, which translates into a higher mortality rate[6,10]. Various traditional and nontraditional cardiovascular risk factors may contribute to these adverse events, including preexisting coronary disease, obesity, reduced cardiovascular reserve, poor response to cardiovascular stress, cirrhotic cardiomyopathy, increased predisposition to arrhythmias, and heart failure exacerbations[12-15]. The prioritization for transplant of sicker

patients with a high burden of critical illness, associated with a higher prevalence of cardiovascular disease, further exacerbates the risk[16]. However, the relative contribution of these factors remains incompletely characterized[7,17,18].

In addition to population aging, there has been a significant change in the most prevalent etiology leading to LT, with an increase in MAFLD observed both in the West and in the East[2,19]. Currently, MAFLD is the fastest-growing indication for LT in Western countries, having become the leading indication for LT waitlisting in the United States[5], as predicted by previous studies[20]. Moreover, MAFLD is strongly associated with a higher prevalence of diabetes mellitus, morbid obesity, and coronary artery disease (CAD)[4,5,8]. This specific population thus requires a detailed pre-LT cardiac evaluation, with particular attention to the increased risk of CAD, as they have a higher risk of cardiac events compared to those without MAFLD[8,21].

The first stage of cardiac evaluation usually involves assessing risk factors and subsequently performing noninvasive stratification. However, this approach is still controversial. In 2014, the American Association for the Study of Liver Diseases updated its guideline, maintaining the recommendation that patients undergoing pre-LT evaluation should complete a noninvasive myocardial stress test[22]. Conversely, the 2012 guideline developed by the American Heart Association in conjunction with the American College of Cardiology[23] suggests performing a noninvasive myocardial stress test only for patients with three or more risk factors for CAD. However, systematic reviews have demonstrated that current noninvasive strategies, such as myocardial perfusion scintigraphy (MPS) and dobutamine stress echocardiography (DSE), are unreliable and inadequate for predicting MACE, mortality, and significant CAD after LT[24-26]. Therefore, there is an unmet need for an alternative approach to accurately predict post-LT MACE in this vulnerable patient population[18,27].

Few models are available to assist clinicians in accurately stratifying the cardiovascular risk of LT candidates, especially those with ESLD[18]. Existing models often rely on traditional logistic regression statistics, making assumptions of independent linear relationships between dependent and independent variables[28]. These models are further constrained by small sample sizes and the limited number of variables for which they can account, primarily due to concerns of overfitting and multicollinearity. They are also unable to accurately consider the small effects of minor variables and their complex correlations[18,28]. Two scores have been developed using such models, the CAD-LT[29], and the CAR-orthotopic liver transplantation (OLT)[30]. The CAD-LT has demonstrated ability to stratify the risk of CAD into low, intermediate, and high categories, while the CAR-OLT point-based prediction model has shown superior performance compared to other existing risk models in predicting post-LT MACE.

In addition, patients with liver cirrhosis exhibit significant peripheral vasodilation, which can alter cardiac function and mask the presence of CAD, leading to what is now termed cirrhotic cardiomyopathy, a distinct pathologic entity for which diagnostic criteria were published in 2020[31]. In the 1990s, a high mortality rate (around 50%) was reported in patients with significant CAD in the peri-LT period[32]. However, in the last decade, with improved pre-LT cardiac therapy, it is believed that the presence of CAD does not significantly alter the post-LT survival of these patients[33].

To overcome these limitations, we propose the use of machine learning, a subarea of computer science that focuses on predicting outcomes using computational models that iteratively learn from data[34,35]. Machine learning models have demonstrated robust performance in various fields in gastroenterology[36], such as the diagnosis of hepatocellular carcinoma[37], prognostication of variceal hemorrhage[38,39], prediction of acute kidney injury after LT[40], short- and long-term post-LT mortality[41], and adverse cardiovascular events in various medical conditions[42]. Unlike conventional statistical models, machine learning models can detect complex patterns and relationships within datasets without relying on fixed assumptions about data behavior or pre-selection of variables, using correlations within variables to determine outcome[43].

The aim of this study is to conduct a comprehensive assessment of the feasibility and accuracy of employing a machine learning model for prediction of MACE following LT. The study focuses on a specific regional cohort to examine the potential of machine learning techniques in effectively forecasting post-LT MACE. By leveraging advanced computational models, this research aims to enhance the predictive capabilities in identifying individuals at higher risk of experiencing MACE after LT, thereby enabling early intervention strategies and optimizing patient care.

MATERIALS AND METHODS

This retrospective cohort study was approved by the Research Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre under protocol no. 07793412.2.3001.5345 on May 22, 2013, and conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The study utilized medical records from Irmandade Santa Casa de Misericórdia de Porto Alegre (Rio Grande do Sul, Brazil).

Inclusion and exclusion criteria

Patients above 18 years of age who underwent their first LT at Irmandade Santa Casa de Misericórdia de Porto Alegre, Guido Cantisani LT Team, Brazil, for cirrhosis, between January 1, 2001, and December 31, 2011, were eligible. Patients without cirrhosis, those with incomplete medical records, those who did not undergo cardiac evaluation prior to LT, retransplantation cases, and living-donor LT recipients were excluded. Patients with 20% or more missing data were excluded.

Outcomes

Data were systematically collected on structured forms encompassing extensive clinical and laboratory variables from the pre-LT, perioperative, and post-LT periods. The primary outcome of interest was any in-hospital MACE, a composite

outcome including stroke, new-onset heart failure, severe arrhythmia, and myocardial infarction. Statistics, including frequency, means, SD, and tests such as Pearson's χ^2 test and linear model analysis of variance (ANOVA), were conducted in R software (version 4.3.2) using the 'readxl' and 'dplyr' packages, with the analysis involving data manipulation and exploration.

Machine learning approach and model definition

We employed the extreme gradient boosting (XGBoost) model, available through the XGBoost package, to construct a classification model aimed at predicting post-LT MACE. XGBoost is particularly effective in handling imbalanced datasets and offers native support for missing data and categorical variables, making it particularly useful for real-world applications. The columns considered to compose the outcome variable were not included in the model to avoid bias and collinearity.

Data pre-processing and feature engineering

The dataset was divided into training (75%) and test (25%) sets, preserving the outcome proportions in both subsets[44]. The training set is used to teach the model, and the test set is used to evaluate how well the model has learned. To mitigate the risk of introducing bias by excluding patients with missing values, we employed a two-step imputation process using the Scikit-Learn package. First, we removed variables that had missing values for more than 20% of the patient population. Following this, we used the k-nearest neighbor (kNN) imputation algorithm to fill in the missing values for the remaining continuous variables, imputing the calculated mean value among the 10 closest neighbors. Of 83 features screened, the model incorporated 50 according to the measure of the impact of each feature on the model's prediction for an instance. This included patient demographics, laboratory data, medical history, and pre-LT cardiac evaluations, selected after an initial screening. Categorical and numerical variables were imputed using mode and kNN imputation, respectively. To avoid data leakage, transformations were first trained on the training dataset, and only then applied to test data. To simulate real-world settings in which missing data are often present, we trained an additional model without the imputation and one-hot step and describe its results following the main model report.

Model training and hyperparameter optimization

Overfitting is a problem that occurs when a machine learning model learns the training data too well and is unable to generalize to new data. This can happen when the model is too complex or when the training dataset is too small or noisy. As a result, the model outputs extremely accurate results in the training set but performs poorly on unseen test-set data. To avoid overfitting, we applied regularization and early-stop techniques during the training of the model, as described in the code. Regularization is a technique that penalizes the model for being too complex; early stopping is a technique that stops training the model when it starts to overfit the training data.

Hyperparameters are external configurations for the model that are not learned from the data and are used to optimize the model's performance. The training set was used for model training, while the test set was reserved for performance evaluation. The Optuna package was used for hyperparameter optimization. Additional information about the model hyperparameter results and training are provided as supplemental material.

Performance assessment

The area under the receiver operating characteristic curve (AUROC) was used as an evaluation metric and reported with a 95% confidence interval (CI). To calculate the AUROC, the true positive rates are compared against the false positive rates at various threshold settings. The AUROC represents the degree or measure of separability, indicating how well the model distinguishes between the classes.

The model's performance in predicting positive cases was also assessed using the area under the precision-recall curve (AUC-PR). The AUC-PR is a graphical representation of a model's precision and recall at different thresholds, which are the points where the model decides which class an instance belongs to. It is particularly useful when the classes are imbalanced. The x-axis represents recall (the proportion of actual positive cases that were correctly classified) and the y-axis represents precision (the proportion of cases classified as positive that are indeed positive). A higher AUC-PR indicates better performance in distinguishing between the classes.

In evaluating the model's ability to predict positive cases, additional metrics were employed, such as recall, precision, sensitivity, specificity, accuracy, and F1-score. Recall measures the model's effectiveness in correctly identifying actual positive cases among all positive instances. It is calculated by dividing the number of true positives by the sum of true positives and false negatives. Precision assesses the accuracy of the model's positive predictions by calculating the proportion of true positives among all instances predicted as positive, determined by dividing the number of true positives by the sum of true positives and false positives. Sensitivity evaluates the model's capability to identify positive cases accurately, similar to recall. Specificity measures the model's ability to correctly identify negative cases by calculating the proportion of true negatives among actual negatives. Accuracy reflects the overall correctness of the model's predictions, considering both true positives and true negatives relative to the total number of predictions. F1-score represents the harmonic mean of precision and recall, providing a balanced assessment of the model's performance. The statistical methods of this study were reviewed by co-author Corso LL.

Calibration assessment

Calibration is the process of refining the model to ensure that the predicted probabilities of an event occurring align well with the actual probabilities. We tested various methods of calibration for the validation model, including sigmoid, isotonic, and Gaussian calibration. We used calibration curves to present the comparison graphically. We used the Brier

score to choose the model with the best calibration for deployment and explanation of feature importance.

Model explanation and interpretation

The Shapley additive explanations (SHAP) framework was used to interpret the output of machine learning models, providing a measure of the impact of each feature on the model's prediction for an instance. SHAP are based on game theory and assign an importance value to each feature in a model. Features with positive SHAP values positively impact the prediction, while those with negative values have a negative impact. The magnitude of the SHAP value is a measure of how strong the effect is. To calculate SHAP values, we consider all possible combinations of features (coalitions) and how they affect the model's prediction. We then average the marginal contribution of each feature across all possible coalitions. This gives us a measure of how much each feature contributes to the model's prediction, taking into account the interactions between features.

Checklist adherence

In accordance with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement, we have followed a comprehensive reporting framework for this study. The TRIPOD statement guided the design, implementation, and reporting of our prediction model for post-LT MACE and the respective checklist for present study is presented as a Supplementary material. The checklist comprised the 22 items outlined in the TRIPOD statement, ensuring transparency and rigor in our methodology and reporting.

Code availability and web deployment

The code employed for data preprocessing, feature engineering, and model development and evaluation is in an accessible public repository (link provided in the supplemental materials). Furthermore, we have deployed our model as a user-friendly MACE prediction calculator, which is now available online at https://huggingface.co/spaces/mmrech/mace-calc. The frontend application was coded with the Streamlit library. The model was originally saved and then loaded as a joblib file, and the backend application was deployed with Hugging Face Spaces. All phases from data preprocessing to model deployment were implemented in Python 3.

RESULTS

A comprehensive search of hospital databases identified a total of 662 patients who had undergone LT during the study period. From this initial cohort, 82 patients were excluded based on specific criteria. The reasons for exclusion were as follows: 19 patients transplanted due to fulminant liver failure, 32 patients who had undergone retransplantation, 7 patients transplanted due to familial amyloid polyneuropathy, 1 patient excluded due to amyloidosis without cirrhosis, 1 patient due to congenital hepatic fibrosis, 27 patients due to insufficient cardiological data, 2 patients who received living-donor grafts, 2 patients with primary hyperoxaluria, 2 patients with polycystic liver disease, and 1 patient with metastasis of a neuroendocrine tumor. Another 38 patients were excluded due to the high rate of missing data among selected variables. The dataset utilized by the final model consisted of 537 samples, with 23 events and 514 non-events (Figure 1). As noted above, the original dataset was split such that 75% was used for training the model and 25% was reserved as unseen data for internal validation. The proportion of outcomes (4.46%) was maintained in both the training and the validation sets.

General cohort

Of the 537 included patients, 23 developed in-hospital MACE, with a mean age at transplantation of 52.9 years. The majority, 66.1%, were male. The overall incidence of the composite variable MACE was 4.46%. The components of this outcome - stroke, new-onset heart failure, severe arrhythmia, and myocardial infarction - had observed rates of 0.19%, 1.3%, 1.3% and 1.67%, respectively. Detailed data on the general population included, the 50 variables used in model construction, and the composite outcomes are available in Table 1, specifying values for the total cohort, for the strata of present and absent MACE, and also their respective missing rates.

Model performance

The XGBoost model demonstrated substantial predictive capability, with an AUROC of 0.89. The classification results showed a precision of 0.89, recall of 0.80, and F1-score of 0.84 for the negative class. The AUROC and AUC-PR, along with their respective 95%CIs, are provided in Figure 2. The hyperparameters utilized for the best-performing model after optimization are provided in the supplementary materials, as is an overview regarding the role of these components in the model functionality.

Calibration

The model achieved optimal calibration with the isotonic method, as evidenced by the lowest Brier score of 0.100. This calibration demonstrated a high level of precision, recall, F1-score, and accuracy for both negative and positive classes, with closer proximity to the diagonal line on the calibration curve (Supplementary Figure 1). Calibration curve is provided as a supplemental material.

Table 1 Cohort patient data

Variable	Total (*n* = 537)	MACE absent (*n* = 520)	MACE present (*n* = 17)	*P* value
Race[1]				0.41
White	463 (90.4%)	447 (90.1%)	16 (100.0%)	
Mixed/other	28 (5.6%)	28 (5.6%)	0 (0.0%)	
Black	21 (4.1%)	21 (4.2%)	0 (0.0%)	
Missing	25	24	1	
Sex[1]				0.26
Male	352 (65.7%)	343 (66.1%)	9 (52.9%)	
Female	184 (34.3%)	176 (33.9%)	8 (47.1%)	
Missing	1	1	0 (0)	
Previous esophageal variceal ligation[1]				0.71
No	327 (64.5%)	318 (64.6%)	9 (60.0%)	
Yes	180 (35.5%)	174 (35.4%)	6 (40.0%)	
Missing	30	28	2	
Portal hypertensive gastropathy[1]				0.48
Mild	229 (49.4%)	220 (49.0%)	9 (60.0%)	
Absent	83 (17.9%)	82 (18.3%)	1 (6.7%)	
Intense	152 (32.8%)	147 (32.7%)	5 (33.3%)	
Missing	73	71	2	
Previous ascites[1]				0.05
Yes	393 (74.3%)	377 (73.6%)	16 (94.1%)	
No	136 (25.7%)	135 (26.4%)	1 (5.9%)	
Missing	8	8	0	
Previous spontaneous bacterial peritonitis[1]				0.92
No	385 (74.3%)	374 (74.4%)	11 (73.3%)	
Yes	133 (25.7%)	129 (25.6%)	4 (26.7%)	
Missing	19	17	2	
Previous hepatopulmonary syndrome[1]				0.31
No	399 (77.3%)	389 (77.6%)	10 (66.7%)	
Yes	117 (22.7%)	112 (22.4%)	5 (33.3%)	
Missing	21	19	2	
Previous use of nonselective beta-blockers[1]				0.01
No	270 (53.9%)	267 (54.8%)	3 (21.4%)	
Yes	231 (46.1%)	220 (45.2%)	11 (78.6%)	
Missing	36	33	3	
Portal vein thrombosis[1]				0.04
No	443 (85.7%)	432 (86.2%)	11 (68.8%)	
Yes	74 (14.3%)	69 (13.8%)	5 (31.2%)	
Missing	20	19	1	
Hepatic encephalopathy[1]				0.44
No	277 (53.2%)	270 (53.5%)	7 (43.8%)	
Yes	244 (46.8%)	9 (56.2%)	235 (46.5%)	

Missing	16	15	1	
Previous hepatorenal syndrome[1]				0.52
No	499 (97.5%)	15 (100.0%)	484 (97.4%)	
Yes	13 (2.5%)	0 (0.0%)	13 (2.6%)	
Missing	25	23	2	
Antibiotic therapy for > 24 h[1]				0.52
No	486 (97.4%)	15 (100.0%)	471 (97.3%)	
Yes	13 (2.6%)	0 (0.0%)	13 (2.7%)	
Missing	38	36	2	
Hospitalized for > 48 h[1]				0.70
No	476 (95.4%)	462 (95.5%)	14 (93.3%)	
Yes	23 (4.6%)	22 (4.5%)	1 (6.7%)	
Missing	38	36	2	
Pre-transplant hemodialysis[1]				0.01
No	486 (97.0%)	473 (97.3%)	13 (86.7%)	
Yes	15 (3.0%)	13 (2.7%)	2 (13.3%)	
Missing	36	34	2	
Hepatocellular carcinoma[1]				0.34
No	330 (63.7%)	318 (63.3%)	12 (75.0%)	
Yes	188 (36.3%)	184 (36.7%)	4 (25.0%)	
Missing	19	18	1	
Blood group[1]				0.39
O	230 (42.9%)	221 (42.6%)	9 (52.9%)	
A	231 (43.1%)	226 (43.5%)	5 (29.4%)	
B	53 (9.9%)	50 (9.6%)	3 (17.6%)	
AB	22 (4.1%)	22 (4.2%)	0 (0.0%)	
Missing	1	1	0	
Congestive heart failure[1]				0.69
No	518 (99.0%)	502 (99.0%)	16 (100.0%)	
Yes	5 (1.0%)	5 (1.0%)	0 (0.0%)	
Missing	14	13	1	
Previous angioplasty[1]				0.75
No	520 (99.4%)	504 (99.4%)	16 (100.0%)	
Yes	3 (0.6%)	3 (0.6%)	0 (0.0%)	
Missing	14	13	1	
Dyslipidemia[1]				0.53
No	512 (97.7%)	496 (97.6%)	16 (100.0%)	
Yes	12 (2.3%)	12 (2.4%)	0 (0.0%)	
Missing	13	12	1	
Systemic arterial hypertension[1]				0.04
No	388 (73.6%)	379 (74.3%)	9 (52.9%)	
Yes	139 (26.4%)	131 (25.7%)	8 (47.1%)	
Missing	10	10	0	

Familiar history of coronary artery disease[1]				0.43
No	416 (79.8%)	402 (79.6%)	14 (87.5%)	
Yes	105 (20.2%)	103 (20.4%)	2 (12.5%)	
Missing	16	15	1	
Previous acute myocardial infarction[1]				0.06
No	515 (98.7%)	501 (98.8%)	14 (93.3%)	
Yes	7 (1.3%)	6 (1.2%)	1 (6.7%)	
Missing	15	13	2	
Previous stroke[1]				0.38
Ischemic	3 (0.6%)	3 (0.6%)	0 (0.0%)	
Hemorrhagic	27 (5.2%)	25 (4.9%)	2 (12.5%)	
No	493 (94.3%)	479 (94.5%)	14 (87.5%)	
Missing	14	13	1	
Diabetes mellitus[1]				0.21
No	396 (75.6%)	386 (76.0%)	10 (62.5%)	
Yes	128 (24.4%)	122 (24.0%)	6 (37.5%)	
Missing	13	12	1	
Valve replacement[1]				0.96
Metallic	1 (0.2%)	1 (0.2%)	0 (0.0%)	
Biological	2 (0.4%)	2 (0.5%)	0 (0.0%)	
No	445 (99.3%)	435 (99.3%)	10 (100.0%)	
Missing	89	82	7	
Mitral insufficiency[1]				0.001
Mild	137 (28.8%)	131 (28.4%)	6 (46.2%)	
Moderate	3 (0.6%)	2 (0.4%)	1 (7.7%)	
Absent	335 (70.5%)	329 (71.2%)	6 (46.2%)	
Missing	62	58	4	
Tricuspid insufficiency[1]				0.88
Mild	23 (4.9%)	22 (4.8%)	1 (7.7%)	
Moderate	1 (0.2%)	1 (0.2%)	0 (0.0%)	
Absent	448 (94.9%)	436 (95.0%)	12 (92.3%)	
Missing	65	61	4	
Noninvasive diagnostic method for myocardial ischemia[1]				0.001
Negative	65 (12.8%)	58 (11.8%)	7 (41.2%)	
Positive	442 (87.2%)	432 (88.2%)	10 (58.8%)	
Missing	30	30	0	
Dynamic myocardial perfusion scintigraphy induced ischemia[1]				0.01
Negative	304 (88.9%)	292 (89.8%)	12 (70.6%)	
Positive	38 (11.1%)	33 (10.2%)	5 (29.4%)	
Missing	195	195	0	
Weight (kg)[2]				0.74
Mean (SD)	74.2 (14.2)	74.2 (14.4)	73.1 (10.2)	
Missing	9	9	0	

Height (cm)[2]				0.38
Mean (SD)	168.0 (9.0)	168.1 (9.1)	166.2 (6.9)	
Missing	12	12	0	
Body mass index (kg/m²)[2]				0.8
Mean (SD)	26.2 (4.3)	26.2 (4.3)	26.5 (3.5)	
Missing	13	13	0	
Hematocrit (%)[2]				0.98
Mean (SD)	36.2 (5.8)	36.2 (5.8)	36.2 (3.8)	
Missing	3	3	0	
White blood cell count (mm³)[2]				0.36
Mean (SD)	5153 (4539)	5185 (4602)	4163 (1473)	
Missing	2	2	0	
Platelets[2]				0.67
Mean (SD)	84524 (55946)	84340 (56004)	90058 (55570)	
Missing	9	0	0	
Total bilirubin (mg/dL)[2]				0.09
Mean (SD)	2.9 (3.4)	2.9 (3.5)	1.5 (0.7)	
Missing	1	0	0	
Creatinine (mg/dL)[2]				0.08
Mean (SD)	1.2 (1.8)	1.2 (1.7)	2.0 (2.8)	
Missing	5	5	0	
International normalized ratio[2]				0.50
Mean (SD)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)	
Missing	32	32	0	
Sodium (mEq/L)[2]				0.91
Mean (SD)	137.7 (4.9)	137.7 (4.9)	137.8 (4.6)	
Missing	37	36	1	
Potassium (mmol/L)[2]				0.90
Mean (SD)	4.4 (0.6)	4.4 (0.6)	4.4 (0.7)	
Missing	44	43	1	
Albumin (g/dL)[2]				0.64
Mean (SD)	3.2 (0.6)	3.2 (0.6)	3.3 (0.4)	
Missing	13	13	0	
Aspartate aminotransferase (U/L)[2]				0.15
Mean (SD)	89.9 (60.1)	90.6 (60.7)	69.0 (34.8)	
Missing	9	8	1	
Alanine aminotransferase (U/L)[2]				0.62
Mean (SD)	74.1 (62.8)	74.3 (62.9)	66.5 (60.7)	
Missing	8	7	1	
Gamma-glutamyl transferase (U/L)[2]				0.56
Mean (SD)	102.8 (131.8)	102.3 (131.5)	122.6 (145.0)	
Missing	33	30	3	
Alkaline phosphatase (U/L)[2]				0.75

Mean (SD)	147.2 (127.9)	146.9 (128.4)	157.6 (113.2)	
Missing	24	22	2	
Alpha-fetoprotein (ng/mL)[2]				0.67
Mean (SD)	49.6 (339.5)	50.8 (345.2)	15.2 (26.7)	
Missing	20	20	0	
Fasting blood glucose (mg/dL)[2]				0.65
Mean (SD)	108.6 (41.7)	108.7 (42.1)	104.1 (29.2)	
Missing	26	26	0	

[1]*n* (%), Pearson's χ^2 test.
[2]Mean (SD), linear model ANOVA.
MACE: Major adverse cardiovascular event.

Model explanations

Figure 3 presents feature importance analysis as per mean SHAP values. It reveals that, at the cohort-wide level, the most significant variables for prediction of postoperative MACE were negative noninvasive cardiac stress testing, use of a nonselective beta-blocker, direct bilirubin levels, blood type O, and dynamic alterations on MPS. SHAP values are averaged, and the impact of each feature on individual predictions may vary. For instance, the feature 'blood type O' may have varying impacts depending on the specific conditions and characteristics of the patient.

DISCUSSION

The aim of the present study was to assess the risk of in-hospital post-LT MACE and identify clinically relevant predictors of such events. In pursuit of this objective, we constructed a machine learning-based risk stratification model which could be made available online to assist clinicians in identifying LT recipients at heightened cardiac risk immediately after LT. These models hold significance due to cardiovascular causes being a leading contributor to post-LT mortality, and the absence of risk prediction models tailored to patients with ESLD.

In this study, various recipient-related factors known prior to LT were thoroughly examined. An optimized clinical model demonstrated predictive capabilities for in-hospital MACE following LT, exhibiting a strong discriminative performance with an area under the curve (AUC) of 0.89. This surpasses the performance reported in a previously published study attempting to predict similar outcomes, which achieved an AUC of 0.71[45].

The present study employed a comprehensive set of candidate variables gathered during the pre-LT evaluation, which encompassed a wide array of cardiovascular risk factors. Notably, the machine learning model consistently demonstrated superior performance across all endpoints, highlighting significant improvements when compared to widely utilized traditional models.

On performance analysis, the XGBoost model demonstrated remarkable predictive capability, achieving an impressive AUROC of 0.89. This performance highlights its effectiveness in predicting postoperative MACE in our cohort of 575 LT patients. Furthermore, our classification results revealed excellent precision (0.89), recall (0.80), and an F1-score of 0.84 for the negative class, underscoring the model's precision in identifying patients at low risk of MACE. The exceptional performance of the model is further substantiated by the calibration results, where the isotonic-calibrated model achieved optimal calibration, as indicated by the lowest Brier score of 0.100. This calibration ensures a high level of precision, recall, F1-score, and accuracy for both negative and positive classes, aligning the model's predictions closely with observed outcomes. The calibration curve (available as supplemental material) visually depicts the model's excellent calibration performance.

To gain insights into the factors influencing postoperative MACE in our cohort, we conducted feature importance analysis, as depicted in Figure 3. Our analysis revealed that several variables - namely, outcomes of noninvasive cardiac stress testing, administration of nonselective beta-blockers, direct bilirubin levels, blood type O, and dynamic alterations on MPS - contributed significantly to prediction of postoperative MACE at the cohort-wide level. These findings emphasize the importance of considering both cardiac and liver-related factors in assessing the risk of post-transplant MACE. It bears stressing that, while these variables hold substantial predictive power at the cohort level, their impact may vary for individual patients, depending on their unique clinical characteristics and conditions.

We also evaluated the performance of our models in comparison to existing cardiovascular disease risk prediction models, such as the Cardiovascular Risk in Orthotopic Liver Transplantation (CVROLT) score, which was derived from a cohort of 1024 first-time LT recipients[8]. The CVROLT score included a multitude of donor- and recipient-related factors and identified pre-transplant heart failure, atrial fibrillation, diabetes, and the presence of respiratory failure at the time of transplantation as the most significant predictors of post-LT adverse cardiovascular events. Notably, our study used similar source variables but employed advanced machine learning techniques, which, uniquely, allowed our models to be internally validated in a series of "blinded" test cohorts, enhancing the generalizability of the results. While the CVROLT score achieved a C statistic of 0.78, our models demonstrated substantial predictive capability, particularly the XGBoost

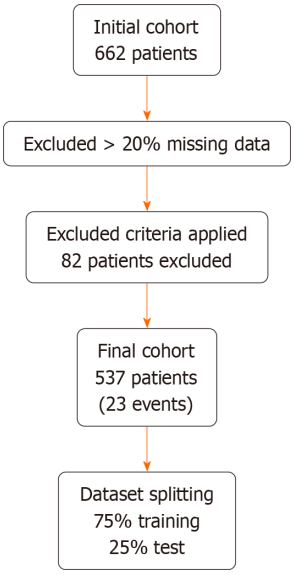

Figure 1 Flow diagram of patient inclusion.

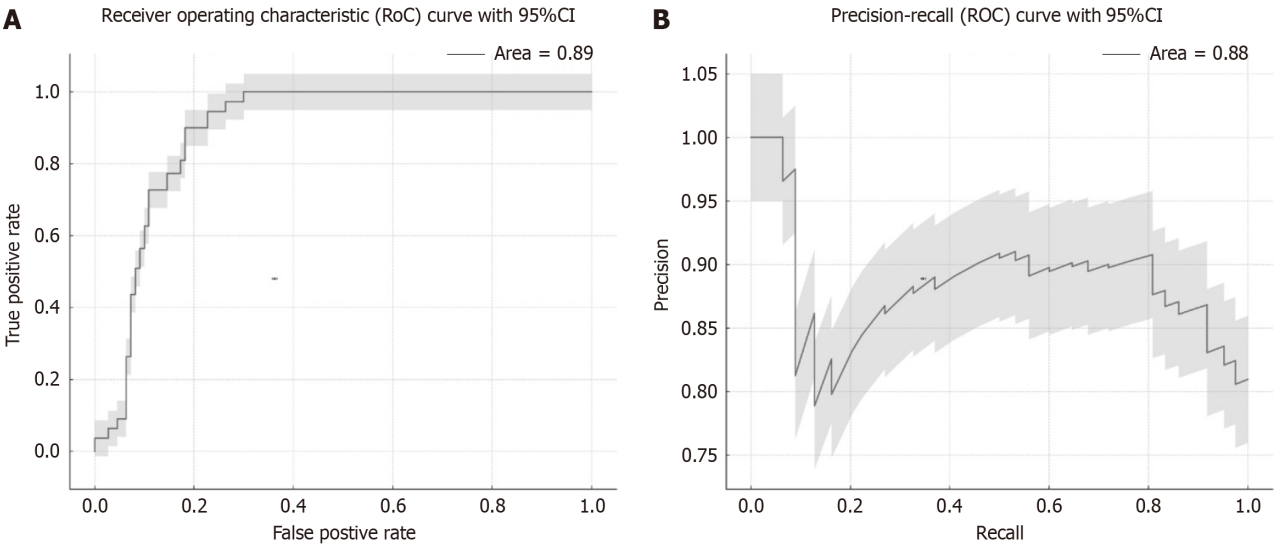

Figure 2 Area under the receiver operating characteristic curve and area under the precision-recall curve for the model on the validation set. A and B: The area under the receiver operating characteristic curve in Figure 2A plots the true positive rate (sensitivity) against the false positive rate (1-specificity) for various threshold values. The area under the precision-recall curve in Figure 2B illustrates precision × recall for different threshold values. The shaded region represents the 95% confidence interval in both figures. ROC: Receiver operating characteristic; CI: Confidence interval.

model (AUC = 0.89). As noted above, this exceptional performance underscores the superiority of our models in predicting postoperative MACE in the context of LT.

The Revised Cardiac Risk Index (RCRI), another model traditionally used for predicting postoperative cardiovascular risk in individuals undergoing noncardiac surgery, has limited applicability in LT candidates[46]. The RCRI derivation cohort excluded patients with ESLD and primarily aimed to detect underlying ischemic heart disease, resulting in a suboptimal tool for risk-stratifying LT candidates for the occurrence of long-term MACE.

Both Josefsson *et al*[47] and Umphrey *et al*[48] reported on smaller cohorts of LT patients (n = 202 and n = 157, respectively). In their study, Josefsson *et al*[47] identified renal impairment, prolonged QTc, and age > 52 years as predictors of 1-year cardiovascular mortality. Similarly, Umphrey *et al*[48] investigated the role of DSE and reported that maximum heart rate achieved during the procedure, together with the model for end-stage liver disease (MELD) score, may predict adverse cardiovascular events up to 4 months post-orthotopic LT. Both of these previous models were limited by relatively small sample sizes, which may have impacted their external validity.

Historically, the assessment of cardiovascular risk in LT candidates has often prioritized the evaluation of CAD using methods such as DSE or coronary artery calcium scoring. This focus was largely driven by the high prevalence of traditional cardiovascular risk factors in LT recipients. However, the landscape is evolving as transplantation is increasingly performed on a medically complex population with higher median age at transplantation and higher MELD

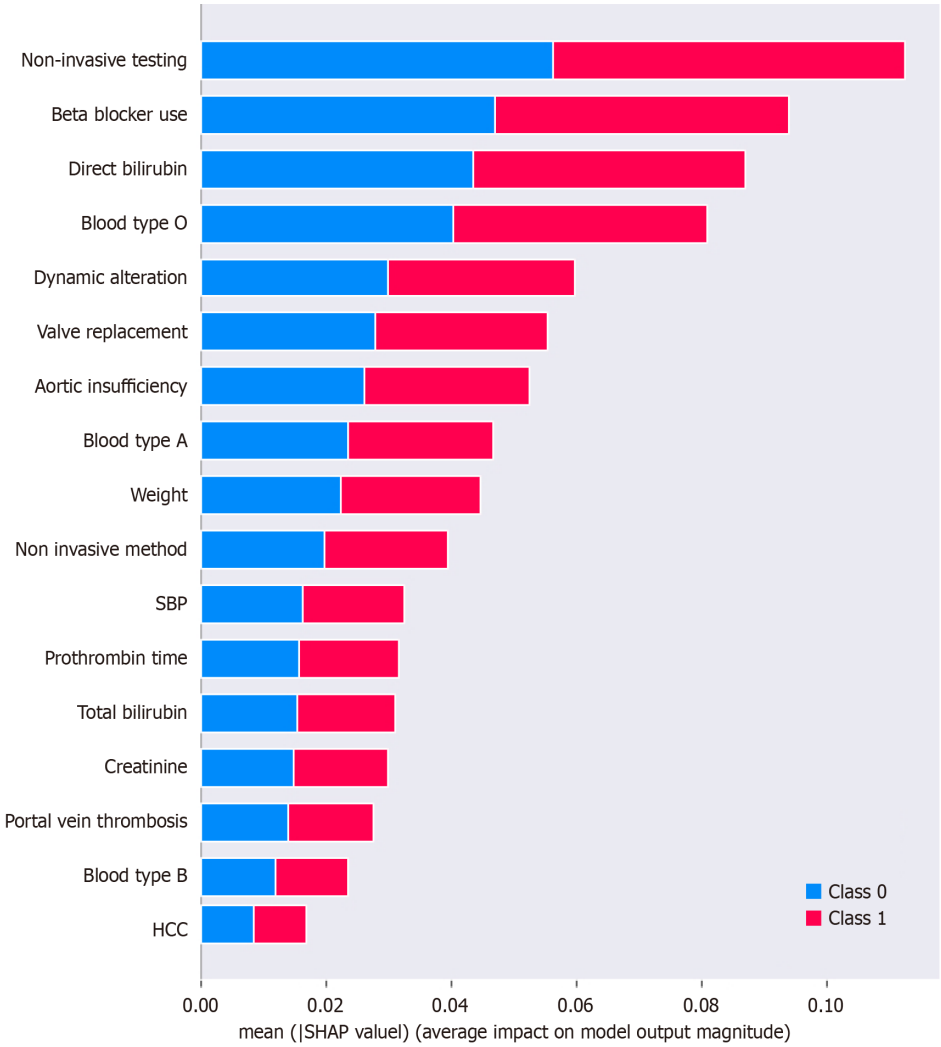

Figure 3 The x-axis represents the mean Shapley additive explanations value, which quantifies the average impact of each feature on the model's output. A higher mean Shapley additive explanations value means that the feature has a more significant influence on model predictions. The bars are color-coded to represent two distinct classes: Class 0 (blue), which represents absent major adverse cardiovascular event (MACE), and Class 1 (red), which represents the occurrence of MACE. The length of the bar in each color indicates the average impact of the corresponding feature on prediction of that specific class. Longer bars (regardless of color) mean that the feature has a greater average impact on model output. The direction of the influence (whether it pushes predictions towards Class 0 or Class 1) is denoted by the color. SBP: Spontaneous bacterial peritonitis; HCC: Hepatocellular carcinoma; Class 0: Major adverse cardiovascular event absent; Class 1: Major adverse cardiovascular event present; SHAP: Shapley additive explanations.

scores. Notably, advanced age alone correlates with cardiovascular comorbidities and independently predicts adverse cardiovascular events[1]. Additionally, ESLD is characterized by a high-output state with compromised ventricular reserve, known as cirrhotic cardiomyopathy, which may be exacerbated by the hemodynamic stress of liver reperfusion.

Recent systematic reviews and meta-analyses have shed light on the value of DSE in patients listed for LT. These studies reported that DSE had variable sensitivity (ranging from 20% to 32%) and specificity (ranging from 78% to 99%) for detecting CAD[25,26,49,50] mixed predictive capabilities for MACE post-LT, with sensitivity ranging from 20% to 28% and specificity as low as 78%[25,26,48,49]. It is evident that, while DSE exhibits a high negative predictive value, it may not be a reliable test for detecting risk of cardiovascular events, mortality, or presence of CAD in LT candidates. Therefore, its use should be reserved for selected intermediate-risk patients[51-53].

Furthermore, Oprea-Lager *et al*[54] demonstrated that the presence of a reversible perfusion defect suggestive of myocardial ischemia on MPS appears to increase all-cause mortality post-LT, with a hazard ratio of 3.17. Regarding MPS, several systematic reviews and meta-analyses have been conducted to evaluate its value in LT candidates. One such analysis, including five studies, found that MPS had a sensitivity of 62% and a specificity of 83% for detecting CAD[50]. Another diagnostic meta-analysis, involving 10 studies, reported a sensitivity of 82% and a specificity of 74% for MPS in CAD detection[25]. Finally, a prognostic meta-analysis revealed that positive MPS was associated with a relative risk of 2.6 (95%CI: 1.09-6.1) for major cardiac events and a relative risk of 2.7 (95%CI: 1.25-5.9) for mortality post-LT[26].

In patients listed for LT, the presence of coronary calcium has been significantly associated with various factors, including age, systolic blood pressure, alcohol-related cirrhosis, fasting blood glucose levels, the number of metabolic syndrome criteria, and the number of affected vessels. Importantly, coronary artery calcium score (CACS) values offer valuable insights into cardiac risk stratification. A CACS below 100 predicts a very low risk of post-LT cardiac events,

while a CACS above 250 suggests the need for coronary angiography[55] and a CACS exceeding 400 identifies patients at risk of MACE for up to 5 years post-LT. A recent study from 2021, comparing the diagnostic accuracy of DSE and CACS in detecting CAD, demonstrated the superiority of CACS over DSE[56].

Currently, it is proposed that coronary computed tomography angiography (CCTA) serves as the initial testing strategy for LT candidates with moderate to high CAD risk, while low-risk patients may not require additional cardiovascular assessment[51]. However, it is essential to acknowledge that CCTA may have limitations in detecting functional microvascular disease, which can contribute to type 2 myocardial infarction post-LT[57].

A recent systematic review has highlighted the promising role of machine learning models in improving prognostication for LT. The authors have found that machine learning models consistently outperformed traditional scoring systems, demonstrating excellent predictive capabilities for various post-transplant complications, including mortality, sepsis, and acute kidney injury. They suggest that machine learning could enhance decision-making related to organ allocation and LT, representing a substantial advancement in prognostication methods[58].

In the future, generalist medical artificial intelligence (GMAI) may bring a paradigm shift in medical AI use. Emphasizing flexibility and reusability, GMAI models can perform diverse tasks with minimal labeled data, developed through self-supervision on extensive datasets[59]. This might cause a shift in this paradigm, driven by hardware advances and the demand for personalized care, emphasizing AI's role in decision-making and improving diagnostic and prognostic performance[60].

In the context of utilizing machine learning to predict major MACE following LT, addressing the ethical implications and challenges that arise when implementing these models in clinical practice is crucial. The integration of machine learning introduces concerns surrounding data privacy, as patient information must be handled securely to protect confidentiality. Additionally, ensuring model transparency is essential, as clinicians need to understand the decision-making process of the machine learning model to trust its predictions. Furthermore, the potential biases embedded in the training data used for these models must be carefully examined and mitigated to avoid disproportionate effects on certain patient populations. By discussing these ethical considerations, the application of machine learning in predicting post-LT MACE can be approached with a well-rounded perspective that prioritizes patient privacy, model transparency, and fairness in healthcare outcomes.

This study is subject to several limitations. The retrospective design introduces inherent biases and data limitations. Significantly, a notable portion of the excluded patients, marked by a substantial volume of missing data, underwent LT with increased celerity attributed to higher MELD scores, and this resulted in an incomplete pre-LT clinical or cardiological evaluation. Second, the single-center setting may limit the generalizability of the findings to broader patient populations. Third, it is important to note that, while the machine learning model provides valuable predictive insights, it should serve as an aid to clinical judgment rather than a replacement, as it is better suited to predict a general rather than an individual risk of MACE. Additionally, the exclusion of certain patient groups based on specific criteria may impact the model's applicability in real-world scenarios. Finally, while the SHAP framework offers insights into feature importance, further investigation is needed to establish clinical relevance. While the study presents a robust predictive model, these limitations should be taken into consideration when interpreting and applying its results; future research with a view to external validation and improvement of clinical utility will be welcome.

The uncertainty surrounding the positive or negative outcomes of noninvasive tests and the prevalence of blood type O as risk factors for MACE highlights a critical aspect of machine learning model interpretability - it is advisable to avoid overestimating the significance and generalization of such information. The limitation of many models, including XGBoost, is the absence of clarity on why negative noninvasive cardiac stress testing correlates with a reduced risk of MACE. While these models excel at identifying statistical patterns, they often fall short in providing explicit explanations for correlations, lacking inherent insights into the biological or clinical reasons behind observed associations. Complementary research to unravel the biological significance of these correlations is required, emphasizing the distinction between mathematical patterns and causal relationships.

In this context, we can only speculate about these variables. Blood type O has shown a negative association with myocardial infarction[61-63], adding an intriguing dimension to the findings of the machine learning model. In patients with ESLD, distinguishing whether chronotropic incompetence results from cirrhosis-related autonomic dysfunction or is solely due to a beta-blocker effect is challenging. This ambiguity leads to numerous false negatives in stress testing, potentially influencing the negative association observed between stress testing and MACE[64]. One particularly intriguing discovery was the correlation between liver function markers and MACE - arguably the most noteworthy among these variables. Often, liver function is underestimated, and its impact on MACE may be overlooked, with attention primarily directed at the heart. Emphasizing the evaluation of both cardiac and hepatic aspects is crucial in pre-LT cardiac assessments[65].

The meticulous evaluation of pre-LT factors, incorporation of advanced machine learning techniques, and the demonstrated superior performance of the XGBoost model in predicting MACE distinguish this study. The model developed outperforms existing risk prediction tools, such as the CVROLT and CAR-OLT scores, and adds significant value to the relevant and current discussion on this topic. Additionally, the insights from this research not only contribute to the current knowledge but also pave the way for more accurate and tailored risk predictions in the context of LT.

CONCLUSION

In conclusion, the outcomes produced by our developed machine learning model are consistent with findings reported in prior literature. The calibration analysis indicates that our efforts to prevent overfitting and data leakage have indeed

been successful, suggesting that results are likely to remain stable when the model is applied to prospective data. Moreover, we have integrated the model into a user-friendly MACE prediction calculator which is now available online. This implementation will enable us to conduct a more comprehensive assessment of its prospective impact on prognosis.

With the increasing volume of LT procedures, the machine learning model presented herein can serve as a valuable resource for patient counseling, shared clinical decision-making with patient consent, quality improvement, and development of risk-reduction strategies. Further validation and application of this machine learning model in other registries and patient populations are essential to better understand its external validity in patients undergoing LT across multiple major transplantation-capable tertiary referral centers.

ARTICLE HIGHLIGHTS

Research background

The landscape of liver transplant (LT) candidates has evolved, with an aging and increasingly morbid population, often linked to metabolic-associated fatty liver disease (MAFLD). MAFLD's rise as a cause of cirrhosis raises concerns about a subsequent increase in major adverse cardiovascular events (MACE) post-LT, a critical complication negatively impacting prognosis. This study is prompted by the growing incidence of post-LT MACE, particularly within the first 6 months, and the complex interplay of traditional and nontraditional cardiovascular risk factors in this vulnerable population. The prevalence shift toward MAFLD as a leading indication for LT necessitates a thorough pre-LT cardiac assessment, demanding a reconsideration of existing noninvasive strategies' reliability. The pressing need for an alternative approach to predict post-LT MACE accurately propels the exploration of machine learning as a transformative tool to navigate the challenges posed by conventional models.

Research motivation

Motivating this research is the imperative to address the limitations of current cardiovascular risk stratification models for LT candidates, especially those with end-stage liver disease. Traditional models exhibit constraints related to assumptions of linear relationships and limited variables, leading to unreliable predictions. The inadequacy of existing noninvasive strategies and the absence of effective models for accurate cardiovascular risk stratification in LT candidates underscore the urgency for a paradigm shift. The study is driven by the aspiration to introduce machine learning as an innovative and more effective approach, leveraging its capacity to discern intricate patterns and relationships within datasets. The ultimate goal is to revolutionize risk prediction, enabling clinicians to identify high-risk individuals with precision, thus optimizing patient care strategies.

Research objectives

The primary objective of this study is to assess the feasibility and accuracy of implementing a machine learning model to predict MACE post-LT. Focusing on a specific regional cohort, the study aims to revolutionize risk assessment by moving beyond the limitations of conventional statistical models. Realizing this objective involves scrutinizing the potential of machine learning techniques to forecast post-LT MACE with enhanced precision. By leveraging advanced computational models, the research seeks to provide a comprehensive evaluation of the predictive capabilities, enabling the early identification of individuals at elevated risk. The ultimate significance lies in facilitating early intervention strategies and refining patient care in the context of the evolving landscape of LT candidates.

Research methods

This retrospective cohort study, approved by the Research Ethics Committee, delves into the cardiovascular risks following LT. Employing a comprehensive approach, medical records from Irmandade Santa Casa de Misericórdia de Porto Alegre were scrutinized for patients undergoing their first LT between 2001 and 2011 due to cirrhosis. Rigorous inclusion and exclusion criteria were applied, focusing on patients above 18 years of age with complete records, cardiac evaluation pre-LT, and no retransplantation. Data encompassed pre-LT, perioperative, and post-LT periods, with the primary outcome being in-hospital MACE. Statistical analyses, including frequency, means, standard deviation, Pearson's χ^2 test, and linear model analysis of variance, were executed using R software. The study introduces a machine learning paradigm, leveraging the XGBoost model, known for handling imbalanced datasets. Feature engineering involved a two-step imputation process, incorporating patient demographics, medical history, and cardiac evaluations. Model training incorporated regularization and early-stop techniques, aiming to prevent overfitting. Hyperparameter optimization using the Optuna package and performance evaluation metrics, including area under the receiver operating characteristic curve (AUROC) and area under the precision-recall curve, ensured robustness. Calibration, model explanation through Shapley additive explanations values, and adherence to the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis statement further enriched the methodological rigor, ultimately culminating in web deployment and code availability for transparency and accessibility.

Research results

The study involved 662 LT patients, with 82 exclusions based on specific criteria. The final dataset included 537 samples, with 23 in-hospital MACE cases. The XGBoost model demonstrated substantial predictive capability, achieving an AUROC of 0.89. Precision, recall, and F1-score for the negative class were 0.89, 0.80, and 0.84, respectively. The overall incidence of MACE was 4.46%, with observed rates for stroke, new-onset heart failure, severe arrhythmia, and

myocardial infarction. The model achieved optimal calibration using the isotonic method with a Brier score of 0.100. Feature importance analysis revealed key predictors, including negative noninvasive cardiac stress testing, use of a nonselective beta-blocker, direct bilirubin levels, blood type O, and dynamic alterations on myocardial perfusion scintigraphy. The findings contribute a valuable machine learning model for predicting post-LT MACE, offering insights into specific risk factors and enhancing precision in identifying at-risk individuals. Remaining challenges involve addressing potential variability in feature impact across patients and further validation in diverse cohorts.

Research conclusions

This study pioneers a novel approach in assessing in-hospital post-LT MACE. The research introduces a machine learning-based risk stratification model, surpassing the predictive performance of existing models, particularly demonstrating an impressive area under the curve of 0.89 using the XGBoost model. The optimized clinical model considers recipient-related factors and provides valuable insights into predicting MACE, crucial for addressing the leading cause of post-LT mortality. The use of machine learning techniques, specifically XGBoost, brings substantial improvements over traditional models, enhancing risk stratification accuracy. This study highlights the importance of comprehensive pre-LT evaluation, considering a wide array of cardiovascular risk factors.

Research perspectives

Future research should focus on refining and expanding the machine learning model's application, considering external validation in diverse patient populations and healthcare settings. Addressing ethical implications and ensuring transparency in model application are imperative for integrating machine learning predictions into clinical practice. The study suggests the need for continued exploration into the biological significance of identified predictors, such as the intriguing correlation between blood type O and reduced MACE risk. The model's implementation in a user-friendly MACE prediction calculator opens avenues for prospective impact assessment, counseling, shared decision-making, and risk reduction strategies in the growing landscape of LT procedures. External validation and application in various transplantation-capable centers will enhance understanding of the model's broader utility.

FOOTNOTES

Co-first authors: Jonathan Soldera and Leandro Luis Corso.

Author contributions: Soldera J, Corso LL, Rech MM, Tomé F, and Moraes N substantially contributed to the conception and design of the work, data collection, and drafting of the manuscript; Corso LL; Rech MM are credited with the development of the algorithm upon which the machine learning model relies; Ballotin VR, Bigarella LG, Balbinot RS, and Rodriguez S substantially contributed to data collection and critical revision of the manuscript; Brandão ABM and Hochhegger B were responsible for supervision, manuscript revision, and additional writing; and all authors have reviewed and approved the final version and agreed to be accountable for the work's integrity.

Institutional review board statement: The study was reviewed and approved for publication by our Institutional Reviewer under protocol no. 07793412.2.3001.5345.

Informed consent statement: The Ethics Committee waived the need for informed consent for this study since it solely utilized data from medical charts without direct patient contact.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The original anonymous dataset is available on request from the corresponding author at jonathansoldera@gmail.com. The code for implementation of the reported pipeline on the present dataset, including data preprocessing, feature engineering, model development, hypermeter optimization, and model assessment, is provided in the GitHub repository, publicly and freely available through the following link: https://github.com/matheus-rech/ML.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

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S-Editor: Wang JJ
L-Editor: A

P-Editor: Zheng XM

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 211-228

DOI: 10.4254/wjh.v16.i2.211

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Effects of SARS-CoV-2 infection on incidence and treatment strategies of hepatocellular carcinoma in people with chronic liver disease

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Elshimi E, Egypt

Received: December 1, 2023
Peer-review started: December 1, 2023
First decision: December 12, 2023
Revised: December 31, 2023
Accepted: January 30, 2024
Article in press: January 30, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

Chronic liver disease (CLD) was associated with adverse clinical outcomes among people with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

AIM

To determine the effects of SARS-CoV-2 infection on the incidence and treatment strategy of hepatocellular carcinoma (HCC) among patients with CLD.

METHODS

A retrospective, territory-wide cohort of CLD patients was identified from an electronic health database in Hong Kong. Patients with confirmed SARS-CoV-2 infection [coronavirus disease 2019 (COVID-19)+CLD] between January 1, 2020 and October 25, 2022 were identified and matched 1:1 by propensity-score with those without (COVID-19-CLD). Each patient was followed up until death, outcome event, or November 15, 2022. Primary outcome was incidence of HCC. Secondary outcomes included all-cause mortality, adverse hepatic outcomes, and different treatment strategies to HCC (curative, non-curative treatment, and palliative care). Analyses were further stratified by acute (within 20 d) and post-acute (21 d or beyond) phases of SARS-CoV-2 infection. Incidence rate ratios (IRRs) were estimated by Poisson regression models.

RESULTS

Of 193589 CLD patients (> 95% non-cirrhotic) in the cohort, 55163 patients with COVID-19+CLD and 55163 patients with COVID-19-CLD were included after 1:1 propensity-score matching. Upon 249-d median follow-up, COVID-19+CLD was not associated with increased risk of incident HCC (IRR: 1.19, 95%CI: 0.99-1.42, $P = 0.06$), but higher risks of receiving palliative care for HCC (IRR: 1.60, 95%CI: 1.46-1.75, $P < 0.001$), compared to COVID-19-CLD. In both acute and post-acute phases of infection, COVID-19+CLD were associated with increased risks of all-cause mortality (acute: IRR: 7.06, 95%CI: 5.78-8.63, $P < 0.001$; post-acute: IRR: 1.24, 95%CI: 1.14-1.36, $P < 0.001$) and adverse hepatic outcomes (acute: IRR: 1.98, 95%CI: 1.79-2.18, $P < 0.001$; post-acute: IRR: 1.24, 95%CI: 1.13-1.35, $P < 0.001$), compared to COVID-19-CLD.

CONCLUSION

Although CLD patients with SARS-CoV-2 infection were not associated with increased risk of HCC, they were more likely to receive palliative treatment than those without. The detrimental effects of SARS-CoV-2 infection persisted in post-acute phase.

Key Words: SARS-CoV-2 infection; Chronic liver disease; Long COVID; Post-COVID-19 syndrome; Cirrhosis; Hepatocellular carcinoma

Core Tip: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in patients with chronic liver disease (CLD) leads to worse adverse clinical outcomes. In our study, we found that although CLD patients with SARS-CoV-2 infection did not have higher risk of developing liver cancer, they are more likely to receive palliative treatment for hepatocellular carcinoma, compared to CLD patients who did not have SARS-CoV-2 infection. Coronavirus disease 2019 also led to increased risks of all-cause mortality and adverse hepatic outcomes. These detrimental effects of SARS-CoV-2 infection were observed in both acute and post-acute phases among CLD patients.

Citation: Mak LY, Chung MSH, Li X, Lai FTT, Wan EYF, Chui CSL, Cheng FWT, Chan EWY, Cheung CL, Au ICH, Xiong X, Seto WK, Yuen MF, Wong CKH, Wong ICK. Effects of SARS-CoV-2 infection on incidence and treatment strategies of hepatocellular carcinoma in people with chronic liver disease. *World J Hepatol* 2024; 16(2): 211-228

URL: https://www.wjgnet.com/1948-5182/full/v16/i2/211.htm

DOI: https://dx.doi.org/10.4254/wjh.v16.i2.211

INTRODUCTION

In the year 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection led to coronavirus disease 2019 (COVID-19) pandemic across the globe. The disruption to the routines due to social distancing measures has affected all sectors of the society. Healthcare systems were particularly stretched by the enormous influx of SARS-CoV-2 infected patients, inevitably leading to change in clinical practice such as adoption of virtual consultations, suspension of healthcare services including cancellation of planned investigations, procedures and treatments. Colorectal cancer and

lung cancer are examples of chronic conditions that were negatively influenced by the pandemic, with significant delays in screening, diagnosis and workup. In subjects with chronic liver disease (CLD), SARS-CoV-2 infection has been associated with an increased risk of short-term mortality, predominantly caused by respiratory failure and observed in cirrhotic patients[1-4]. In comparison, it remains controversial whether SARS-CoV-2 infection increases the risk of non-respiratory causes of death among non-cirrhotic CLD, a condition that affects 1.5 billion persons globally[5,6]. There is no data regarding how COVID-19 interplay with the risk of hepatocellular carcinoma (HCC) and subsequent treatment strategy, which not only depends on the general performance status of the subject, the liver reserve, and the tumor status [7], but also the relative resource allocation within the health care system in the event of the pandemic.

Much is unknown regarding precisely how COVID-19 affects prognosis and liver outcomes in CLD. In particular, the detrimental effects of SARS-CoV-2 infection seem to linger beyond the acute phase of infection and are associated with a number of conditions, collectively termed 'post-acute sequelae of SARS-CoV-2 infection' (PASC), also known as 'long COVID' or 'post-COVID-19 syndrome'[8,9]. Among the numerous conditions associated with PASC (*e.g.*, pulmonary, neuropsychiatric, gastrointestinal, endocrine, renal, *etc.*), hepatic effects of recent SARS-CoV-2 infection have not been well-characterized[10]. In addition, it was hypothesized that SARS-CoV-2 infection will lead to long-lasting impacts on the quality of cirrhosis care, resulting from the initial intense period of prioritization of healthcare services with delays in routine care, and subsequent return of backlog presentations of illness and protracted period of suboptimal outcomes [11]. Therefore, it is important to understand the immediate and long-term consequences of SARS-CoV-2 infection in patients with CLD, and how they affect the incidence and oncological treatment for HCC.

In this study, we determined the risk of incident HCC, all-cause mortality, adverse hepatic outcomes, and the impact on treatment strategies for patients with liver cancer in a large cohort of patients with CLD and laboratory proven SARS-CoV-2 infection, in comparison to a contemporaneous cohort of patients with CLD who did not have SARS-CoV-2 infection in Hong Kong.

MATERIALS AND METHODS

Data source and study population

Our data were extracted from territory-wide cohort of patients with anonymized electronic health records provided by the Hong Kong Hospital Authority (HA), and COVID-19 vaccination records were available from the Department of Health (DH), The Government of Hong Kong Special Administrative Region. Electronic medical records of patients with COVID-19 were retrieved from the HA, and included demographics, disease diagnoses, drug prescriptions, laboratory tests, hospital admissions, emergency departments, and inpatient procedures. The HA data were linked to the COVID-19 vaccination records provided by the DH using the unique identification numbers. This linked database has been used extensively for studies on COVID-19 vaccine safety[12-14] and PASC[15].

This study included patients with CLD between January 1, 2020 and November 15, 2022. CLD was defined as patients having any of the following diagnoses: (1) Viral hepatitis B (HBV) infection; (2) viral hepatitis C; (3) chronic hepatitis; (4) fatty liver disease; (5) alcoholic liver disease (ALD); (6) alcoholic hepatitis; (7) Wilson's disease, (8) autoimmune hepatitis; and (9) primary biliary cholangitis and primary sclerosing cholangitis. Each of the above diseases was identified by International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) diagnosis code, prescription of hepatitis antivirals, or positive hepatitis B surface antigen test (Supplementary Table 1). Patients who were identified by a positive result on the SARS-CoV-2 reverse transcription polymerase chain reaction test or rapid antigen test during the observational period were classified into the COVID-19+CLD group. The index date was set at the first date of SARS-CoV-2 infection for patients in the COVID-19 group (*i.e.* only the first infection was eligible for analysis). Patients who did not have confirmed SARS-CoV-2 infection during the observational period were classified into the control group, *i.e.* COVID-19-CLD. The pseudo-index date of COVID-19-CLD patients was set at the first date of the respective year (*i.e.*, January 1, 2020, January 1, 2021, or January 1, 2022) to maintain a similar follow-up period between the COVID-19+CLD and COVID-19-CLD groups. Patients in the COVID-19-CLD group were matched 1:1 by propensity-score with patients in the COVID-19+CLD group of each index year sequentially starting from 2020 until 2022, and without replacement. Unmatched control patients were eligible for matching with COVID-19+CLD patients in the subsequent index year, with the baseline characteristics of COVID-19-CLD groups updated using the new pseudo-index date (*i.e.* January 1 of the following year). Each patient was observed from the index or pseudo-index date to the occurrence of outcomes, death, or the end of observational period (*i.e.*, November 15, 2022), whichever occurred earlier.

Patients who died on or before the index date, or had less than 21 d of follow-up (*i.e.*, patients with COVID-19 diagnosed on or after October 26, 2022) were further excluded[16].

Outcomes definition

The primary study outcome was HCC incidence. The secondary outcomes included: (1) All-cause mortality; (2) adverse hepatic outcomes cirrhosis, HCC, liver decompensation (composite outcome including hepatorenal syndrome, liver failure, hepatic coma/encephalopathy, ascites, and variceal bleeding); (3) curative treatment to HCC (hepatic resection, liver transplantation, radiofrequency ablation of liver); (4) non-curative treatment to HCC (transarterial chemoembolization, radiotherapy to liver, systemic chemotherapy or immunotherapy); and (5) palliative care.

Each of the above outcomes was identified by ICD-9-CM diagnosis and procedure code, prescription of antivirals for hepatitis, and fibrosis-4 index (FIB-4)[17] (Supplementary Table 1).

Acute and post-acute phases of SARS-CoV-2 infection

The risks of study outcomes during the acute and post-acute phase of SARS-CoV-2 infection were further stratified and analyzed among those with index date at 2022 amid the Omicron predominance period. The acute phase of infection was defined as the first 20 d after the index date[18] and the post-acute phase of infection was defined from 21 d of the index date onwards. For the analysis of the post-acute phase of infection when the index date was set to 21 d after COVID-19 diagnosis, patients who died within 20 d of the index date, or had less than 21 d of follow-up were excluded.

Definition of covariates

Baseline characteristics were captured based on ICD-9-CM diagnosis, procedure codes, and treatment records as follows: age, sex, pre-existing comorbidities [Charlson Comorbidity Index (CCI), cirrhosis, HCC, liver decompensation], oncological treatment received prior to the index date (curative treatment to HCC, non-curative treatment to HCC, palliative care), and COVID-19 vaccination status (Supplementary Table 1). The FIB-4[17] was also used to enhance case identification for cirrhosis. Fully vaccinated patients were defined as those with at least two doses of BNT162b2 (Comirnaty) or three doses of COVID-19 Vaccine (Vero Cell), Inactivated (CoronaVac)[19].

Statistical analysis

Descriptive statistics of baseline characteristics between the COVID-19 groups and matched control groups were presented as mean and standard deviation, or median and interquartile range (IQR) for continuous variables, and count and proportion for categorical variables.

We constructed propensity-score models conditional on age, sex, CCI, and COVID-19 vaccination status in a logistic regression model. We performed 1:1 propensity-score matching using a caliper width of 0.05. Standardized mean differences (SMDs) of each covariate between the groups after propensity-score matching were calculated, which was interpreted as balanced when the SMD was below the threshold of 0.1[20]. The incidence rate ratio (IRR) and corresponding 95% confidence intervals (CIs) were estimated using the Poisson regression model.

Subgroup analyses were performed on several patient groups, including age groups (≤ 50 *vs* > 50 years), causes of CLD (HBV *vs* other causes), the presence of cirrhosis, the presence of multiorgan dysfunction, COVID-19 vaccination status (fully vaccinated *vs* not fully vaccinated), respective years of COVID-19 diagnosis (year of 2020 *vs* 2021 *vs* 2022). Multiorgan dysfunction was defined as patients having any 3 or more organ system malfunctions in the following categories, including: (1) Neurological; (2) psychiatric; (3) respiratory; (4) cardiovascular; (5) hematologic; (6) endocrine; (7) nephrological; (8) hepatic; (9) gastrointestinal; and (10) dermatologic disorder. Each subgroup analysis was re-constructed with a new propensity-score model, and the pairs of patients with COVID-19 and their respective controls were rematched. Furthermore, subgroup analyses among COVID-19+CLD patients were performed on two patient groups, including hospitalization groups (hospitalized *vs* non-hospitalized) and receipt of antiviral medications for COVID-19 infection. COVID-19+CLD patients were identified as antiviral users if they received any of the following antiviral medications, including: (1) Molnupiravir; (2) nirmatrelvir/ritonavir; and (3) remdesivir. Each subgroup analysis among COVID-19+CLD patients was also re-constructed with a new propensity-score model, and rematched between hospitalized and non-hospitalized patients or antiviral users and non-users, respectively.

All statistical analyses were performed using Stata (version 17). The analyses were conducted by Chung MSH and analyzed independently by Xi X and Au ICH for quality assurance. All significance tests were two-tailed, where *P* values < 0.05 were considered statistically significant.

Role of the funding source

This work was supported by a research grant from Collaborative Research Fund, University Grants Committee, HKSAR Government (principal investigator, Wong ICK; reference no. C7154-20GF). Lai FTT, Wong CKH, and Wong ICK are partially supported by the Laboratory of Data Discovery for Health (D24H) funded by AIR@InnoHK administered by Innovation and Technology Commission. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

RESULTS

Baseline characteristics

A total of 193589 CLD patients of whom 57323 patients had confirmed SARS-CoV-2 infection between January 1, 2020 and November 15, 2022 in Hong Kong, and 136266 CLD patients without SARS-CoV-2 infection were identified (Figure 1). After applying the exclusion criteria followed by 1:1 propensity-score matching, 55163 patients with COVID-19 and CLD (COVID-19+CLD) and 55163 matched controls (COVID-19-CLD) were included in the present study. The baseline characteristics are presented in Table 1. Baseline age (58.8 *vs* 58.7), gender (male gender: 51.2% *vs* 51.6%), medical comorbidities (CCI: 3.2 *vs* 3.1), and COVID-19 vaccination status (fully vaccinated: 50.1% *vs* 50.0%) were balanced between the two groups. Additionally, underlying cirrhosis (3.4% *vs* 3.1%), decompensated liver disease (2.8% *vs* 2.6%), HCC (2.2% *vs* 2.2%), and previous treatment for HCC (curative: 2.0% *vs* 1.9%; non-curative: 4.3% *vs* 3.9%; palliative care: 3.3% *vs* 3.0%) were matched between COVID-19 patients with CLD and controls with CLD (all SMD < 0.1). Of note, the majority (95.0%) of included subjects came from year 2022 when the omicron strain of SARS-CoV-2 was ubiquitous.

Table 1 Baseline characteristics of chronic liver disease patients with coronavirus disease 2019 and chronic liver disease patients without coronavirus disease 2019 (matched controls) after 1:1 propensity score matching

Baseline characteristics	After matching																			
	2020-2022					2020					2021					2022				
	COVID-19 patients (*n* = 55163)		Control (*n* = 55163)		SMD	COVID-19 patients (*n* = 2397)		Control (*n* = 2397)		SMD	COVID-19 patients (*n* = 375)		Control (*n* = 375)		SMD	COVID-19 patients (*n* = 52391)		Control (*n* = 52391)		SMD
	n/Mean	%/SD	*n*/Mean	%/SD		*n*/Mean	%/SD	*n*/Mean	%/SD		*n*/Mean	%/SD	*n*/Mean	%/SD		*n*/Mean	%/SD	*n*/Mean	%/SD	
Age, yr1	58.8	14.7	58.7	13.9	0.01	54.8	17.2	54.5	16.2	0.02	57.6	15.6	57.5	14.2	0	59	14.5	58.9	13.8	0.01
≤ 50	15482	-28.1	14659	-26.6	0.03	878	-36.6	906	-37.8	0.02	127	-33.9	110	-29.3	0.1	14477	-27.6	13643	-26	0.04
> 50	39681	-71.9	40504	-73.4		1519	-63.4	1491	-62.2		248	-66.1	265	-70.7		37914	-72.4	38748	-74	
Sex					0.01					0.03					0.05					0.01
Male	28244	-51.2	28484	-51.6		1274	-53.1	1244	-51.9		192	-51.2	183	-48.8		26778	-51.1	27057	-51.6	
Female	26919	-48.8	26679	-48.4		1123	-46.9	1153	-48.1		183	-48.8	192	-51.2		25613	-48.9	25334	-48.4	
Fully vaccinated	27633	-50.1	27576	-50	0	0	0	0	0	NA	0	0	0	0	NA	27633	-52.7	27576	-52.6	0
Pre-existing comorbidities																				
Charlson's Comorbidity Index1,2	3.2	2.3	3.1	2.2	0.04	2.3	1.9	2.4	1.9	0	2.5	1.8	2.5	1.6	0	3.2	2.3	3.1	2.2	0.05
0-4	42972	-77.9	44994	-81.6	0.09	2153	-89.8	2145	-89.5	0.01	334	-89.1	340	-90.7	0.11	40485	-77.3	42509	-81.1	0.1
5-6	8338	-15.1	7168	-13		191	-8	200	-8.3		33	-8.8	32	-8.5		8114	-15.5	6936	-13.2	
7-16	3853	-7	3001	-5.4		53	-2.2	52	-2.2		8	-2.1	3	-0.8		3792	-7.2	2946	-5.6	
Cirrhosis	1898	-3.4	1737	-3.1	0.02	11	-0.5	41	-1.7	0.12	2	-0.5	4	-1.1	0.06	1885	-3.6	1692	-3.2	0.02
HCC	1199	-2.2	1189	-2.2	0	5	-0.2	24	-1	0.1	0	0	2	-0.5	0.1	1194	-2.3	1163	-2.2	0
Liver decompensation	1563	-2.8	1420	-2.6	0.02	14	-0.6	41	-1.7	0.11	1	-0.3	4	-1.1	0.1	1548	-3	1375	-2.6	0.02
Hepator	158	-0.3	160	-0.3	0	2	-0.1	3	-0.1	0.01	0	0	0	0	0.07	156	-0.3	157	-0.3	0

enal syndrome																			
Liver failure	298	-0.5	303	-0.5	0	5	-0.2	15	-0.6	0.06	0	0	1	-0.3	0.07	293	-0.6	287	-0.5	0
Hepatic coma/encephalopathy	218	-0.4	159	-0.3	0.02	0	0	0	0	NA	1	-0.3	0	0	0.07	217	-0.4	159	-0.3	0.02
Ascites	394	-0.7	330	-0.6	0.01	5	-0.2	5	-0.2	0	0	0	0	0	NA	389	-0.7	325	-0.6	0.01
Variceal bleeding	973	-1.8	842	-1.5	0.02	6	-0.3	24	-1	0.1	0	0	3	-0.8	0.13	967	-1.8	815	-1.6	0.02
Treatment for HCC used before index date																			
Curative treatment to HCC	1111	-2	1065	-1.9	0.01	4	-0.2	25	-1	0.11	0	0	3	-0.8	0.13	1107	-2.1	1037	-2	0.01
Non-curative treatment to HCC	2388	-4.3	2125	-3.9	0.02	19	-0.8	53	-2.2	0.12	0	0	4	-1.1	0.15	2369	-4.5	2068	-3.9	0.03
Palliative care	1811	-3.3	1675	-3	0.01	20	-0.8	43	-1.8	0.08	1	-0.3	8	-2.1	0.17	1790	-3.4	1624	-3.1	0.02

[1]Age, and Charlson Comorbidity index are presented in mean ± SD.

[2]The calculation of Charlson Comorbidity Index does not include Acquired Immune Deficiency Syndrome.

COVID-19: Coronavirus disease 2019; HCC: Hepatocellular carcinoma; NA: Not available; SMD: Standardized mean difference.

Incidence rates of clinical sequelae

The median follow-up duration was 249 (IQR: 108-259) days in the COVID-19+CLD group and 318 (IQR: 318-318) days in the COVID-19-CLD group. The crude incidence rates of HCC were 64.2 and 54.0 events per 10000 person-years for COVID-19+CLD and COVID-19-CLD, respectively. There was a trend for increased risk of HCC among COVID-19+CLD group compared to COVID-19-CLD group (IRR: 1.19, 95%CI: 0.99-1.42, P = 0.06) but did not reach statistical significance. There were 2273 and 1600 events of all-cause mortality, for COVID-19+CLD group and COVID-19-CLD group, respectively. The crude incidence rates of all-cause mortality were 676.5 (95%CI: 648.9-704.9) events per 10000 person-years (2273 events/33601 person-years) for COVID-19+CLD group, and 306.2 (95%CI: 291.4-321.6) events per 10000 person-years (1600 events/52258 person-years) for COVID-19-CLD group. The cumulative incidence of HCC, all-cause

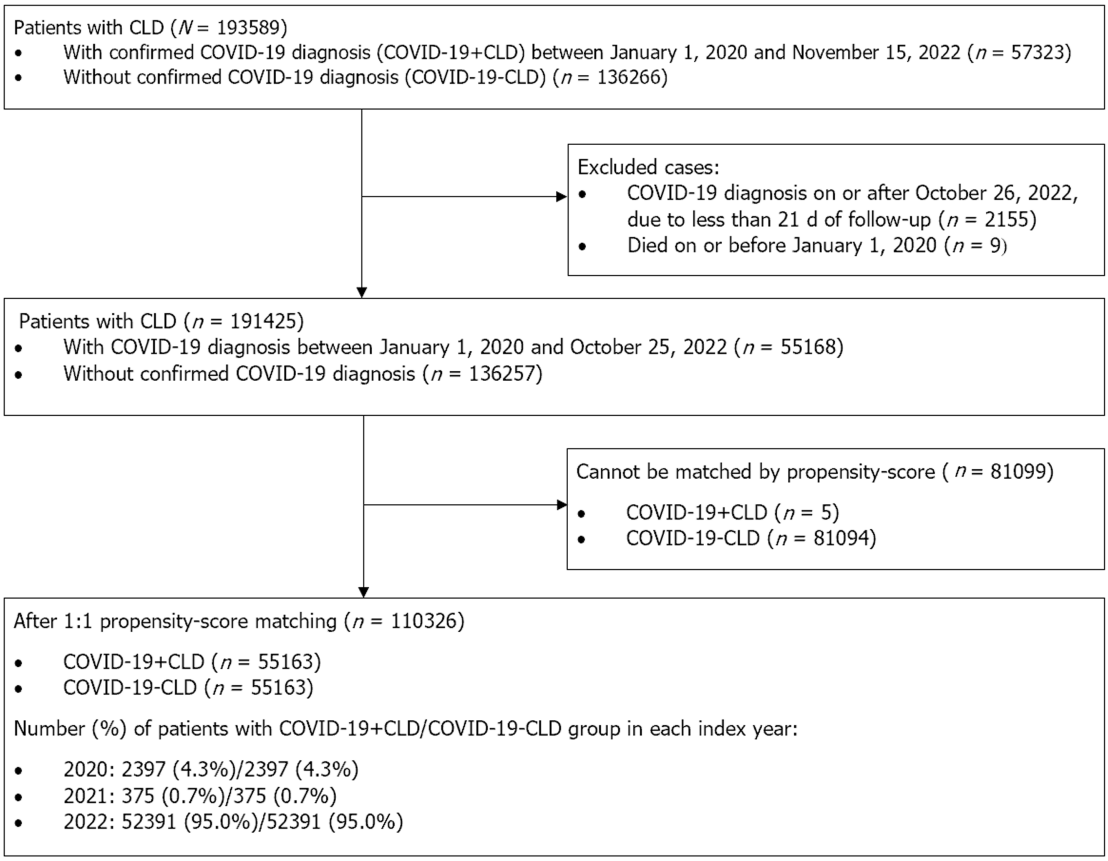

Figure 1 Study flowchart of eligible chronic liver diseases patients with and without confirmed coronavirus disease 2019 diagnosis for analysis. CLD: Chronic liver disease; COVID-19: Coronavirus disease 2019.

mortality, adverse hepatic outcomes, and palliative care among COVID-19+CLD patients and COVID-19-CLD are shown in Figure 2. COVID-19+CLD patients were associated with significantly higher risks of all-cause mortality (IRR: 2.21, 95%CI: 2.07-2.36, $P < 0.001$), adverse hepatic outcomes (IRR: 1.74, 95%CI: 1.64-1.85, $P < 0.001$), which were predominantly contributed by incident cirrhosis (IRR: 1.79, 95%CI: 1.68-1.89, $P < 0.001$), followed by liver decompensation (IRR: 1.36, 95%CI: 1.17-1.57, $P < 0.001$), compared to the COVID-19-CLD (Table 2).

Among patients with CLD, there were no significant differences in the risks of receiving curative (IRR: 1.16, 95%CI: 0.93-1.46, $P = 0.20$) or non-curative (IRR: 0.98, 95%CI: 0.86-1.11, $P = 0.70$) treatment to HCC. COVID-19+CLD patients were at higher chance of receiving palliative care (IRR: 1.60, 95%CI: 1.46-1.75, $P < 0.001$) compared to COVID-19-CLD patients (Table 2).

Incidence rates of clinical sequelae according to the phase of infection

During the acute phase of infection, patients with CLD who had confirmed SARS-CoV-2 infection in 2022 were associated with significantly higher risks of HCC (IRR: 1.89, 95%CI: 1.03-3.47, $P = 0.04$) and all-cause mortality (IRR: 7.06, 95%CI: 5.78-8.63, $P < 0.001$). The risks of adverse hepatic outcomes were increased (IRR: 1.98, 95%CI: 1.79-2.18, $P < 0.001$), not only contributed by an increased risk of HCC, but also cirrhosis (IRR: 1.88, 95%CI: 1.71-2.06, $P < 0.001$) and liver decompensation (IRR: 2.85, 95%CI: 1.77-4.58, $P < 0.001$). There were no significant differences in the incidence of receiving curative treatment (IRR: 0.57, 95%CI: 0.25-1.28, $P = 0.17$) or non-curative treatment (IRR: 1.24, 95%CI: 0.77-2.01, $P = 0.38$), but a significantly higher chance of receiving palliative care (IRR: 4.46, 95%CI: 3.28-6.06, $P < 0.001$) for the COVID-19 patients, compared to the controls (Table 3).

In the post-acute phase of infection, CLD patients with SARS-CoV-2 infection were still associated with significantly higher risks of HCC (IRR: 1.24, 95%CI: 1.00-1.53, $P = 0.05$), all-cause mortality (IRR: 1.24, 95%CI: 1.14-1.36, $P < 0.001$) and adverse hepatic outcomes (IRR: 1.24, 95%CI: 1.13-1.35, $P < 0.001$), but the risk ratios were numerically diminished compared to the acute phase. Risk of incident cirrhosis (IRR: 1.28, 95%CI: 1.17-1.39, $P < 0.001$) and liver decompensation (IRR: 1.26, 95%CI: 1.05-1.52, $P = 0.01$) in CLD patients with COVID-19 were maintained compared to controls. There were no significant differences in the incidence of receiving curative treatment (IRR: 1.20, 95%CI: 0.92-1.57, $P = 0.18$), non-curative treatment (IRR: 1.02, 95%CI: 0.88-1.18, $P = 0.82$), and palliative care (IRR: 1.10, 95%CI: 0.98-1.24, $P = 0.11$) for HCC (Table 3). Figure 3 shows the cumulative incidence of HCC, all-cause mortality, adverse hepatic outcomes, and palliative care in the acute and post-acute phases of SARS-CoV-2 infection and Figure 4 shows the proportion of treatment modalities of HCC stratified by the presence and phase of SARS-CoV-2 infection.

Table 2 Incidence rates of clinical sequelae of chronic liver disease patients with coronavirus disease 2019 diagnosis in 2020-2022 and controls after 1:1 propensity score matching

Outcomes	2020-2022												
	COVID-19 patients (*n* = 55163)					Control (*n* = 55163)					COVID-19 patients *vs* control		
	Cumulative incidence		Crude incidence rate (Events/10000 person-yr)			Cumulative incidence		Crude incidence rate (Events/10000 person-yr)					
	New events	Rate, %	Estimate	95%CI	Person-years	New events	Rate, %	Estimate	95%CI	Person-years	IRR[1]	95% CI	*P* value
HCC	211	0.39	64.2	(55.8, 73.5)	32870	276	0.51	54	(47.8, 60.8)	51087	1.19	(0.99, 1.42)	0.06
All-cause mortality	2273	4.12	676.5	(648.9, 704.9)	33601	1600	2.9	306.2	(291.4, 321.6)	52258	2.21[b]	(2.07, 2.36)	< 0.001
Adverse hepatic outcomes	2407	4.62	789.3	(758.1, 821.5)	30495	2183	4.19	453.5	(434.7, 473.0)	48133	1.74[b]	(1.64, 1.85)	< 0.001
Cirrhosis	2493	4.68	803.8	(772.6, 836.0)	31014	2206	4.14	450.1	(431.5, 469.2)	49015	1.79[b]	(1.68, 1.89)	< 0.001
Liver decompensation hepatorenal syndrome	88	0.16	26.3	(21.1, 32.4)	33468	106	0.19	20.4	(16.7, 24.6)	52070	1.29	(0.97, 1.71)	0.08
Liver failure	71	0.13	21.3	(16.6, 26.8)	33371	91	0.17	17.5	(14.1, 21.5)	51902	1.21	(0.89, 1.66)	0.22
Hepatic coma/encephalopathy	60	0.11	17.9	(13.7, 23.1)	33454	61	0.11	11.7	(9.0, 15.1)	52033	1.53[a]	(1.07, 2.18)	0.02
Ascites	129	0.24	38.7	(32.3, 46.0)	33341	154	0.28	29.7	(25.2, 34.8)	51882	1.30[a]	(1.03, 1.65)	0.03
Variceal bleeding	150	0.28	45.5	(38.5, 53.4)	32977	159	0.29	31	(26.4, 36.2)	51316	1.47[b]	(1.17, 1.84)	< 0.001
Curative treatment to HCC	130	0.24	39.5	(33.0, 46.9)	32892	174	0.32	34	(29.1, 39.5)	51156	1.16	(0.93, 1.46)	0.2
Hepatic resection	86	0.16	25.9	(20.7, 32.0)	33206	109	0.2	21.1	(17.3, 25.5)	51664	1.23	(0.93, 1.63)	0.16
Liver transplantation	13	0.02	3.9	(2.1, 6.6)	33442	20	0.04	3.8	(2.3, 5.9)	52028	1.01	(0.50, 2.03)	0.97
Radiofrequency ablation of liver	51	0.09	15.3	(11.4, 20.1)	33294	75	0.14	14.5	(11.4, 18.2)	51784	1.06	(0.74, 1.51)	0.76

Non-curative treatment to HCC	394	0.75	122.5	(110.7, 135.3)	32151	627	1.19	125.6	(116.0, 135.8)	49916	0.98	(0.86, 1.11)	0.7
Transarterial chemoembolization	83	0.15	25	(19.9, 31.0)	33239	125	0.23	24.2	(20.1, 28.8)	51691	1.03	(0.78, 1.36)	0.82
Radiotherapy to liver	3	0.01	0.9	(0.2, 2.6)	33543	3	0.01	0.6	(0.1, 1.7)	52189	1.56	(0.31, 7.71)	0.59
Systemic chemotherapy or immunotherapy	351	0.66	108.2	(97.2, 120.2)	32434	547	1.03	108.6	(99.7, 118.1)	50363	1	(0.87, 1.14)	0.96
Palliative care	924	1.73	285.8	(267.7, 304.8)	32332	904	1.7	179	(167.5, 191.0)	50510	1.60[b]	(1.46, 1.75)	< 0.001

[1]IRR > 1 (or < 1) indicates COVID-19 patients had higher (lower) risk of clinical outcome compared to the matched control group.

[a]$P < 0.05$.

[b]$P < 0.01$. IRR is considered statistically significant, with indicates [a]$P < 0.05$ and indicates [b]$P < 0.01$. COVID-19: Coronavirus disease 2019; HCC: Hepatocellular carcinoma; IRR: Incidence rate ratio.

Subgroup analysis

Results of HCC among most subgroups showed that there were no significant differences between the subgroups compared, which were generally consistent with the main results. Meanwhile, results of the subgroups of patients with cirrhosis, HBV, multi-organ dysfunction, and patients in the year 2022 showed that COVID-19+CLD was associated with significantly higher risks of HCC, while result of subgroup of patients in the year 2020 showed a significantly lower risk of HCC, compared to COVID-19-CLD. Results of all-cause mortality outcome among subgroups were mostly consistent with the main results, except for the subgroup of younger patients (age ≤ 50) and patients in the year 2020, 2021. The increased risks of adverse hepatic outcomes in CLD patients with SARS-CoV-2 infection were mostly consistent with the main results, regardless of causes of CLD, presence of multi-organ dysfunction, COVID-19 vaccination status, or the time period. For the observed heightened risks of liver decompensation in SARS-CoV-2 infected patients with CLD compared to uninfected patients with CLD, the results in the subgroups were also mostly consistent for older patients (age > 50), HBV causes of CLD, patients with multi-organ dysfunction, fully vaccinated individuals, and patients who were diagnosed with COVID-19 in the year 2022 (Supplementary Table 2). The observed higher risks of palliative care in all subgroups were consistent with the main results, regardless of cirrhosis, etiology of CLD, multi-organ dysfunction, and COVID-19 vaccination status (Supplementary Table 2). The results of subgroup analyses among COVID-19+CLD patients showed no significant differences in the incidence of HCC, all-cause mortality, adverse hepatic outcomes, and receiving palliative care in the hospitalization subgroup. Nevertheless, antiviral users were associated with significantly higher risk of adverse hepatic outcomes, compared to patients who did not receive any antiviral medications (Supplementary Table 3).

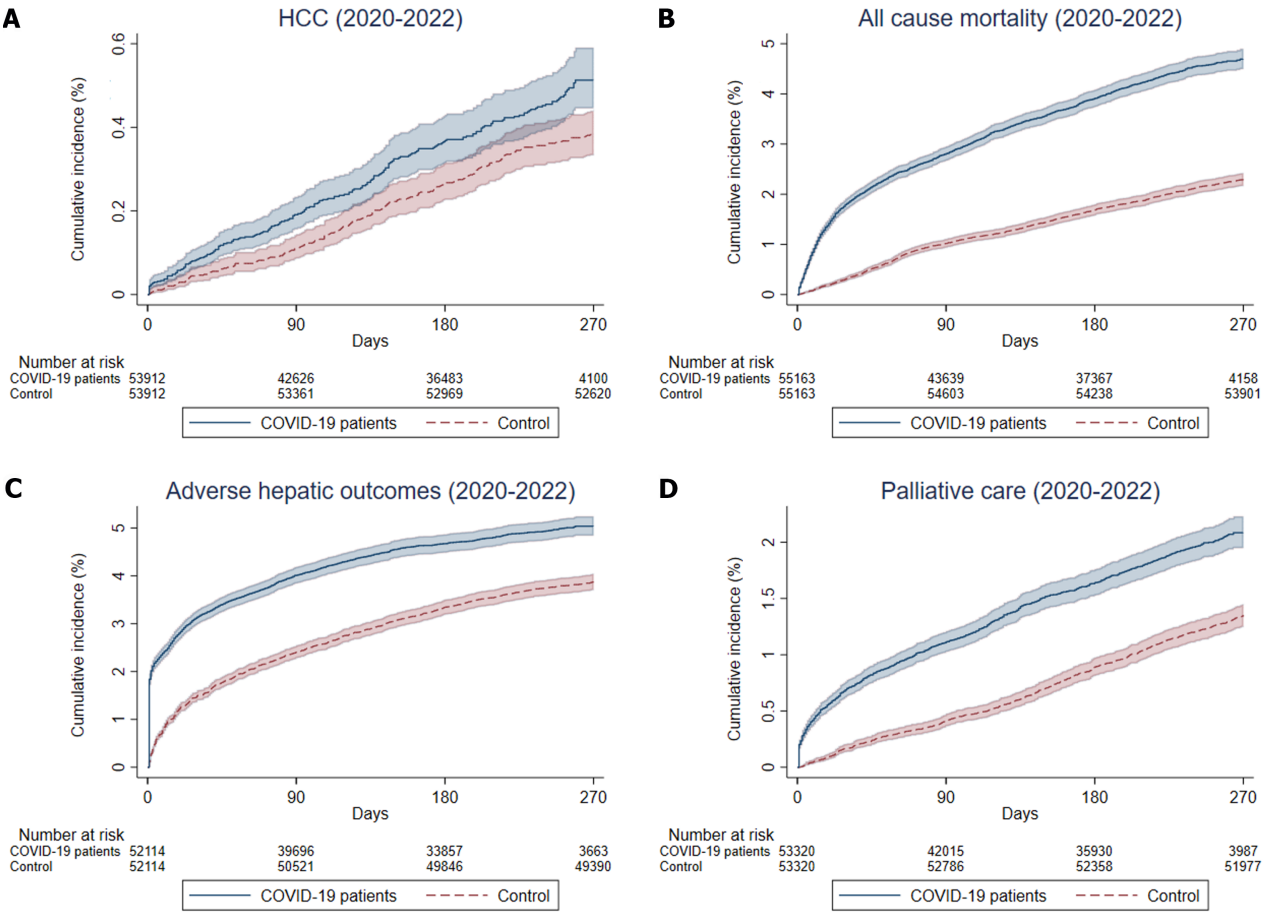

Figure 2 The cumulative incidence of study outcomes among chronic liver disease patients with and without severe acute respiratory syndrome coronavirus 2 infection. A: Hepatocellular carcinoma; B: All-cause mortality; C: Adverse hepatic outcomes; D: Palliative care. HCC: Hepatocellular carcinoma; COVID-19: Coronavirus disease 2019.

DISCUSSION

In this large real-world cohort of patients with pre-existing CLD, we demonstrated that SARS-CoV-2 infection was significantly associated with an increased risk of all-cause mortality and adverse hepatic outcomes, which is consistent with the literature. We observed that while the overall risk of incident HCC was not increased, alterations in treatment strategies for HCC were inevitable following COVID-19 in patients with CLD, with an increased risk of receiving palliative care as the definitive treatment for HCC. The negative influence of SARS-CoV-2 infection on patients with CLD observed during the acute phase persisted through to the post-acute phase, albeit in a diminished manner. Our cohort is further distinguished from other published studies by the inclusion of mostly (> 95%) non-cirrhotic patients, whose underlying CLD was due to HBV in 40% of the cohort (Supplementary Table 2), in contrast to other published studies that investigated individuals with cirrhosis[1-3], with ALD as the predominant etiology[21]. Importantly, instead of uninfected healthy controls, historic cohorts or SARS-CoV-2 infected patients without CLD, we compared the risk against contemporaneous CLD patients without SARS-CoV-2 infection, after matching for age, gender, comorbidity, COVID-19 vaccination status, and observation period. In addition, we demonstrated that the increased risk of all-cause mortality in COVID-19+CLD was at least contributed by adverse hepatic outcomes, namely incident cirrhosis and liver decompensation (hepato-renal syndrome, liver failure, hepatic encephalopathy, ascites, and variceal bleeding). For the first time, the risk of adverse hepatic outcomes in the acute and post-acute phase of COVID-19 among patients with CLD was reported. We showed that the risk of incident cirrhosis persisted in the post-acute phase among COVID-19+CLD patients. Similarly, the risk of liver decompensation was most pronounced in the acute phase of SARS-CoV-2 infection, but was maintained in a diminished manner in the post-acute phase. Although the exact mechanisms are not known, one can postulate that SARS-CoV-2 infection and the resultant cytokine activation[22,23] and immune perturbations[24] resulted in further liver injury, and accelerated liver fibrogenesis due to activation of hepatic stellate cells responsible for fibrogenesis[25] in CLD subjects who are already predisposed to cirrhosis, leading to earlier onset of this complication. Even after the resolution of SARS-CoV-2 infection, which is a predominantly extra-hepatic acute illness, the risk of new-onset cirrhosis and liver decompensation remains exaggerated compared to uninfected controls. This finding carries potential implications on enhanced surveillance and monitoring of patients with CLD who have recovered from SARS-CoV-2 infection.

Table 3 Incidence rates of clinical sequelae of chronic liver disease patients with coronavirus disease 2019 in 2022 and matched controls in the acute and post-acute phases of infection

Outcomes	Acute phase of infection (within 21 d)													Post-acute phase of infection (beyond 21 d)				
	COVID-19 patients in 2022 (*n* = 52391)					Control (*n* = 52391)					COVID-19 patients in 2022 *vs* control			COVID-19 patients in 2022 (*n* = 47986)				
	Cumulative incidence		Crude incidence rate (Events/10000 person-yr)			Cumulative incidence		Crude incidence rate (Events/10000 person-yr)						Cumulative incidence		Crude incidence rate (Events/10000 person-yr)		
	New events	Rate	Estimate	95%CI	Person-yr	New events	Rate	Estimate	95%CI	Person-yr	IRR[1]	95%CI	*P* value	New events	Rate	Estimate	95%CI	Person-yr
HCC	30	0.06	107.9	(72.8, 154.0)	2781	16	0.03	57.1	(32.6, 92.8)	2,801	1.89[a]	(1.03, 3.47)	0.04	151	0.32	66.2	(56.1, 77.7)	22,797
All-cause mortality	764	1.46	2683.7	(2496.7, 2880.9)	2847	109	0.21	380.1	(312.1, 458.5)	2868	7.06[b]	(5.78, 8.63)	< 0.001	854	1.78	365.6	(341.5, 391.0)	23356
Adverse hepatic outcomes	1180	2.39	4469.6	(4218.2, 4732.1)	2640	607	1.23	2259.6	(2083.4, 2446.7)	2686	1.98[b]	(1.79, 2.18)	< 0.001	895	1.99	415	(388.3, 443.1)	21564
Cirrhosis	1260	2.49	4676.2	(4421.6, 4941.8)	2694	683	1.35	2490	(2306.7, 2683.9)	2743	1.88[b]	(1.71, 2.06)	< 0.001	900	1.96	409.6	(383.2, 437.2)	21974
Liver decompensation hepatorenal syndrome	20	0.04	70.5	(43.1, 108.9)	2837	6	0.01	21	(7.7, 45.7)	2859	3.36[b]	(1.35, 8.36)	0.009	54	0.11	23.2	(17.4, 30.3)	23278
Liver failure	14	0.00%	49.5	(27.0, 83.0)	2831	4	0.01	14	(3.8, 35.9)	2852	3.53[a]	(1.16, 10.71)	0.03	43	0.09	18.5	(13.4, 24.9)	23217
Hepatic coma/encephalopathy	8	0.02	28.2	(12.2, 55.6)	2835	6	0.01	21	(7.7, 45.7)	2856	1.34	(0.47, 3.87)	0.59	38	0.08	16.3	(11.6, 22.4)	23261
Ascites	22	0.04	77.9	(48.8, 117.9)	2826	10	0.02	35.1	(16.8, 64.6)	2847	2.22[a]	(1.05, 4.68)	0.04	82	0.17	35.4	(28.1, 43.9)	23176
Variceal bleeding	37	0.07	132.5	(93.3, 182.6)	2793	12	0.02	42.6	(22.0, 74.5)	2,815	3.11[b]	(1.62, 5.96)	< 0.001	85	0.18	37.1	(29.7, 45.9)	22898
Curative treatment to HCC	9	0.02	32.3	(14.8, 61.3)	2786	16	0.03	57	(32.6, 92.6)	2807	0.57	(0.25, 1.28)	0.17	93	0.2	40.7	(32.9, 49.9)	22,828
Hepatic resection	4	0.01	14.2	(3.9, 36.4)	2815	10	0.02	35.3	(16.9, 64.8)	2836	0.4	(0.13, 1.28)	0.12	64	0.13	27.7	(21.4, 35.4)	23072
Liver transplantation	3	0.01	10.6	(2.2, 30.9)	2835	1	0	3.5	(0.1, 19.5)	2856	NA	NA	NA	6	0.01	2.6	(0.9, 5.6)	23268

Radiofrequency ablation of liver	2	0	7.1	(0.9, 25.6)	2822	5	0.01	17.6	(5.7, 41.0)	2,843	0.4	(0.08, 2.08)	0.28	41	0.09	17.7	(12.7, 24.0)	23137
Non-curative treatment to HCC	37	0.07	136.1	(95.8, 187.6)	2719	30	0.06	109.6	(73.9, 156.4)	2738	1.24	(0.77, 2.01)	0.38	289	0.63	129.5	(115.0, 145.4)	22309
Transarterial chemoembolization	9	0.02	31.9	(14.6, 60.6)	2817	11	0.02	38.8	(19.4, 69.4)	2837	0.82	(0.34, 1.99)	0.67	64	0.13	27.7	(21.3, 35.4)	23096
Radiotherapy to liver	0	0	0	NA	2845	1	0	3.5	(0.1, 19.4)	2866	NA	(0.00, 0.00)	NA	3	0.01	1.3	(0.3, 3.8)	23338
Systemic chemotherapy or immunotherapy	28	0.06	102	(67.8, 147.5)	2744	21	0.04	76	(47.0, 116.2)	2763	1.34	(0.76, 2.36)	0.31	261	0.56	115.9	(102.2, 130.8)	22527
Palliative care	221	0.44	805	(702.3, 918.4)	2745	50	0.1	180.5	(134.0, 238.0)	2770	4.46[b]	(3.28, 6.06)	< 0.001	447	0.96	198.1	(180.2, 217.3)	22565

[1]IRR > 1 (or < 1) indicates COVID-19 patients had higher (lower) risk of clinical outcome compared to the matched control group. IRR was estimated only if the total number of events in the two comparison groups were greater than 1.
[a]$P < 0.05$.
[b]$P < 0.01$. IRR is considered statistically significant, with indicates [a]$P < 0.05$ and indicates [b]$P < 0.01$. HCC: Hepatocellular carcinoma; HR: Hazard ratio; IRR: Incidence rate ratio; COVID-19: Coronavirus disease 2019.

Although the risk of HCC was not found to be significantly increased among COVID-19+CLD patients in the overall cohort (IRR 1.19, 95%CI: 0.99-1.42, $P = 0.06$), there was an increased risk of HCC in both acute (IRR 1.89, 95%CI: 1.03-3.47, $P = 0.04$) and post-acute phase (IRR 1.24, 95%CI: 1.00-1.53, $P = 0.05$). This phenomenon cannot be explained by the higher risk of cirrhosis and liver decompensation following SARS-CoV-2 infection as the time window was too short for hepatocarcinogenesis. Although SARS-CoV-2 has been suggested to demonstrate liver tropism as confirmed by viral RNA and spike protein detection in autopsy liver specimens[26,27], it is not known to cause carcinogenic mutations or induce pro-oncogenic proteins like what hepatitis B virus does[28,29]. Therefore, non-biological mechanisms likely exist to account for the observed increased risk of HCC in CLD patients infected by SARS-CoV-2 infection. We hypothesized that it might be related to paradoxically earlier detection of tumors in patients with COVID-19 who are also known to have increased risk of acute liver injury[30,31], that triggered off imaging workups for abnormal liver enzymes. Importantly, the chance of receiving palliative care was markedly increased in the acute phase (IRR 4.46) but not in the post-acute phase of SARS-CoV-2 infection. Understandably, during the acute phase of infection, patients might be too sick to receive more aggressive treatment such as surgical resection, and the common association with abnormal liver enzymes would have precluded these subjects from medical oncological treatment such as immunotherapy or targeted therapy[7]. During the initial phase of COVID-19 pandemic, there was implementation of lockdown strategies and prioritization of healthcare services to prevention and management of SARS-CoV-2 in virtually all health care facilities[32]. It inevitably led to delays

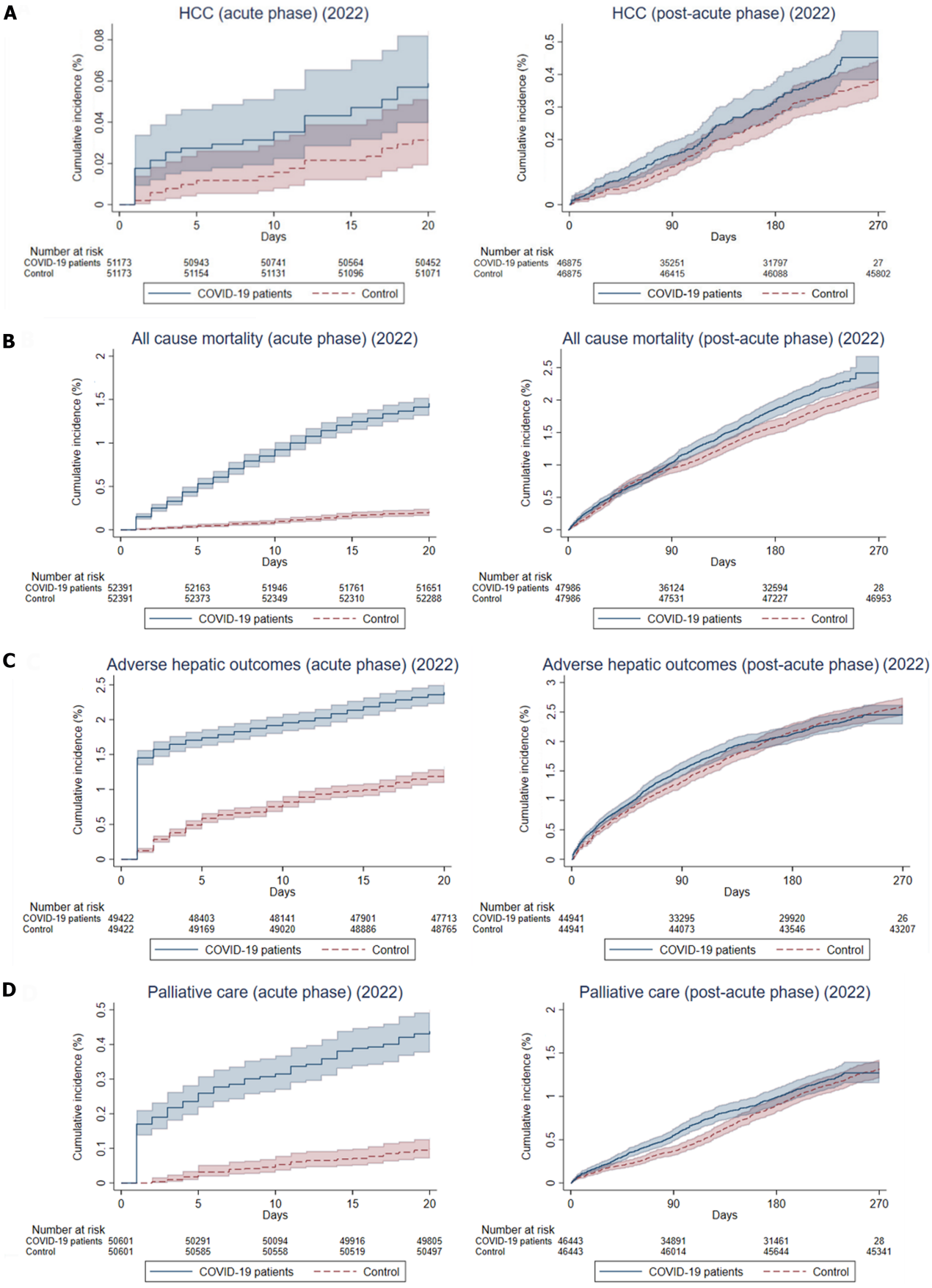

Figure 3 The cumulative incidence of study outcomes in the acute phase *vs* post-acute phase of infection. A: Hepatocellular carcinoma; B: All-cause mortality; C: Adverse hepatic outcomes; D: Palliative care. HCC: Hepatocellular carcinoma; COVID-19: Coronavirus disease 2019.

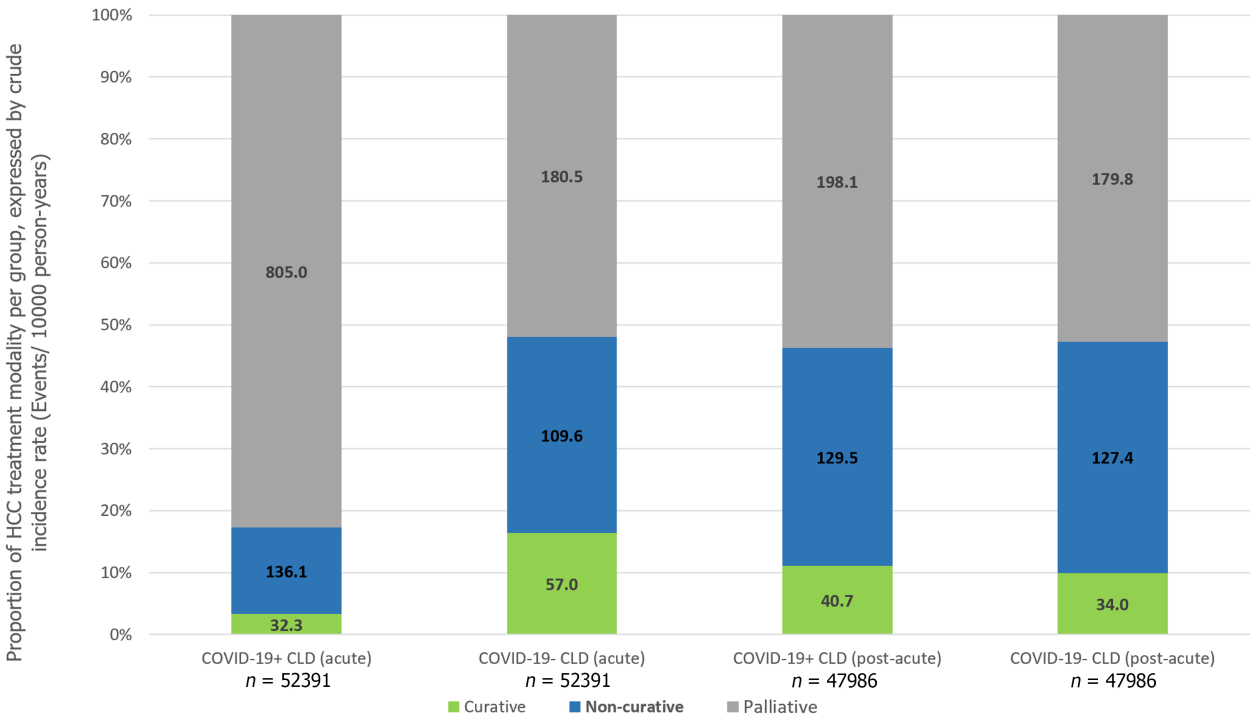

Figure 4 Proportion of hepatocellular carcinoma treatment modality in different groups, expressed by crude incidence rate (events/10000 person-years). HCC: Hepatocellular carcinoma; COVID-19: Coronavirus disease 2019.

in routine care, such as patient follow-up[33], HCC surveillance and priority referrals to relevant disciplines to manage HCC.[11] Even for subjects with milder disease course of COVID-19 and preserved liver function, because of such disruption in the routine clinical service, essential abdominal imaging such as ultrasound scans and computed tomography scans[34,35] was not performed for CLD patients in a capacity similar to pre-COVID era[36]. This would inevitably lead to delays in HCC diagnosis, causing these patients to be diagnosed at a more advanced stage of cancer and eventually became ineligible for loco-regional oncological treatments for HCC even when their medical condition was otherwise stable[7]. This hypothesis is further supported by the fact that patients had a paradoxically 'reduced' risk of HCC during earlier period of COVID-19 (*i.e.*, year 2020) coinciding with lockdown and suspension of services, but an increased risk of HCC towards the later stages of the COVID-19 pandemic (*i.e.* year 2022) when healthcare services gradually resumed (Supplementary Table 2). In the post-acute phase, when the infection resolved, regardless of whether there was COVID-19 induced abnormal liver biochemistry, these patients would be re-evaluated for eligibility to receive oncological treatment, thus contributing to the resolved risk of receiving palliative care.

In the subgroup analysis, we showed that the increased risks of all-cause mortality, liver decompensation, and palliative strategy for HCC were more pronounced among older subjects, cirrhotic patients, HBV-related CLD, presence of multi-organ dysfunction, and unvaccinated/non-fully vaccinated subgroups. We observed no increased risk for these adverse outcomes in year 2020 and 2021. Intriguingly, there was a significantly reduced risk of liver decompensation among COVID-19 subjects with CLD during year 2020 compared to uninfected CLD subjects. In the early stage of the pandemic, when vaccination and antiviral treatment were unavailable, intensive monitoring and supportive treatment were the only measures that could be taken. In addition, every confirmed case of COVID-19 infection was hospitalized regardless of severity. These practices might have paradoxically led to heightened vigilance, allowing opportunistic surveillance for laboratory abnormalities and optimization of the underlying CLD, which in turn lowered the risk of liver decompensation. Importantly, full vaccination was associated with a numerically lower risk of all-cause mortality and adverse hepatic outcomes in COVID-19 subjects with CLD (IRR 1.53 and 1.42, respectively) compared to non-fully vaccinated COVID-19 subjects with CLD (IRR 2.37 and 1.73, respectively). Similarly, full vaccination was associated with numerically lower chance of palliative care in COVID-19 subjects with CLD (IRR 1.41, 95%CI: 1.15-1.71, $P < 0.001$) compared to non-fully vaccinated COVID-19 subjects with CLD (IRR 1.68, 95%CI: 1.50-1.89, $P < 0.001$). Although the immediate threats of the COVID-19 pandemic is waning with the widespread adoption of vaccination and availability of antiviral therapies, the pandemic is not yet over[37] and vigilance should be maintained to protect vulnerable subjects from the adverse effects of COVID-19. Booster doses for COVID-19 vaccine for the general population are recommended [38], due to rapid emergence of variant strains and to maintain immunological memory. As COVID-19 vaccines have been proven safe to use without increasing risk of acute liver injury[12], the findings from our current study further supports the uptake of the COVID-19 vaccine among patients with CLD.

Our study has some limitations. Firstly, we did not adjust for the severity of SARS-CoV-2 infection during the acute phase, and further stratification of the risks of adverse outcomes based on disease severity was not possible. Disease severity might also have confounded the observed higher risk of adverse hepatic outcomes among antiviral users (Supplementary Table 3), compared to no antiviral use, because antivirals were mainly indicated among those with, or at

risk of more severe COVID-19 infection[39]. Secondly, the diagnosis and outcomes were based on coding, and might have detected fewer events than expected due to non-coded conditions. This might have contributed to the small sample size in certain subgroups, leading to under-powered issue for the statistical analysis.

CONCLUSION

In conclusion, this large cohort consisting of 110,326 patients with CLD demonstrated SARS-CoV-2 infection was not associated with increased risk of HCC, but significantly higher risk of all-cause mortality, adverse hepatic outcomes, and with negative effect in treatment strategy for HCC. We found that although CLD patients with SARS-CoV-2 infection were not associated with increased risk of liver cancer, they are more likely to receive palliative treatment for HCC, compared to CLD patients who did not have SARS-CoV-2 infection. We showed for the first time that these detrimental effects of SARS-CoV-2 infection are observed in both the acute and post-acute phases among patients with CLD. Specifically, new-onset cirrhosis and liver decompensation are shown to be a type of clinical presentation of PASC, with a persisting risk of these hepatic PASCs even after the resolution of acute SARS-CoV-2 infection. These findings have important implications for monitoring and surveillance strategies for patients with CLD who have recovered from SARS-CoV-2 infection, and vaccination against SARS-CoV-2 infection should continue to be advocated among patients with CLD.

ARTICLE HIGHLIGHTS

Research background

Chronic liver disease (CLD) was associated with adverse clinical outcomes among people with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Research motivation

There is no data regarding how coronavirus disease 2019 (COVID-19) interplay with the risk of hepatocellular carcinoma (HCC) and subsequent treatment strategy. In addition, much is known about the immediate and long-term consequences of SARS-CoV-2 infection in CLD patients, and how they affect the incidence and oncological treatment for HCC.

Research objectives

We determined the effects of SARS-CoV-2 infection on the incidence and treatment strategy of HCC among patients with CLD.

Research methods

A retrospective, territory-wide cohort of CLD patients was identified from an electronic health database in Hong Kong. Patients with confirmed SARS-CoV-2 infection (COVID-19+CLD) between January 1, 2020 and October 25, 2022 were identified and matched 1:1 by propensity-score with those without (COVID-19-CLD). Each patient was followed up until death, outcome event, or November 15, 2022. Primary outcome was incidence of HCC. Secondary outcomes included all-cause mortality, adverse hepatic outcomes, and different treatment strategies to HCC (curative, non-curative treatment, and palliative care). Analyses were further stratified by acute (within 20 d) and post-acute (21 d or beyond) phases of SARS-CoV-2 infection. Incidence rate ratios (IRRs) were estimated by Poisson regression models.

Research results

Of 193589 CLD patients (> 95% non-cirrhotic) in the cohort, 55163 patients with COVID-19+CLD and 55163 patients with COVID-19-CLD were included after 1:1 propensity-score matching. Upon 249-d median follow-up, COVID-19+CLD was not associated with increased risk of incident HCC (IRR: 1.19, 95%CI: 0.99-1.42, $P = 0.06$), but higher risks of receiving palliative care for HCC (IRR: 1.60, 95%CI: 1.46-1.75, $P < 0.001$), compared to COVID-19-CLD. In both acute and post-acute phases of infection, COVID-19+CLD were associated with increased risks of all-cause mortality (acute: IRR: 7.06, 95%CI: 5.78-8.63, $P < 0.001$; post-acute: IRR:1.24, 95%CI: 1.14-1.36, $P < 0.001$) and adverse hepatic outcomes (acute: IRR: 1.98, 95%CI: 1.79-2.18, $P < 0.001$; post-acute: IRR: 1.24, 95%CI: 1.13-1.35, $P < 0.001$), compared to COVID-19-CLD.

Research conclusions

Although CLD patients with SARS-CoV-2 infection were not associated with increased risk of HCC, they were more likely to receive palliative treatment than those without. We showed for the first time that the detrimental effects of SARS-CoV-2 infection persisted in post-acute phase.

Research perspectives

Our findings have important implications for strategies of monitoring and surveillance for patients with CLD who have recovered from SARS-CoV-2 infection, and vaccination against SARS-CoV-2 infection should continue to be advocated among CLD patients.

ACKNOWLEDGEMENTS

The authors thank the Department of Health and Hospital Authority for the generous provision of data for this study. Lai FTT, Wong CKH and Wong ICK's post were partly funded by D24H. Hence this work was partly supported by AIR@InnoHK administered by Innovation and Technology Commission.

FOOTNOTES

Co-first authors: Lung-Yi Mak and Matthew Shing Hin Chung.

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Author contributions: Mak LY, Chung MSH, Wong CKH reviewed the literature, conducted analyses, contributed to the interpretation of the analysis, and wrote the manuscript; Mak LY, Li X, Wong CKH reviewed the literature, designed the study and statistical analysis. Chung MSH, Au ICH, Xiong X conducted analyses. Mak LY, Li X, Lai FTT, Wan EYF, Chui CSL, Cheng FWT, Chan EW, Cheung CL, Seto WK, Yuen MF, Wong CKH, Wong ICK contributed to the interpretation of the analysis. Mak LY, Wong CKH and Wong ICK were responsible for the study concept. Both Wong CKH and Wong ICK have played important and indispensable roles in the study design, data interpretation and manuscript preparation as the co-corresponding authors. Wong CKH and Wong ICK conceptualized, designed, and supervised the whole process of the project. Wong CKH reviewed the literature and was instrumental for statistical analysis. Wong ICK applied for and obtained the funds for this research project, and contributed to the interpretation of the analysis. This collaboration between Wong CKH and Wong ICK is crucial for the publication of this manuscript. All authors contributed to the interpretation of the analysis, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Supported by Collaborative Research Fund Scheme, University Grants Committee, No. C7154-20GF; and Data Discovery for Health (D24H); Innovation and Technology Commission, AIR@InnoHK.

Institutional review board statement: The study protocol was approved by the Institutional Review Board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (UW 20-556, UW 21-149 and UW 21-138); and the Department of Health Ethics Committee (LM 21/2021 and LM 175/2022).

Informed consent statement: Informed patient consent was not required as the data used in this study were anonymised.

Conflict-of-interest statement: Lung-Yi Mak is an advisory board member for Gilead Sciences. Xue Li has received research grants from the Health Bureau of the Government of the Hong Kong SAR, research and educational grants from Janssen and Pfizer; internal funding from the University of Hong Kong; consultancy fee from Merck Sharp & Dohme, unrelated to this work. Francisco Tsz Tsun Lai has been supported by the RGC Postdoctoral Fellowship under the Hong Kong Research Grants Council and has received research grants from the Health Bureau of the Government of the Hong Kong SAR, outside the submitted work. Eric Yuk Fai Wan has received research grants from the Health Bureau of the Government of the Hong Kong SAR, and the Hong Kong Research Grants Council, outside the submitted work. Celine Sze Ling Chui has received grants from the Health Bureau of the Hong Kong Government, Hong Kong Research Grant Council, Hong Kong Innovation and Technology Commission, Pfizer, IQVIA, and Amgen; personal fee from Primevigilance Ltd.; outside the submitted work. Esther Wai Yin Chan reports honorarium from the Hospital Authority, grants from the Research Grants Council (RGC, Hong Kong), Research Fund Secretariat of the Health Bureau, National Natural Science Fund of China, Wellcome Trust, Bayer, Bristol-Myers Squibb, Pfizer, Janssen, Amgen, Takeda, and Narcotics Division of the Security Bureau of the Hong Kong SAR, outside the submitted work. Ching Lung Cheung received research grants and honorarium from Amgen, research grant support from HMRF, and honorarium from Abbott. Wai Kay Seto received speaker's fees from AstraZeneca and Mylan, is an advisory board member of CSL Behring, is an advisory board member and received speaker's fees from AbbVie, and is an advisory board member, received speaker's fees, and researching funding from Gilead Sciences. Man-Fung Yuen serves as advisor/consultant for AbbVie, Assembly Biosciences, Aligos Therapeutics, Arbutus Biopharma, Bristol Myer Squibb, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, GlaxoSmithKline, Gilead Sciences, Immunocore, Janssen, Merck Sharp and Dohme, Hoffmann-La Roche, and Springbank Pharmaceuticals, Vir Biotechnology and receives grant/research support from Assembly Biosciences, Aligos Therapeutics, Arrowhead Pharmaceuticals, Bristol Myer Squibb, Fujirebio Incorporation, Gilead Sciences, Immunocore, Merck Sharp and Dohme, Hoffmann-La Roche, Springbank Pharmaceuticals, and Sysmex Corporation, outside the submitted work. Carlos King Ho Wong reports receipt of research funding from the EuroQoL Group Research Foundation, the Hong Kong Research Grants Council, the Hong Kong Health and Medical Research Fund, AstraZeneca and Boehringer Ingelheim, all outside of the submitted work. Ian Chi Kei Wong reports research funding outside the submitted work from Amgen, Bristol-Myers Squibb, Pfizer, Janssen, Bayer, GSK, Novartis, the Hong Kong RGC, and the Hong Kong Health and Medical Research Fund, National Institute for Health Research in England, European Commission, National Health and Medical Research Council in Australia. All other authors declare no competing interests.

Data sharing statement: The data that support the findings of this study were provided by the Hong Kong Hospital Authority. Restrictions apply to the availability of these data, which were used under license for this study.

STROBE statement: The authors have read the STROBE Statement – checklist of items, and the manuscript was prepared and revised according to the STROBE Statement – checklist of items.

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S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

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World J Hepatol 2024 February 27; 16(2): 229-240

DOI: 10.4254/wjh.v16.i2.229

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Epidemiological survey of cystic echinococcosis in southwest China: From the Qinghai-Tibet plateau to the area of Yunnan

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Amante MF, Argentina

Received: October 5, 2023
Peer-review started: October 5, 2023
First decision: October 28, 2023
Revised: November 18, 2023
Accepted: December 14, 2023
Article in press: December 14, 2023
Published online: February 27, 2024

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Abstract

BACKGROUND

Echinococcosis is prevalent in 9 provinces in Western and Northern China. An epidemiological survey of echinococcosis in 2012 and 2016 showed cases of echinococcosis in Yunnan Province.

AIM

To understand the spatial distribution and epidemiological characteristics of echinococcosis in Yunnan for the prevention and control of echinococcosis and to reduce the risk of infection in Yunnan Province.

METHODS

Based on the China Information System for Disease Control and Prevention (CISDCP), echinococcosis cases reported from 36 hospitals and 34 Centers for Disease Control were investigated and epidemiologically analyzed from 2021 to 2022. The exclusion criteria included suspected cases, same case only counted once and cases not from Yunnan. A total of 705 cases were investigated, of which 397 cases were suitable for statistical analysis. In these 397 cases, epidemiological investigation was tracked in 187 cases. All data were inputted using double entry in the Excel database, with error correction by double-entry comparison. The data

on echinococcosis cases in Yunnan Province were analyzed by ArcGIS 10.1 software to generate a density map of echinococcosis distribution. All statistical analyses were conducted using SPSS 17.0, including the chi-square test, linear regression test and logistic univariate and multivariate regression analyses.

RESULTS

A total of 397 cases were found in 89 counties in Yunnan Province. The number of cases in the top three prefectures were Dali (38.1%), Diqing (10.1%), and Kunming (8.3%), and the top five counties were Jianchuan (9.1%), Shangri La (8.3%), Eryuan (7. 6%), Heqing (6.9%), and Dali Districts (5.0%). There were significant differences between the different areas. The case reporting rate by CISDCP (33.8%) was low; the first case was reported by CISDCP in 2002, and the highest number of cases was 50 (2017). Confirmed and clinical cases accounted for 62.5% and 37.5%, respectively. However, 90.9% of the cases of hydatid disease were reported by the hospital system, and only 9.1% of the cases of hydatid disease were found in the community through active screening. The difference between the two methods of case detection was statistically significant. Most of the cases of echinococcosis were found in farmers/herdsmen (75.1%) and students (9.1%). In addition, Han (43.6%) and Bai (26.2%) had a higher incidence of infection than other nationalities, and the liver (87.7%) and lung (6.8%) were the most common sites of cyst formation. Among the analyzed cases, 187 were epidemiologically analyzed and the clinical symptoms were not obvious in the early stage in 47.1% of cases. The results of logistic regression analysis showed that the age group, education level, presence of dogs in the family (either previously or currently), and handwashing (occasionally or not) were factors related to echinococcosis infection. 55.6% of cases were in endemic areas, and 44.4% of cases were in non-endemic areas. Among 83 cases in non-endemic areas, only 4 cases had been to endemic areas and had a history of living, working, travelling, or hunting in echinococcosis epidemic areas.

CONCLUSION

Cases of echinococcosis were reported throughout the entire Yunnan province, with the majority distributed in Western Yunnan, suggesting that echinococcosis control should be strengthened in this area. We suggest that an epidemiological investigation should be carried out in the future, based on the clues from newly discovered cases in hospitals or from the CISDCP. The newly discovered cases in the hospital provided clues to comprehensively determine the location of cases and where epidemic spot investigation should be conducted.

Key Words: Echinococcosis; Cases; Epidemiological analysis; Yunnan province

Core Tip: Seventy medical institutions, including 36 hospitals, 34 Centers for Disease Control (CDCs), and China Information System for Disease Control and Prevention, were surveyed from 2021 to 2022. The 36 hospitals included 4 provincial hospitals (the First and Second-People's Hospital of Yunnan, and the First and Second Affiliated Hospitals of Kunming Medical University), 8 prefectural hospitals, and 24 county hospitals. The 34 CDCs included the Yunnan Institute of Parasitic Diseases, 9 prefectural CDCs, and 24 county CDCs. Information on echinococcosis cases from the ultrasound department, hepatobiliary surgery department, and case archives in the hospitals was retrospectively surveyed and recorded. The registration and management information on echinococcosis was consulted through the CDCs. The collected information on cases was organized and summarized before being entered into the database. Patient-privacy data were not included.

Citation: Zi JR, Xiao D, Peng J, Wu FW, Li JX, Yan XL, Wang ZQ, Cai X, Xu Q, Li BF, Yang YM. Epidemiological survey of cystic echinococcosis in southwest China: From the Qinghai-Tibet plateau to the area of Yunnan. *World J Hepatol* 2024; 16(2): 229-240
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/229.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.229

INTRODUCTION

Cystic echinococcus (CE) is a zoonotic parasitic disease caused by the larval stage of the parasite *Echinococcus granulosus* (*E. granulosus*), it parasitizes humans or animals and is distributed globally[1-4]. Two main types of echinococcosis are observed in humans: CE and alveolar echinococcosis (AE). People in epidemic areas commonly become poor or return to poverty due to echinococcosis, which is a major burden on communities in endemic areas of China and poses an important public health problem[5-8]. China has the highest prevalence of echinococcosis in the world. According to the prevalence of echinococcosis in the population and the infection rate of echinococcosis in dogs, the endemic counties were divided into the following 4 categories: Class I counties: The prevalence rate of humans ≥ 1%, or the infection rate of dogs ≥ 5%; class II counties: 0.1% ≤ the human prevalence rate < 1%, or 1% ≤ the canine infection rate < 5%; class III counties: 0 <the prevalence rate of people < 0.1%, or 0 < dog infection rate < 1%; class IV counties: There have been reports of local

cases of echinococcosis in the past, but no local cases have been found in 3 years, and other counties are non-endemic areas. There were 368 counties identified as endemic echinococcosis counties in China, the case detection rate was 0.51%. The prevalence was estimated to be 0.28% in the whole endemic area, and the number of patients was estimated to be 166098 with a distribution in 9 provinces/autonomous regions in Western and Northern China, including Xinjiang, Yunnan and Qinghai province[9,10]. Human echinococcosis was first reported in 1978 in Yunnan province. Liu *et al*[11] screened surgical patient data from the First and Second Affiliated Hospitals of Kunming Medical University and showed that there were 24 cases of hydatid disease in Yunnan Province from 1981 to 2001, with most cases distributed in Northwest Yunnan[12]. An epidemiological survey of echinococcosis in Yunnan Province in 2012 and 2016 showed that the detection rate of echinococcosis in the population was 0.06%, all of which had CE. This survey indicates that Yunnan Province is a low prevalence area of CE, mainly distributed in the northwest of 25° north latitude, including 24 counties of the 9 prefectures such as Diqing and Dali[13]. Understanding the epidemiological status, regional distribution, and epidemic characteristics of echinococcosis will assist with the provision of better control strategies and measures for echinococcosis in Yunnan Province. To fill these knowledge gaps, we aim to describe the demographic characteristics of people with echinococcosis in Yunnan Province, to map the prevalence of CE at high spatial resolution, and to assess the impact of risk factors.

MATERIALS AND METHODS

Data collection

Seventy medical institutions, including 36 hospitals, 34 Centers for Disease Control (CDCs), and the China Information System for Disease Control and Prevention (CISDCP), were surveyed from 2021 to 2022. The 36 hospitals included 4 provincial hospitals (the First and Second-People's Hospital of Yunnan, and the First and Second Affiliated Hospitals of Kunming Medical University), 8 prefectural hospitals, and 24 county hospitals. The 34 CDCs included the Yunnan Institute of Parasitic Diseases, 9 prefectural CDCs, and 24 county CDCs. Information on echinococcosis cases from the ultrasound department, hepatobiliary surgery department, and case archives in the hospitals was retrospectively surveyed and recorded. The registration and management information on echinococcosis was consulted through the CDCs. The collected information on cases was organized and summarized before being entered into the database. Patient-privacy data were not included.

Inclusion and exclusion criteria

Diagnosed cases were defined as confirmed, clinical, and suspected according to the "Diagnostic Criteria for Echinococcosis" (WS257-2006). Suspected cases were defined as those who had visited an echinococcosis epidemic area and presented with clinical characteristics and space-occupying lesions; clinical cases were defined as those with characteristic imaging of echinococcosis (by ultrasonography, computed tomography scan, magnetic resonance imaging, chest-X ray, bronchoscopy, or radiology) and with blood samples positive for anti-echinococcus antibodies (diagnosed by enzyme-linked immunosorbent assay, indirect hemagglutination antibody test, or indirect fluorescent antibody test); and confirmed cases were defined as those with an echinococcosis cyst wall, protoscolices, or ascocyst found on pathological and etiological examination[14]. The information collected from these cases was used to set up the database. The included cases were confirmed and clinical cases from Yunnan. The exclusion of cases included suspected cases, cases with duplicate diagnoses, and when the same case of echinococcosis was only counted once. Additionally, cases that were not from Yunnan were also excluded.

Epidemiological investigation of individual cases and analysis

Among the 397 cases, the complete epidemiological investigation was tracked in 187 cases and included epidemiologically related information such as past medical history, living history, exposure history, *etc*., to determine the epidemic characteristics of CE in Yunnan Province, and identify the source of the infection, in order to provide a scientific basis for CE prevention and control decisions. The 210 cases that could not be contacted or did not cooperate in the investigation were not included in the epidemiological investigation.

The survey included questions pertaining to basic information, clinical symptoms, epidemiological history, and personal behavior. Basic case information included name, sex, age group, ethnic group, occupation, education level, case category, parasite site, and network reports. The clinical symptoms included abdominal pain, cough, headache, and seizures. The questions relating to epidemiological history were as follows: "Have you travelled, worked, or stayed in echinococcosis endemic areas? (YTWSEA)"; "When did you visit the epidemic areas?"; "When you stayed in the echinococcosis epidemic area, did you contact or touch dogs?" and "Have you eaten half cooked or raw food?". The questions relating to personal behavior were as follows: "Do you, or have you ever had dogs in your family? (YHDIYF)"; "Did your family use cow dung to burn? (YUCDB)"; "Do you wash your hands before preparing meals or eating? (YWHPME)" and "Have you drunk wild water? (HYDWW)".

Therefore, we used negative binomial regression to analyze the factors influencing the prevalence of CE from the Qinghai-Tibet Plateau to Yunnan Province. The number of CE cases was set as the dependent variable.

Both univariate analysis and multiple collinearity tests were performed. The sex, age groups, ethnic group, occupation, education level, YTWSEA, YHDIYF, YUCDB, YWHPME and HYDWW, which may affect the development of echinococcosis, and these 10 factors were the independent variables. Spearman correlation analysis was used for preliminary exploration of the relationship between the prevalence of CE and the variables. Logistic single factor analysis showed the associations with echinococcosis, which were included in the multivariate analysis.

Statistical analysis

All data were inputted using double entry in the Excel database with error correction by double-entry comparison. All cases meeting the inclusion criteria were used for statistical analysis, but suspected cases were not used in the analysis of disease factors. All statistical analyses were conducted using SPSS 17.0 (IBM, New York, United States), and mapping was performed using ArcGIS 10.1 (ESRI, RedLands, United States). The data are expressed as frequency and percentage, and the comparison of rates between groups was tested using the chi-square test, linear regression tests and logistic regression analysis were performed. The significance level was set at $P < 0.05$.

RESULTS

Case characteristics

A total of 705 cases from 70 survey sites, including hospitals, CDCs and CISDCP, were recorded in the previously mentioned electronic database (Figure 1). A total of 397 cases found in 89 counties in Yunnan Province met the inclusion criteria, of which only 187 cases were subjected to individual epidemiological investigation. According to diagnostic criteria, the confirmed and clinical cases accounted for 62.4% (248/397) and 37.5% (149/397), respectively. Moreover, CE accounted for 99.8% (396/397) and AE accounted for 0.3% (1/397) of the total cases. According to the infection site, hepatic echinococcosis accounted for 87.7% (348/397), lung echinococcosis accounted for 6.8% (27/397), renal echinococcosis accounted for 1.3% (5/397), abdominal echinococcosis accounted for 1.0% (4/397), splenic echinococcosis accounted for 0.5% (2/397), cerebral echinococcosis accounted for 0.50% (2/397), and other sites accounted for 2.3% (9/397). The difference between the parasitic sites was statistically significant ($\chi^2 = 1051.170$, $P < 0.05$). Furthermore, there were significantly more hospital diagnosed cases (90.93%, 361/397) than investigation detected cases (9.1%, 36/397) ($\chi^2 = 298.870$, $P < 0.05$). The incidence rates were 0.002, 0.01, 0.01, 0.03, 0.03, 0.04, 0.04, 0.04, 0.07, 0.06, 0.07, 0.1, 0.08, 0.09, 0.08 and 0.06 per 100000 from 2006 to 2021, respectively. The incidence rate of this disease has been maintained at a low level.

Density map of echinococcosis distribution

The data relating to the echinococcosis epidemic in Yunnan Province were used to generate a density map of echinococcosis distribution using ArcGIS 10.1 software (Figure 2). Echinococcosis was found in 89 of the 129 counties in the province, accounting for 68.99% of all counties. The density map showed that echinococcosis was mainly distributed in nine prefectures (cities) of Northwest Yunnan, including Diqing, Dali, Nujiang, Lijiang, Chuxiong, Baoshan, Zhaotong, and Kunming, while sporadic cases were found in other prefectures.

Geographical distribution

Cases of echinococcosis were found in 16 prefectures of the province (Figure 3), with the highest number of cases in Dali (38.3%, 152/397), followed by Diqing (10.1%, 40/397), while the lowest number of cases was in Wenshan (0.8%, 3/397). There were significant differences between the different areas ($\chi^2 = 810.303$, $P< 0.05$). By county, there were 3 counties with more than 30 cases, namely, Jianchuan County (9.1%, 36/397), Shangri-La city (8.3%, 33/397), and Eryuan County (7.7%, 30/397). Two counties had 20-29 cases, namely, Heqing County (6.6%, 26/397) and Dali City (5.0%, 20/397), while 2 counties had 10-19 cases, including Binchuan County (3.3%, 13/397) and Yongsheng County (2.5%, 10/397). The number of cases in the other 82 counties was < 10.

Time distribution

A total of 397 cases were found in Yunnan Province from 2002 to 2021 (Figure 4), of which the case reporting rate of CISDCP (33.8%, 134/397) was low; the first case was reported by CISDCP in 2002, and the highest number of reported cases was 50 (2017). With implementation of the echinococcosis prevention and control project in Yunnan Province, governments at all levels have increased efforts to conduct population echinococcosis screening, and the number of cases has increased annually.

Distribution of echinococcosis by sex, age, ethnicity, and occupation

The constituent echinococcosis ratios of male and female were 46.60% and 53.4%, respectively, and there was no significant difference between them ($\chi^2 = 3.673$, $P > 0.05$). Among all age groups, most cases were 40-49 years old, accounting for 18.4%, and there was a significant difference among different ages ($\chi^2 = 118.105$, $P < 0.05$). Most cases were Han (43.6%) and Bai (26.2%), with a significant difference among nationalities ($\chi^2 = 667.763$, $P < 0.05$). Among the occupation groups, echinococcosis was more common in farmers/herdsmen (75.1%) and students (9.1%), with a significant difference among occupations ($\chi^2 =1645.711$, $P < 0.05$) (Table 1).

Epidemiological characteristics of individual cases

A total of 187 cases were epidemiologically investigated, including clinical symptoms, epidemiological history, and personal behavior of the cases. Investigation into clinical symptoms included the presence or absence of abdominal pain, cough, headache, and seizures. Symptoms were not obvious in the early stage in 47.1% (88/187). 31.6% (59/187) of cases had abdominal pain, 60.4% (113/187) of cases had no pain, and 8.0% (15/187) of cases were unclear. The cases with cough accounted for 11.8% (22/187), those with no cough accounted for 78.6% (147/187), and 9.63% (18/187) of cases were unclear. Cases with headache, none, and unclear accounted for 17.1% (32/187), 73.3% (137/187), and 9.6% (18/187), respectively. With regard to epilepsy, 1.6% (3/187) had epilepsy, 89.8% (168/187) did not, and 8.6% (16/187) were

Table 1 Distribution of echinococcosis by sex, age, ethnicity, and occupation in Yunnan

Features	Number of cases	Constituent ratio (%)
Sex		
Male	185	46.6
Female	212	53.4
Age (yr)		
1-9	15	3.8
10-19	29	7.3
20-29	43	10.8
30-39	69	17.4
40-49	73	18.4
50-59	66	16.6
60-69	58	14.6
70-79	37	9.3
≥ 80	7	1.8
Ethnicity		
Han	173	43.6
Bai	104	26.2
Tibetan	36	9.1
Yi	31	7.8
Lisu	11	2.8
Naxi	10	2.5
Dai	6	1.5
Hani	6	1.5
Others	20	5.0
Occupation		
Farmers and herders	298	75.1
Students	36	9.1
Cadre	28	7.1
Businessmen/individual	10	2.5
Children	10	2.5
Retired personnel	5	1.3
Guides	4	1.0
Others	6	1.5

unknown. The results of the survey on the epidemiological history of cases showed that 13.9% (26/187) of cases had stayed, worked, travelled, or hunted in echinococcosis endemic areas, 78.6% (147/187) of cases had not been to endemic areas, and 7.5% (14/187) of cases did not know whether they had been to endemic areas. In terms of personal behavior, 74.9% (140/187) of cases stated that their household currently or previously had a dog, 20.9% (39/187) of cases did not have a dog, and 4.3% (8/187) had never had a dog. Among the cases, those who drank wild raw water, did not drink wild water, and those who could not remember whether they had drunk wild water accounted for 66.8% (125/187), 14.4% (28/187), and 18.2% (34/187), respectively. Among all cases, the proportions of frequent hand washing, occasional hand washing, and no hand washing before meals were 22.3% (42/187), 53.5% (100/187), and 24.1% (45/187), respectively.

Of the 187 cases, 26 had been to endemic areas and had a history of living, working, travelling, or hunting in epidemic areas of echinococcosis, 7.7% (2/26) of cases stayed perennially in an epidemic area, 15.4% (4/26) of cases visited an epidemic area 1 year previously, 3.9 (1/26) of cases visited an epidemic area 3 years previously, 7.7% (2/26) of cases visited an epidemic area 6 years previously, and 65.4% (17/26) of cases did not remember the time at which they visited

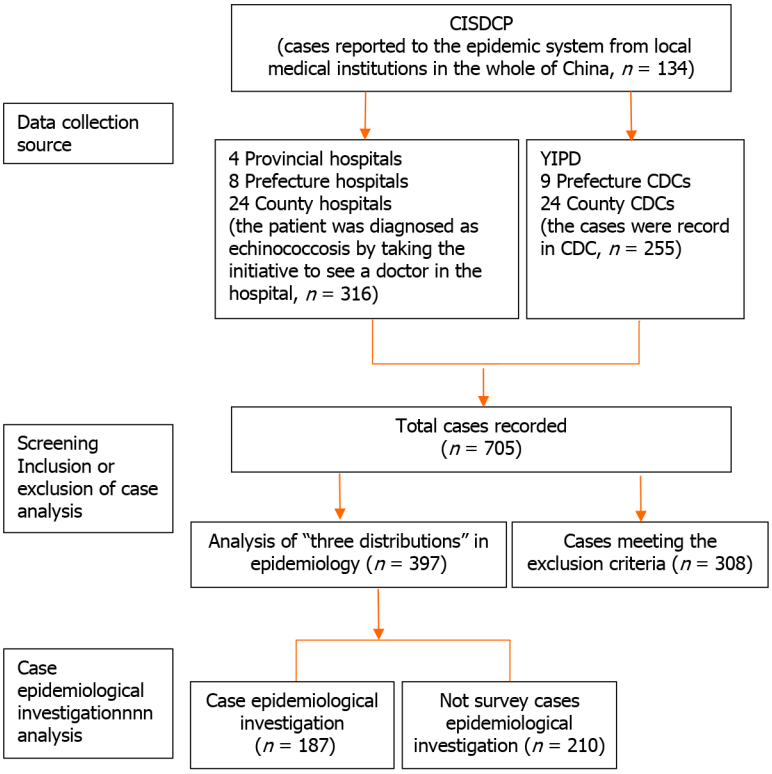

Figure 1 Summary of echinococcosis cases: search and study selection. CISDCP: China Information System for Disease Control and Prevention; YIPD: Yunnan Institute of Parasitic Diseases; CDC: Centers for Disease Control.

an epidemic area. During their stay in an epidemic area of echinococcosis, 46.2% (12/26) of cases had contacted dogs, 11.5% (3/26) had not, and 43.3% (11/26) of cases were unclear. Moreover, 11.5% (3/26) of cases had eaten undercooked or raw food, 34.6% (9/26) of cases had not, and 53.9% (14/26) of cases did not remember. The cases that had drunk wild water, had not drunk wild water, and who were unclear as to whether they had drunk wild water accounted for 7.7 (2/26), 26.92% (7/26), and 65.4% (17/26), respectively. Of the 187 cases, 104 cases (55%) were in endemic areas and 83 cases (44.4%) were in non-endemic areas. Among 83 cases in non-endemic areas, 4 had been to endemic areas and had a history of living, working, travelling, or hunting in epidemic areas of echinococcosis. 79 cases lived in non-endemic areas.

Correlation analysis

Linear regression tests were performed on the following factors: Sex, age groups, ethnic group, occupation, education level, YTWSEA, YHDIYF, YUCDB, YWHPME and HYDWW, and the regression model showed statistical significance ($F = 5.227$, $P < 0.05$). The logistic analysis of the above 10 factors showed that the prevalence of human CE was positively correlated with age group, education level, YHDIYF and YWHPME (Table 2).

Analysis of risk factors

Logistic analysis of single factors was conducted on 10 variables that may affect echinococcosis; these included sex, age, ethnicity, occupation, education level, YTWSEA, YHDIYF, YUCDB, YWHPME, and HYDWW. Among them, age, education level, YHDIYF, and YWHPME were significantly associated with echinococcosis ($P < 0.05$). Age as a risk factor was 1.672 (95%CI: 1.301-2.149) times greater than that of the other variables. Education level as a risk factor was 1.891 (95%CI: 1.091-3.279) times greater than that of the other variables, and the prevalence in the population with a low education level was higher than that in those with a high education level. YHDIYF as a risk factor was 2.830 (95%CI: 1.809-4.429) times greater than that of the other variables. YWHPME as a risk factor was 1.877 (95%CI: 1.109-3.177) times greater than that of the other variables (Table 2).

Logistic multiple factor analysis was conducted on age group, education level, YHDIYF and YWHPME which showed statistical significance in the single factor analysis. For age group, age from 20 to 29 years was 40.846 (95%CI: 3.855-432.769) times, 30 to 39 years was 40.846 (95%CI: 3.855-432.769) times, and 40 to 49 years was 8.302 (95%CI: 1.014-67.938) times that of the other variables. Where there was a dog in family, YHDIYF was 24.112 (95%CI: 2.347-247.666) times, and washing hands occasionally was 10.38 (95%CI: 3.464-31.101) times that of the other variables (Table 3).

DISCUSSION

Echinococcosis is a chronic zoonotic parasitic disease caused by the larval stages of cestodes of the genus Echinococcus [3]. Four species of *E. granulosus* cause human infection, namely, CE, AE, polycystic echinococcosis (*E. vogeli Rausch*), and

Table 2 Logistic regression analysis of single risk factors of infection

Factors	Regression coefficient	Standard error	*P* value	Risk ratio (95%CI)
Sex	0.627	0.402	0.119	1.872 (0.852-4.115)
Age group	0.514	0.128	0.000	1.672 (1.301-2.149)
Ethnicity	0.077	0.118	0.514	1.080 (0.857-1.361)
Occupation	-0.043	0.445	0.923	0.958 (0.400-2.292)
Education level	0.637	0.281	0.023	1.891 (1.091-3.279)
YTWSEA	-0.008	0.081	0.926	0.992 (0.846-1.164)
YHDIYF	1.040	0.228	0.000	2.830 (1.809-4.429)
YUCDB	-1.029	0.717	0.151	0.357 (0.088-1.457)
YWHPME	0.630	0.269	0.019	1.877 (1.109-3.177)
HYDWW	-0.122	0.235	0.602	0.885 (0.559-1.402)

CI: Confidence interval; YTWSEA: Have you travelled, worked, or stayed in echinococcosis endemic areas; YHDIYF: Do you, or have you ever had dogs in your family? YUCDB: Did your family use cow dung to burn? YWHPME: Do you wash your hands before preparing meals or eating? HYDWW: Have you drunk wild water?

E. oligarthrus. Echinococcosis in humans presents predominantly as CE and AE types. *E. vogeli Rausch* and *E. oligarthrus* cause polycystic echinococcosis, but the incidence in humans is very rare[15]. Echinococcosis is globally distributed[4], and it is estimated that 91% of all AE cases worldwide occur in China[8]. Echinococcosis is endemic in China, mainly in the Western regions, including Inner Mongolia, Tibet, Gansu, Qinghai, Ningxia, and Xinjiang Production and Construction Corps, Sichuan, Yunnan, and Xi'an provinces[16,17].

The results of this survey demonstrated that sporadic cases of echinococcosis were reported throughout Yunnan Province, with a low prevalence, while most cases were distributed in 24 endemic counties in 9 prefectures, including Diqing and Dali in the Northwest. The prevalence of echinococcosis varied significantly in different areas. As there are many intermediate hosts suitable for echinococcus infection in Yunnan Province, the infection rate of animal hosts is high and there are natural foci with a potential risk of transmission and epidemics[13]. Yunnan Province closely neighbors Tibet and Sichuan provinces with a high prevalence of echinococcosis and is at risk of becoming an epidemic area[18-24]. According to the survey results, there was no significant difference in the constituent ratio of male and female; which may be related to the geographical characteristics of the combination of agricultural and pastoral areas, the mode of production in local residents, and their living habits in Yunnan. The first case of echinococcosis was reported by CISDCP in 2002, and screening has been strengthened since the implementation of the echinococcosis control project in 2012. As a result, the number of cases has increased annually, with the highest number of cases in 2017 (50), followed by 2019 (45) and then declined. The reduced number of cases in 2020 and 2021 was probably due to the coronavirus disease 2019 pandemic, and requires further investigation. According to the parasitic sites, hepatic (87.66%) and lung (6.80%) CE were the main types, which is in agreement with previous studies conducted in Yunnan[12,13]. The infection rate of pulmonary echinococcosis in Yunnan Province is as low as 6.8%. This may be related to geographical characteristics, echinococcosis species and ethnicity, which requires further research. Significantly more cases were diagnosed in hospital (90.93%) compared to those found by investigation (9.07%) (χ^2 = 298.870, P < 0.05). This result shows that most cases were identified in hospital and few cases were found by active investigation or screening, likely due to the high cost of investigation and screening when the prevalence of echinococcosis in the population was reduced to a low level. Age group analysis showed that most cases were > 10 years old, with the highest proportion of cases aged 40-49 years. Logistic analysis showed that age was significantly associated with the prevalence of echinococcosis (P < 0.05). As echinococcosis is a chronic infectious disease with a long disease course, symptoms may occur more than 5 years after infection and patients may survive for many years after exposure. In line with this, the older age group may have been exposed to an environment contaminated by echinococcus eggs for an extended time, with the cumulative risk increasing with age[22]. Furthermore, most of the cases were Han (43.58%) and Bai (26.20%) nationalities. The constituent ratio of farmers/herdsmen (75.06%) and students (9.07%) was higher than that of other occupational groups, with significant differences among nationalities and occupations.

A total of 187 cases were investigated and basic information, including clinical symptoms, epidemiological history, and personal behavior, was collected. The survey results showed that the clinical symptoms in 47.1% of cases were not obvious in the early stage. Echinococcosis, is a chronic infectious disease with a long disease course, and the symptoms may occur 5-20 years after infection, leading to patients surviving for many years after exposure. The epidemiological history analysis showed that 78.61% of cases had not stayed, worked, travelled, or hunted in echinococcosis endemic areas, and that YTWSEA was not a risk factor for echinococcosis, indicating that there were local cases of infection in Yunnan Province. Regarding personal behavior, 74.87% (140/187) of households currently or previously had a dog. The proportions of occasional hand washing and no hand washing before meals were 53.48% and 24.06%, respectively, with the results of logistic analysis showing that the above two variables were factors influencing the prevalence of echino-

Table 3 Logistic regression analysis of multiple factors

Factors	Regression coefficient	Standard error	*P* value	Risk ratio (95%CI)
Age group				
1-9	19.167	0.000		2.11E+08 (2.11E + 08-2.11E + 08)
10-19	19.576	5647.366	0.997	3.18E + 08 (0-.b)
20-29	3.534	1.298	0.006	34.26 (2.689-436.552)
30-39	3.710	1.204	0.002	40.846 (3.855-432.769)
40-49	2.116	1.073	0.048	8.302 (1.014-67.938)
50-5	1.776	0.937	0.058	5.908 (0.941-37.081)
60-69	0.976	0.892	0.274	2.654 (0.462-15.253)
70-79	0.971	0.914	0.288	2.642 (0.441-15.834)
≥ 80	0c	0.000		
Education level				
Illiterate	2.059	3.242	0.525	7.836 (0.014-4503.762)
Elementary school	0.763	3.212	0.812	2.145 (0.004-1161.921)
Junior high school	1.123	3.225	0.728	3.073 (0.006-1708.471)
High school	2.636	3.676	0.473	13.953 (0.010-18788.338)
College and above	0c	0.00		
YHDIYF				
Yes	3.183	1.188	0.007	24.112 (2.347-247.666)
No	-0.013	1.260	0.992	0.987 (0.084-11.661)
Previously	2.066	1.247	0.098	7.894 (0.685-90.92)
Never	0c	0.000		
YWHPME				
Often	1.176	0.636	0.064	3.241 (0.932-11.270)
Occasionally	2.340	0.560	0.000	10.38 (3.464-31.101)
Not	0c	0.000		

CI: Confidence interval; YTWSEA: Have you travelled, worked, or stayed in echinococcosis endemic areas; YHDIYF: Do you, or have you ever had dogs in your family? YUCDB: Did your family use cow dung to burn? YWHPME: Do you wash your hands before preparing meals or eating? HYDWW: Have you drunk wild water?

coccosis.

CONCLUSION

The prevalence of echinococcosis in Yunnan Province was low, and most of the cases were distributed in Northwest Yunnan. The prevention and control of echinococcosis should be strengthened in Diqing, Dali, and other areas in Northwest Yunnan. Based on the discovery of new cases, a case report is required by CISDCP within 24 h, and an epidemiological investigation of the individual cases should be conducted within 2 wk. According to the results, investigations should be conducted in the villages that are comprehensively judged as local infection sites within 2 mo. Together, this forms a comprehensive prevention and control strategy focused on the control of infectious sources, the prevention and control of intermediate hosts, and the combination of patient investigation, treatment, health education and publicity, and is referred to as the "1-2-2" prevention and control mode. Promoting changes in the behavior of the population, such as not raising dogs and washing hands frequently, through health education will serve to control endemic echinococcosis and reduce the medical burden on the population.

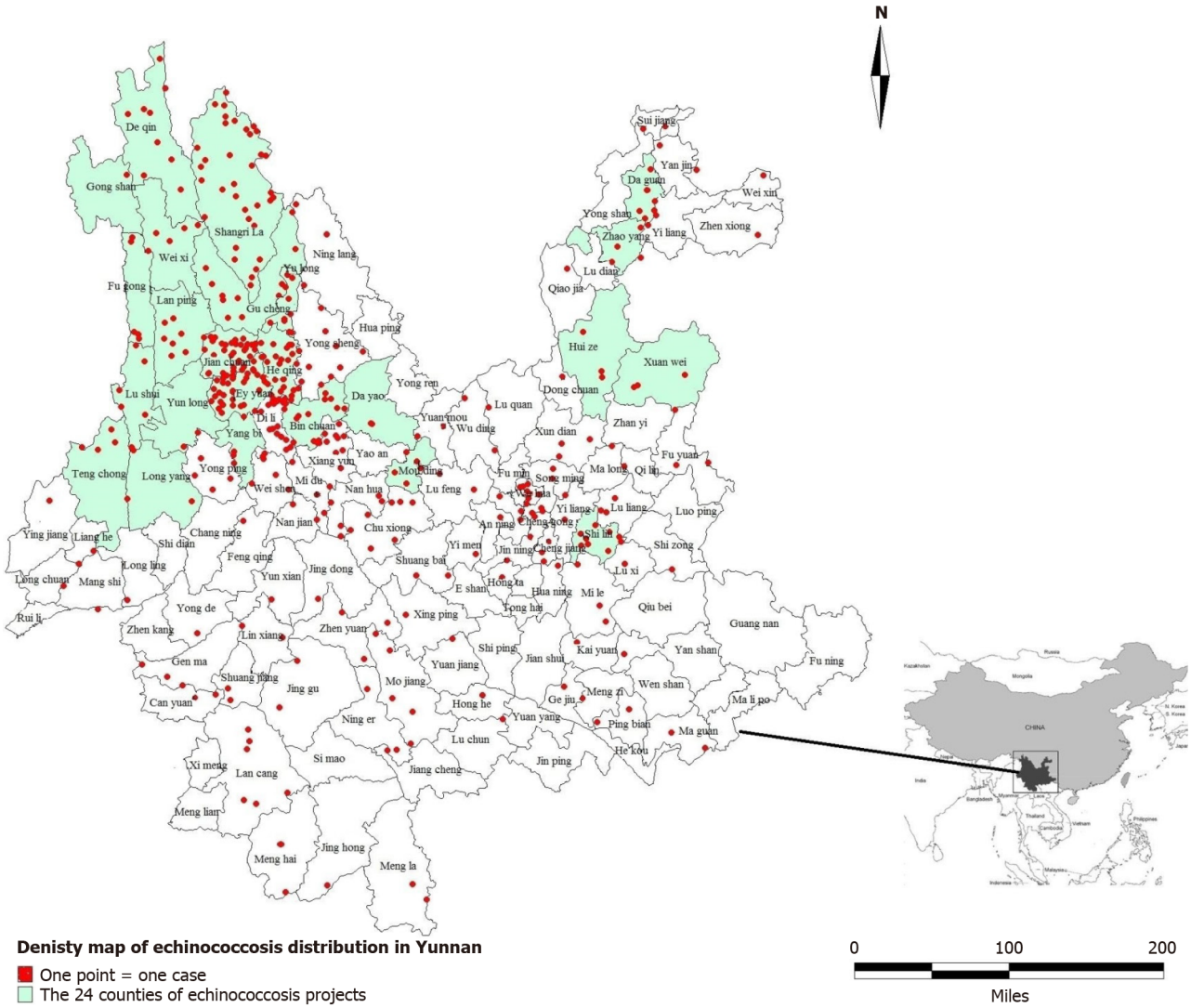

Figure 2 Density map of echinococcosis distribution in Yunnan Province.

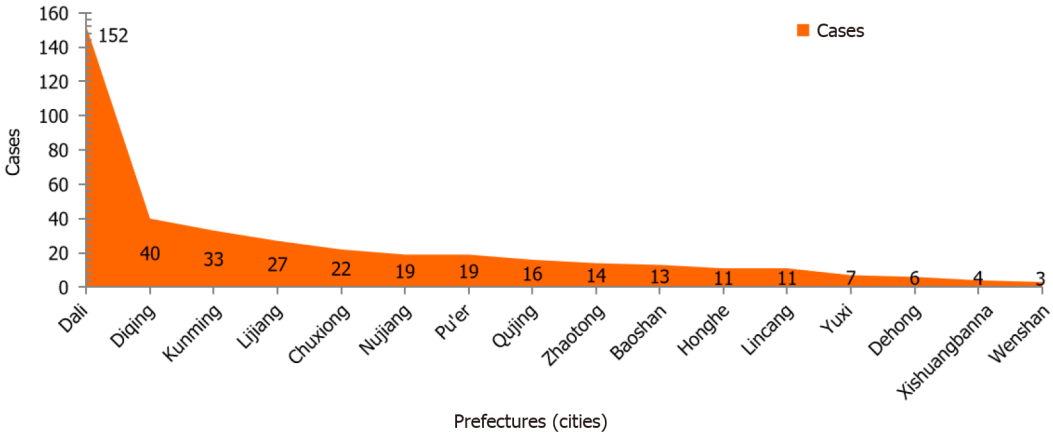

Figure 3 Geographical distribution of echinococcosis in Yunnan.

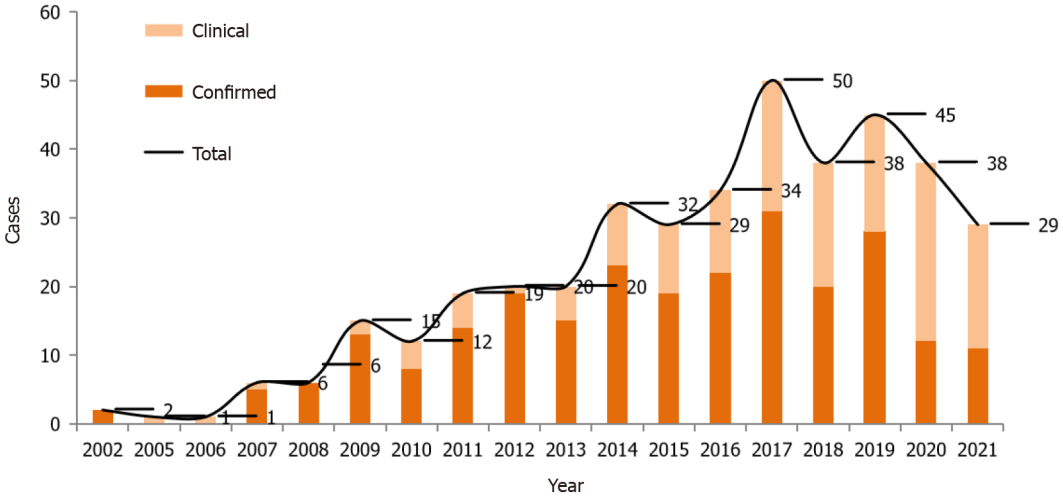

Figure 4 Time distribution of echinococcosis in Yunnan.

ARTICLE HIGHLIGHTS

Research background

Ten cases of echinococcosis were in hospital in 1978, and surgical cases were 24 from 1981 to 2001 in Yunnan Province. An epidemiological survey of echinococcosis in 2012 and 2016 showed cases of echinococcosis in Yunnan Province. It is important to understand the spatial distribution and epidemiological characteristics of echinococcosis in Yunnan for the prevention and control of the disease.

Research motivation

To understand the species and sources of echinococcosis in Yunnan Province using a retrospective investigation and epidemiological analysis. Based on the local epidemic species of echinococcosis corresponding prevention and control measures can then be taken to effectively control the spread and prevalence of echinococcosis in Yunnan Province.

Research objectives

Cystic echinococcus is the main hydatid disease that has a medium prevalence. The aim of this study was to prevent and control echinococcosis and to reduce the risk of infection in Yunnan Province.

Research methods

The cases were retrospectively epidemiologically investigated and analyzed in hospitals, Centers for Disease Control, and China Information System for Disease Control and Prevention.

Research results

A total of 397 cases were found in 89 counties in Yunnan Province, 55.6% of cases were in endemic areas, and 44.4% of cases were in non-endemic areas. The highest number of cases was 50 (2017). Confirmed and clinical cases accounted for 62.5% and 37.5%, respectively. However, 90.9% of the cases with hydatid disease were reported by the hospital system, and only 9.1% of the cases with hydatid disease were found in the community through active screening.

Research conclusions

Cases of echinococcosis were reported throughout the entire Yunnan Province, with the majority distributed in Western Yunnan, suggesting that echinococcosis control should be strengthened in this area. Tracing hydatid disease cases, taking local infection cases as clues, and investigating local infection in villages were carried out, which effectively blocked the spread of hydatid disease.

Research perspectives

Trace the source of cases, block the source of infection, and effectively control hydatid disease.

ACKNOWLEDGEMENTS

This survey was conducted by the Yunnan Institute of Parasitic Diseases and Tibet Centers for Disease Control and Prevention. We sincerely thank all the participants who were involved in this investigation, including the Centers for Disease Control (Institute/Station of Schistosomiasis Control) and related hospitals in Dali, Diqing, Kunming, Lijiang,

Chuxiong, Nujiang, Zhaotong, Qujing, and Baoshan.

FOOTNOTES

Co-first authors: Jin-Rong Zi and Dan Xiao.

Co-corresponding authors: Ben-Fu Li and Ya-Ming Yang.

Author contributions: Yang YM, Li BF and Wu FW designed the research study; Zi JR and Xiao D contributed equally to this work, analyzed the data and wrote the manuscript; Peng J, Li JX, Yan XL, Wang ZQ, Cai X and Xu Q analyzed and interpreted the data; contributed analytical tools or data; all authors have read and approved the final manuscript. Li BF and Yang YM designed the research study, investigation and analysis. Zi JR and Xiao D analyzed and interpreted the data; contributed analytical tools or data; wrote the paper. Li BF and Yang YM conceived and designed the experiments; analyzed and interpreted the data. Li BF and Yang YM proposed the concept and design of the epidemiological investigation of echinococcosis in Yunnan Province, wrote investigation plans, coordinated with investigated hospitals, guided on-site work, and reviewed investigation data. Co-first authors Zi JR and Xiao D are responsible for specific work, including on-site investigation, data organization, database entry, summarization, analysis, and writing of the manuscript.

Supported by Central Government Transfer Payment of China; Open Project of Key Laboratory of Echinococcosis Control and Research of the National Health Commission of People's Republic of China, No. 2021WZK1001.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of Yunnan Institute of Parasitic Diseases.

Informed consent statement: All study participants or their legal guardian provided informed written consent in relation to personal and medical data collection prior to study enrollment.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

ORCID number:

S-Editor: Qu XL
L-Editor: Webster JR
P-Editor: Yuan YY

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 241-250

DOI: 10.4254/wjh.v16.i2.241

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Predictors of portal vein thrombosis after splenectomy in patients with cirrhosis

Ting Li, Li-Li Wang, Ya-Ping Li, Jian Gan, Xi-Sheng Wei, Xiao-Rong Mao, Jun-Feng Li

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: El-Bendary M, Egypt

Received: October 22, 2023
Peer-review started: October 22, 2023
First decision: December 8, 2023
Revised: December 21, 2023
Accepted: January 8, 2024
Article in press: January 8, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

Portal vein thrombosis (PVT) is a commonthsn complication after splenectomy in patients with cirrhosis. However, the predictors of postoperative PVT are not known.

AIM

To investigate the predictors of PVT after splenectomy in patient with cirrhosis.

METHODS

A total of 45 patients with cirrhosis who underwent splenectomy were consecutively enrolled from January 2017 to December 2018. The incidence of PVT at 1 months, 3 months, and 12 months after splenectomy in patients with cirrhosis was observed. The hematological indicators, biochemical and coagulation parameters, and imaging features were recorded at baseline and at each observation point. The univariable, multivariable, receiver operating characteristic curve and time-dependent curve analyses were performed.

RESULTS

The cumulative incidence of PVT was 40.0%, 46.6%, and 48.9% at 1 months, 3 months, and 12 months after splenectomy. Multivariable analysis showed that portal vein diameter (PVD) ≥ 14.5 mm and monthsdel end-stage liver disease

(MELD) score > 10 were independent predictors of PVT at 1 months, 3 months, and 12 months after splenectomy (P < 0.05). Time-dependent curve showed that the cumulative incidence of PVT was significantly different between patients with MELD score ≤ 10 and > 10 (P < 0.05). In addition, the cumulative incidence of PVT in the PVD ≥ 14.5 mm group was significantly higher than that in the PVD < 14.5 mm group (P < 0.05).

CONCLUSION

Wider PVD and MELD score > 10 were independent predictors of PVT at 1 months, 3 months, and 12 months after splenectomy in patient with cirrhosis.

Key Words: Cirrhosis; Splenectomy; Portal vein thrombosis; Predictors

Core Tip: Portal vein diameter (PVD) ≥ 14.5 mm was independent predictors of portal vein thrombosis (PVT) at 1-months, 3-months, and 12-months after splenectomy. End-stage liver disease score > 10 was independent predictors of PVT at 1-months, 3-months, and 12-months after splenectomy. The patients with PVD ≥ 14.5 mm and/or end-stage liver disease score > 10 in preoperative, preoperative treatment of reducing portal vein pressure and improving liver function may help to reduce the incidence of PVT after splenectomy.

Citation: Li T, Wang LL, Li YP, Gan J, Wei XS, Mao XR, Li JF. Predictors of portal vein thrombosis after splenectomy in patients with cirrhosis. *World J Hepatol* 2024; 16(2): 241-250
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/241.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.241

INTRODUCTION

Portal vein thrombosis (PVT) involves the portal vein and its main branches, and cirrhosis is one of the monthsst commonthsn causes. The natural incidence of PVT in cirrhosis is 3.7%-24.4%[1], and the incidence of advanced cirrhosis is 10%-15%[2]. In cirrhosis, PVT is often latent, and is only discovered accidentally. The treatment of PVT with cirrhosis is controversial.

Currently, splenectomy is one of the main methods of treatment for portal hypertension, hypersplenism and upper gastrointestinal bleeding. Splenectomy can significantly improve the prognosis and survival of patients with cirrhosis[3,4]. Splenectomy decreases portal hypertension, improves liver function, and reduces fibrosis[5-7]. It also improves liver regeneration. A 10-year retrospective follow-up study based on the inverse probability of treatment weighting method found that splenectomy decreased the risk of hepatocellular carcinoma in cirrhosis patients with portal-hypertension-related bleeding[8]. Splenectomy has been considered an effective option to reverse thrombocytopenia in cirrhosis patients with splenomegaly. Thus, splenectomy may be beneficial for treatment of liver cirrhosis with hypersplenism. Splenectomy have been widely used in Asia for the treatment of esophagogastric variceal hemonthsrrhage and hypersplenism caused by cirrhotic portal hypertension. However, splenectomy can increase the risk of PVT at least 10 times[9]. The incidence of PVT was 18.9%-57.0% after splenectomy, which was significantly higher than the natural incidence in patients with cirrhosis without surgery[2,10]. PVT can induce or aggravate upper gastrointestinal bleeding, hepatic encephalopathy, and ascites, increase the risk of intestinal ischemia or intestinal necrosis, reduce the survival of patients and grafts after liver transplantation, and result in chronic cavernous transformation of the portal vein system in the long term[11-14].

Alteration in blood flow, hypercoagulability and vascular endothelial injury are the main risk factors for PVT[2]. PVT is associated with preoperative slower portal vein velocity, wider portal vein diameter (PVD) and splenic vein, and lower preoperative and higher postoperative platelet counts[15-18]. A higher monthsdel end-stage liver disease (MELD) score is associated with hepatic encephalopathy, variceal bleeding, refractory ascites, and spontaneous peritonitis[19-21]. Higher MELD score corresponds with higher monthsrtality in liver transplantation[20]. A higher of MELD score may be associated with postoperative PVT. The mechanisms of PVT after splenectomy are still unclear. Our study aimed to establish the risk factors for PVT after splenectomy and early sensitive indicators, to provide a predictive basis for early PVT.

MATERIALS AND METHODS

Patients

We enrolled 45 consecutive patients with cirrhosis who underwent splenectomy between January 2017 and December 2018 at the First Hospital of Lanzhou University. The flow diagram of the study population is shown in Figure 1. The

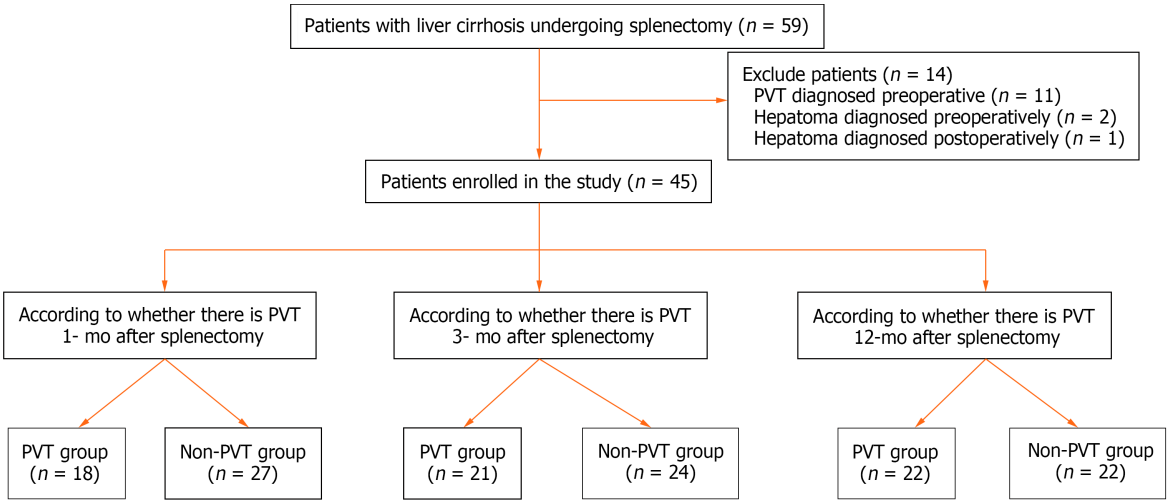


Figure 1 Flowchart of the study population. PVT: Portal vein thrombosis.

study was approved by the ethics committee of the first hospital of Lanzhou University (LDYYLL2019-209) and informed consent was obtained from the patients.

Inclusion criteria were histologically proven cirrhosis or cirrhosis diagnosed by a history of liver disease, clinical manifestations, laboratory tests, and imaging studies, and the patients underwent splenectomy. The indications for splenectomy included: Endoscopic treatment-resistant esophagogastric varices with or without variceal hemonthsrrhage; history of esophageal variceal bleeding or potential bleeding; infection caused by hypersplenism and thrombocytopenia (platelet count < 50 × 10^9/L); and upper abdominal discomfort caused by an enlarged spleen.

The exclusion criteria were as follows: (1) Age > 18 years or < 70 years; (2) patients who developed PVT preoperatively; (3) patients who presented preoperatively with hepatic carcinoma, hepatic encephalopathy, or preoperative Child-Pugh class C, or other tumonthsrs; (4) patients with cirrhosis who underwent liver transplantation; (5) patients who underwent transjugular portal systemic shunt; (6) patients who underwent abdominal surgery; (7) splenectomy for hematological diseases and other reasons (such as trauma); (8) vascular malformation and idiopathic portal hypertension; (9) incomplete clinical data (without hemonthscytes, imaging and other relevant data); (10) coexistence of other serious diseases (shock, multiple organ failure, uremia, and severe infection); and (11) loss to follow-up.

Diagnosis of PVT

PVT was detected by duplex ultrasonography, computed tomonthsgraphy, or computed tomonthsgraphy angiography. They were performed within 1 wk before the operation to exclude preoperative PVT. Re-examination was performed at 1 months, 3 months, 6 months, and 12 months postoperatively.

Laboratory tests

Routine blood, parameters and coagulation parameters were measured within 3 d before the operation and used as baseline data. Re-examinations were performed at 1 months, 3 months, 6 months, and 12 months postoperatively in the outpatient or inpatient department of the First Hospital of Lanzhou University. BC-5390 CRP automatic blood cell analyzer (Mindray Bio-Medical Electronics Co. Ltd., Shenzhen, Guangdong Province, China) was used for routine blood testing. The AU400 automatic biochemical analyzer (Olympus Optics Co. Ltd., Japan) was used to detect biochemical parameters. Coagulation parameters were detected by PrecilC3510 automatic coagulation analyzer (Mindray). PVD was measured using Doppler ultrasound (GE Logic E9).

Statistical analysis

An independent *t* test or single factor analysis was used to analyze the difference in data in accordance with a normal distribution, and the Mann-Whitney test was used to analyze non-normally distributed data. The χ^2 test or Fisher's exact test was used to analyze categorical variables. A logistic regression monthsdel analyzed the multivariable data. A receiver operating characteristic (ROC) curve was used to evaluate the specificity and sensitivity of PVD and MEDL score for predicting PVT. The Kaplan-Meier method was used to calculate the cumulative incidence of PVT, and the log-rank test was used to compare the difference in the cumulative incidence of PVT between the groups.

RESULTS

Basic characteristics

We included 45 consecutive patients with cirrhosis who underwent splenectomy (Table 1). The mean age was 47.62 years ± 11.16 years, and 53.3% were female. In terms of etiology, 84% of patients with cirrhosis had hepatitis B, 4.0% hepatitis C,

Table 1 Demonthsgraphic and clinical characteristics of the study population at baseline

Variables	Data
Gender	
Men, *n* (%)	21 (46.7)
Female, *n* (%)	24 (53.3)
Age (yr)	47.62 ± 11.16
Etiology	
HBV, *n* (%)	37 (74.0)
HCV, *n* (%)	2 (4.0)
AIH, *n* (%)	4 (8.0)
Unexplained cirrhosis, *n* (%)	2 (4.0)
WBC (10^9/L)	2.21 ± 1.18
RBC (10^{12}/L)	3.68 ± 0.78
PLT (10^9/L)	44.09 ± 28.54
AST (U/L)	35.18 ± 14.82
ALT (U/L)	26.89 ± 14.30
TBIL (μmol/L)	26.77 ± 15.97
ALB (g/L)	38.81 ± 4.16
INR	1.30 ± 0.19
PT (s)	14.23 ± 1.94
PTA (%)	68.71 ± 13.45
D-D2 (mg/L)	0.56 ± 1.03
MELD	10.76 ± 3.30
PVD (mm)	13.66 ± 2.49
PV (cm/s)	19.87 ± 5.88
SVD (mm)	10.35 ± 2.61
Ascites, *n* (%)	19 (42.2)
Child-Pugh grade	
A, *n* (%)	32 (71.1)
B, *n* (%)	13 (28.9)
C, *n* (%)	0 (0.0)
Operation ways	1.30 ± 0.19
Open splenectomy, *n* (%)	35 (78.8)
Laparoscopic splenectomy, *n* (%)	10 (21.2)
Spleen volume (cm^3)	2349.28 (531.12-13080.00)

HBV: Hepatitis B virus; HCV: Hepatitis C virus; AIH: Autoimmune hepatitis; WBC: White blood cell counts; RBC: Red blood cell counts; PLT: Platelet counts; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TBIL: Total bilirubin; ALB: Albumin; INR: International normalized ratio; PT: Prothrombin time; PTA: Prothrombin activity; MELD: Monthsdel for end-stage liver disease; PVD: Portal vein diameter; PV: Portal vein velocity; SVD: Splenic vein.

8.0% autoimmune hepatitis, and 4.0% unexplained cirrhosis. Thirty-five (78.8%) liver cirrhosis patients underwent open splenectomy, and 10 (21.2%) underwent laparoscopic splenectomy.

There were 18 (40.0%) patients with PVT and 27 (60.0%) without PVT at 1 months after splenectomy; 21 (46.6%) with PVT and 27 (53.4%) without PVT at 3 months after splenectomy; and 22 (48.9%) with PVT and 27 (61.1%) without PVT at 12 months after the operation.

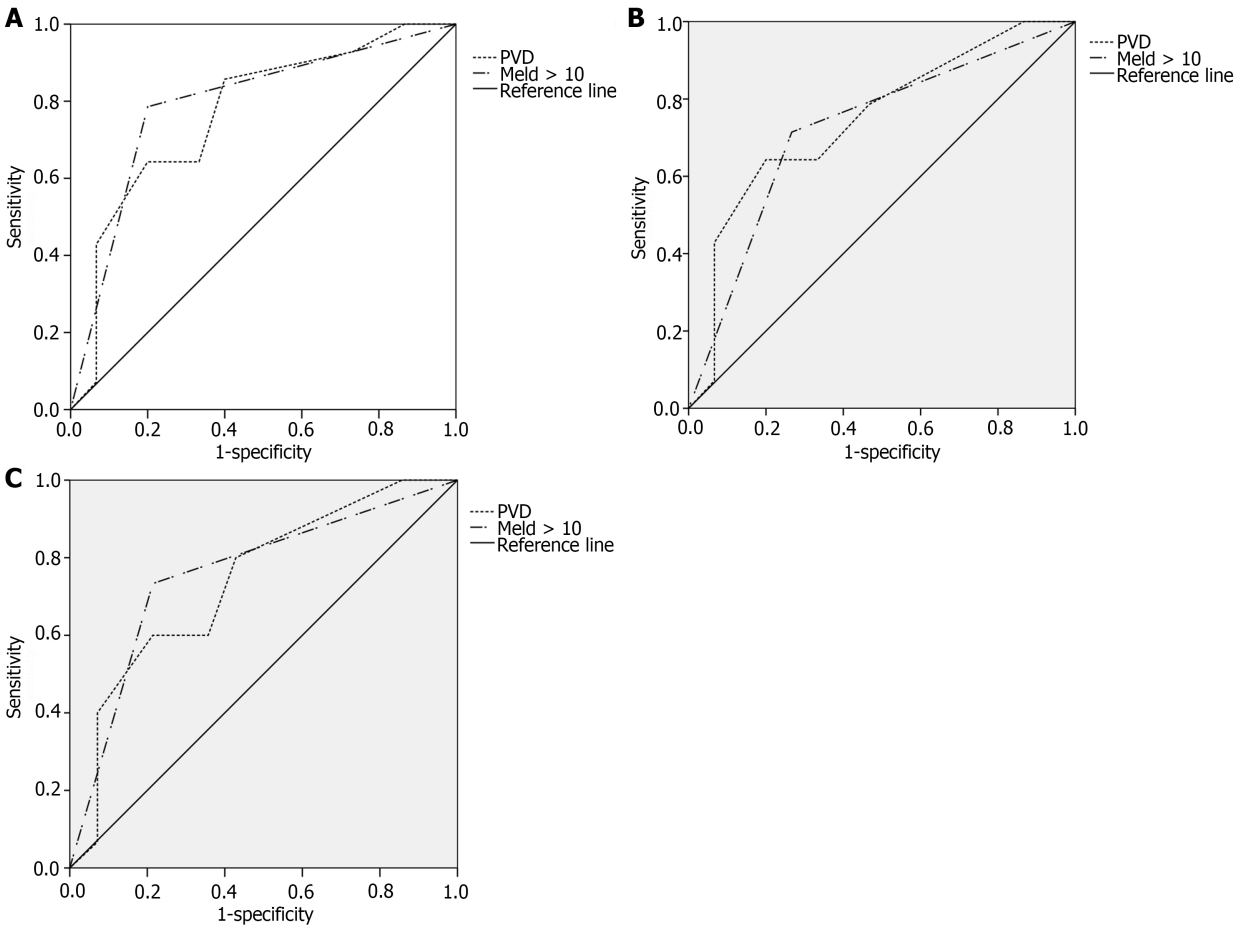


Figure 2 Receiver operating characteristic curve. A: Receiver operating characteristic (ROC) curve of portal vein thrombosis (PVT) in patients with cirrhosis after splenectomy predicted by independent predictors (postoperative 1 months); B: ROC curve of PVT in patients with cirrhosis after splenectomy predicted by independent predictors (postoperative 3 months); C: ROC curve of PVT in patients with cirrhosis after splenectomy predicted by independent predictors (postoperative 12 months). PVT: Portal vein thrombosis; MELD: Monthsdel end-stage liver disease.

There were 18 (40.0%) patients with ascites at 1 wk after splenectomy; six (13.3%) patients with ascites at 1 months after splenectomy; one (2.2%) patient with hepatic encephalopathy, four (8.9%) with ascites, and one (2.2%) with upper gastrointestinal hemonthsrrhage at 3 months after splenectomy; one (2.2%) patient with upper gastrointestinal hemonthsrrhage at 6 months after splenectomy; and no decompensation occurred 1 year after the operation. During 1-year follow-up, there was no postoperative monthsrtality.

Risk factors of development of PVT after splenectomy in patients with cirrhosis

The demonthsgraphic and laboratory data were compared in patients who developed PVT at 1 months, 3 months, and 12 months postoperatively. Univariable analysis revealed that the incidence of PVT at 1 months, 3 months, and 12 months postoperatively in the MEDL score > 10 group was significantly higher than in the MELD score ≤ 10 group ($P < 0.05$). Patients with PVT had a wider PVD than those without PVT ($P < 0.05$). At 3 months postoperatively, the PVT group had a longer prothrombin time (PT) ($P < 0.05$).

Independent predictors of PVT after splenectomy in cirrhotic patients

Multivariable analysis identified the following as independent predictors of PVT at 1 months, 3 months, and 12 months postoperatively: Wider preoperative PVD [odds ratio (OR): 2.194, 95% confidence interval (CI): 1.090-4.415, $P = 0.028$; OR: 1.70, 95%CI: 1.052-2.746, $P = 0.030$; OR: 1.776, 95%CI: 1.036-3.046, $P = 0.037$]; and MELD score > 10 (OR: 76.215, 95%CI: 2.534-2287.318, $P = 0.013$; OR: 12.392, 95%CI: 1.318-116.548, $P = 0.028$; OR: 23.925, 95%CI: 1.875-305.323, $P = 0.015$) (Table 2).

To evaluate the ability of independent predictors to predict PVT after splenectomy, ROC curve analysis was performed (Figure 2). The AUCs of PVD were 0.769, 0.745, and 0.738, respectively ($P < 0.05$). The AUCs of MELD score > 10 was 0.793, 0.724, and 0.760, respectively ($P < 0.05$).

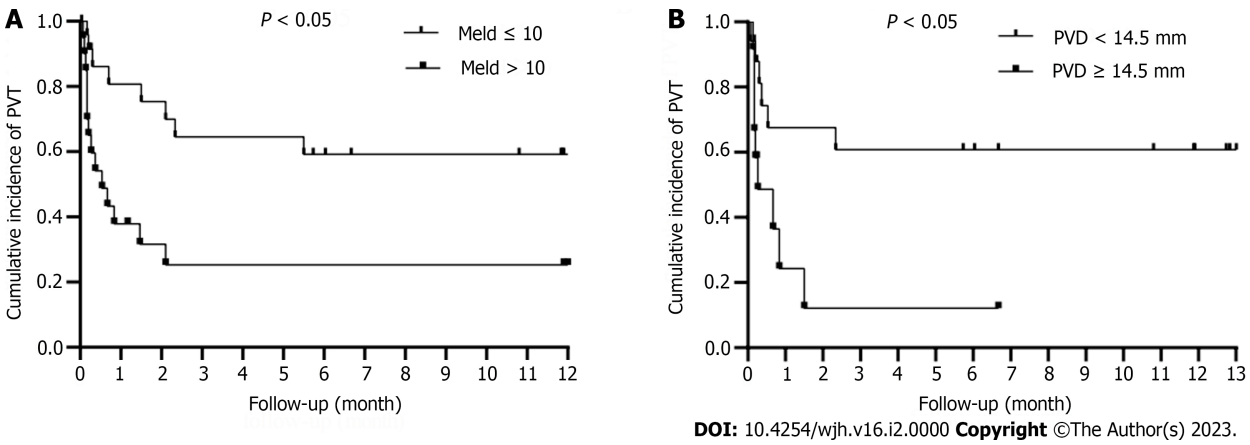
Cumulative incidence of PVT

The mean time to occurrence of PVT after splenectomy was 27 d. The optimal cut-off value of PVD was 14.5 mm. The time-dependent curve analysis is shown (Figure 3).

Table 2 Independent risk factors of portal vein thrombosis formation in patients with liver cirrhosis after splenectomy

Variable	PVT	Non-PVT	*P* value	OR	95%CI
Postoperative 1-month					
Age	45.72 ± 7.84	48.89 ± 12.91			
PVD	14.79 ± 2.08	12.60 ± 2.38	0.028	2.194	1.090-4.415
MELD > 10	12 (70.6)	8 (32.0)	0.013	76.215	2.534-2287.318
PLT	47.00 ± 41.96	42.15 ± 14.78			
Postoperative 3-months					
Age	44.71 ± 7.96	44.71 ± 7.96			
PT	14.69 ± 1.94	14.69 ± 1.94			
PVD	14.71 ± 2.16	14.71 ± 2.16	0.03	1.7	1.052-2.746
Meld > 10	12 (70.6)	8 (32.0)	0.028	12.392	1.318-116.548
PLT	47.57 ± 38.80	41.04 ± 15.11			
Postoperative 12-months					
Age	44.64 ± 7.77	50.4 ± 13.19			
PVD	14.60 ± 2.13	12.64 ± 2.47	0.037	1.776	1.036-3.046
MELD > 10	14 (63.6)	6 (26.1)	0.015	23.925	1.875-305.323
PLT	46.55 ± 38.17	41.74 ± 15.05			

PVT: Portal vein thrombosis; OR: Odd ratio; CI: Confidence interval; PVD: Portal vein diameter; MELD: Monthsdel for end-stage liver disease; PLT: Platelet counts.



DOI: 10.4254/wjh.v16.i2.0000

Figure 3 Time-dependent curve analysis. A: Cumulative incidence of portal vein thrombosis in the groups with monthsdel end-stage liver disease score ≤ 10 and > 10 was significantly different ($P < 0.05$); B: Cumulative incidence of PVT in patients with portal vein diameter (PVD) < 14.5 mm and PVD ≥ 14.5 mm group was significantly different ($P < 0.05$). PVT: Portal vein thrombosis; MELD: Monthsdel end-stage liver disease.

DISCUSSION

In our observational study, the cumulative incidence of PVT after splenectomy in patients with cirrhosis was 40.0%, 46.6%, 46.6%, and 48.9% at 1 months, 3 months, 6 months, and 12 months, respectively. Wider preoperative PVD and MELD score > 10 may predict the development of PVT after splenectomy. The time-dependent curve analyzed that the development of PVT in patients with MELD score ≤ 10 was lower than in those with MELD score > 10 ($P < 0.05$). And in the PVD ≥ 14.5 mm group was significantly higher than that in the PVD < 14.5 mm group ($P < 0.05$).

The cumulative postoperative incidence of PVT was 40.0% at 1 months, 46.6% at 3 months, 46.6% at 6 months, and 48.9% at 12 months. This was similar to the previous study. There are several potential causes of postoperative PVT. Firstly, the occlusion of splenic portal vessels resulted in a reduction in blood flow around the ligation area and enhanced the venous stasis at the splenic vein stump. Secondly, patients with liver cirrhosis are often complicated with changes in blood coagulation proteins, including factor VIII, von Willebrand factor fibrinogen, and tissue factor, putting the blood in

a hypercoagulable state[22], which is involved in venous thrombosis. Thirdly, splenectomy can reduce the portal vein flow velocity[23]. The lack of the portal vein flow velocity in our study, we did not obtain a similar conclusion. Previous reports found that wider preoperative splenic vein diameter was an independent predictor of the development of PVT[15,24]. In our study, we found that diameter of the splenic vein in the PVT group was wider than that in the non-PVT group, but there was no significant difference.

Zhang *et al*[25] considered that the main cause of PVT was the change in portal vein blood flow and not the change in PT or platelet count. We found that a lower preoperative platelet count was not associated with the postoperative development of PVT. In our study, wider PVD was an independent predictor of PVT at 1 months, 3 months, and 12 months after splenectomy. The optimal cut-off value was 14.5 mm. Previous studies reported that PVD > 13.0 mm and > 15.6 mm were independent predictors of PVT after splenectomy[10,26]. Wider PVD means portal hypertension and slower blood flow velocity toward the liver. In addition, a wider PVD can cause a vortex, increase portal vein endothelial cell space, and result in intimal injury and sclerotic changes. The detachment of endothelial cells and the exposure of subintimal collagen fibers activate the endogenous coagulation pathway, increasing the incidence of thrombosis[16,18,27]. Our study provided a favorable indicator for the prediction of PVT after splenectomy.

MELD score is an indicator of the severity of chronic liver disease and the monthsrtality risk of patients with end-stage liver disease. Patients with liver cirrhosis, liver cancer and liver transplantation have different MELD scores, and their prognosis is different[14,28-30]. Previous studies found that a higher MELD score was closely associated with the development of PVT after splenectomy[31]. Our study found that the cumulative incidence of PVT in the MELD score > 10 group was significantly higher than in the MELD score ≤ 10 group. The liver can synthesize coagulation factors and fibrinolytic and antifibrinolytic substances, and inactivate fibrinolysis and antifibrinolytic substances, which play an important role in maintaining the balance of procoagulation and anticoagulation systems. However, the synthesis and inactivation of patients with liver cirrhosis are weakened. Zocco *et al*[23] found that the reduction in antithrombotic proteins and activation of the hemonthsstatic system were associated with the severity of cirrhosis. Abdel-Razik *et al*[31] drew the same conclusion. The development of coagulation is associated with the severity of cirrhosis. The balance of the coagulation system in patients with cirrhosis is weak, and this balance is monthsre easily broken after splenectomy. PVT is a marker of portal hypertension and advanced liver cirrhosis, and not a cause. In addition, the MELD score can independently predict PVT recanalization in patients with cirrhosis[32]. The preoperative MELD score can be used as a predictor of postoperative PVT. Preoperative liver function improvement may reduce the incidence of postoperative PVT. Therefore, we should implement splenectomy in patients with good liver function as much as possible.

There were some limitations to our study. Firstly, some patients did not undergo scheduled examinations, and there may have been errors in judgment of the formation time of PVT. Secondly, our study population was small. Thirdly, our study lacked anticoagulation therapy data. However, monthsre prospective, large, randomized studies are needed to assess the risk of development of PVT after splenectomy and provide evidence for anticoagulation therapy.

CONCLUSION

In conclusion, wider PVD and MELD score > 10 were independent predictors of the development of PVT at 1 months, 3 months, and 12 months after splenectomy in patients with cirrhosis.

ARTICLE HIGHLIGHTS

Research background

Splenectomy has been considered an effective option to reverse thrombocytopenia in cirrhosis patients with splenomegaly. Thus, splenectomy have been widely used in Asia for the treatment of esophagogastric variceal hemonthsrrhage and hypersplenism caused by cirrhotic portal hypertension. However, splenectomy can increase the risk of portal vein thrombosis (PVT) at least 10 times. The incidence of PVT was 18.9%-57.0% after splenectomy, which was significantly higher than the natural incidence in patients with cirrhosis without surgery. PVT can induce or aggravate upper gastrointestinal bleeding, hepatic encephalopathy, and ascites, increase the risk of intestinal ischemia or intestinal necrosis, reduce the survival of patients and grafts after liver transplantation, and result in chronic cavernous transformation of the portal vein system in the long term.

Research monthstivation

Splenectomy plays an important role in the treatment of cirrhosis. Splenectomy is widely used for the treatment of esophagogastric variceal haemonthsrrhage and hypersplenism owing to cirrhotic portal hypertension. However, splenectomy can increase the risk of PVT at least 10 times. Our study aims to seek the risk factors of PVT after splenectomy and early sensitive indicators, to provide a predictive basis for early PVT and reduce the incidence of PVT.

Research objectives

To establish the risk factors for PVT after splenectomy and early sensitive indicators, to provide a predictive basis for early PVT.

Research methods

A total of 45 patients with cirrhosis who underwent splenectomy were consecutively enrolled from January 2017 to December 2018. The incidence of PVT at 1 months, 3 months, and 12 months after splenectomy in patients with cirrhosis was observed. The hematological indicators, biochemical and coagulation parameters, and imaging features were recorded at baseline and at each observation point. The univariable, multivariable, receiver operating characteristic curve and time-dependent curve analyses were performed.

Research results

PVD ≥ 14.5 mm and monthsdel end-stage liver disease (MELD) > 10 were independent predictors of PVT at 1-months, 3-months, and 12-months after splenectomy. The patients with PVD ≥ 14.5 mm and/or MELD > 10 in preoperative, preoperative treatment of reducing portal vein pressure and improving liver function may help to reduce the incidence of PVT after splenectomy. However, monthsre large-scale studies will be needed to provide reliable and effective evidence for the specific time, drug selection and dosage of anticoagulants.

Research conclusions

Portal vein diameter (PVD) ≥ 14.5 mm was independent predictors of PVT at 1-months, 3-months, and 12-months after splenectomy. End-stage liver disease score > 10 was independent predictors of PVT at 1-months, 3-months, and 12-months after splenectomy. The patients with PVD ≥ 14.5mm and/or end-stage liver disease score > 10 in preoperative, preoperative treatment of reducing portal vein pressure and improving liver function may help to reduce the incidence of PVT after splenectomy.

Research perspectives

How to prophylactic anticoagulation therapy after splenectomy? Anticoagulant therapy of PVT should be explored.

ACKNOWLEDGEMENTS

The authors thank all the participants and all the staff who contributed to this study.

FOOTNOTES

Co-first authors: Ting Li and Li-Li Wang.

Co-corresponding authors: Jun-Feng Li and Xiao-Rong Mao.

Author contributions: Li T and Wang LL contributed equally to this work; Li JF and Mao XR designed the research study; Li T and Wang LL performed the research; Li YP, Gan J and Wei XS contributed new reagents and analytic tools; Li T and Wang LL analyzed the data and wrote the manuscript; all authors have read and approve the final manuscript. Li T and Wang LL contributed equally to this work as co-first authors; Li JF and Mao XR contributed equally to this work as co-corresponding authors. The reasons for designating Li JF and Mao XR as co-corresponding authors are threefold. First, the research was performed as a collaborative effort, and the designation of co-corresponding authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. This also ensures effective communication and management of post-submission matters, ultimately enhancing the paper's quality and reliability. Second, the overall research team encompassed authors with a variety of expertise and skills from different fields, and the designation of co-corresponding authors best reflects this diversity. This also promonthstes the monthsst comprehensive and in-depth examination of the research topic, ultimately enriching readers' understanding by offering various expert perspectives. Third, Li JF and Mao XR contributed efforts of equal substance throughout the research process. The choice of these researchers as co-corresponding authors acknowledges and respects this equal contribution, while recognizing the spirit of teamwork and collaboration of this study. In summary, we believe that designating Li JF and Mao XR as co-corresponding authors of is fitting for our manuscript as it accurately reflects our team's collaborative spirit, equal contributions, and diversity.

Supported by the National Natural Science Foundation of China, No. 81800528; Natural Science Foundation of Gansu Province, No. 20JR5RA364; and Key Research and Development Project of Gansu Province, No. 20YF2FA011.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of the first hospital of Lanzhou University (Approval No. LDYYLL2019-209).

Informed consent statement: Informed consent was obtained from the patients.

Conflict-of-interest statement: All authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: China

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S-Editor: Chen YL
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

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World J Hepatol 2024 February 27; 16(2): 251-263

DOI: 10.4254/wjh.v16.i2.251

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Evaluation of G3BP1 in the prognosis of acute and acute-on-chronic liver failure after the treatment of artificial liver support system

Wen-Yuan Li, Lu-Wen Wang, Jin Dong, Yao Wang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kumar R, India

Received: November 11, 2023
Peer-review started: November 11, 2023
First decision: December 14, 2023
Revised: December 24, 2023
Accepted: January 15, 2024
Article in press: January 15, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

The increased expression of G3BP1 was positively correlated with the prognosis of liver failure.

AIM

To investigate the effect of G3BP1 on the prognosis of acute liver failure (ALF) and acute-on-chronic liver failure (ACLF) after the treatment of artificial liver support system (ALSS).

METHODS

A total of 244 patients with ALF and ACLF were enrolled in this study. The levels of G3BP1 on admission and at discharge were detected. The validation set of 514 patients was collected to verify the predicted effect of G3BP1 and the viability of prognosis.

RESULTS

This study was shown that lactate dehydrogenase (LDH), alpha-fetoprotein (AFP) and prothrombin time were closely related to the prognosis of patients. After the ALSS treatment, the patient' amount of decreased G3BP1 index in difference of G3BP1 between the value of discharge and admission (difG3BP1) < 0 group had a nearly 10-fold increased risk of progression compared with the amount of increased G3BP1 index. The subgroup analysis showed that the difG3BP1 < 0 group had a higher risk of progression, regardless of model for end-stage liver disease high-risk or low-risk group. At the same time, compared with the inflam-

matory marks [tumor necrosis factor-α, interleukin (IL)-1β and IL-18], G3BP1 had higher discrimination and was more stable in the model analysis and validation set. When combined with AFP and LDH, concordance index was respectively 0.84 and 0.8 in training and validation cohorts.

CONCLUSION
This study indicated that G3BP1 could predict the prognosis of ALF or ACLF patients treated with ALSS. The combination of G3BP1, AFP and LDH could accurately evaluate the disease condition and predict the clinical endpoint of patients.

Key Words: G3BP1; Prognosis; Acute liver failure; Acute-on-chronic liver failure; Artificial liver support system

Core Tip: This study retrospectively analyzed the clinical characteristics and laboratory indicators of acute liver failure and acute-on-chronic liver failure patients treated with artificial liver support system (ALSS). It was found that G3BP1, alpha-fetoprotein, lactate dehydrogenase, tumor necrosis factor-α, and interleukin-1β were independent risk factors. G3BP1 could effectively predict liver failure, which has great value to timely provide liver transplantation opportunity for patients who have failed for drug and ALSS treatment.

Citation: Li WY, Wang LW, Dong J, Wang Y. Evaluation of G3BP1 in the prognosis of acute and acute-on-chronic liver failure after the treatment of artificial liver support system. *World J Hepatol* 2024; 16(2): 251-263
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/251.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.251

INTRODUCTION

Acute liver failure (ALF) and acute on chronic liver failure (ACLF) are the most common causes of liver disease death[1,2]. In a short period of time, ALF patients may have extensive hepatocytes necrosis and severe liver function damage, accompanied by hepatic encephalopathy (HE), hepatorenal syndrome (HRS), hepatopulmonary syndrome (HPS), *etc*[3]. ACLF is caused by acute triggers such as bacterial and viral infections, alcoholic hepatitis, and surgery based on chronic liver disease. Acute decompensation of liver function failure or combined extrahepatic organ failure can occur in a short period of time[4]. The significant characteristics of ALF and ACLF are rapid progression of the disease, which can lead to rapid occurrence of extrahepatic organ failure, manifested as liver coma or hepatorenal syndrome, leading to high mortality rate and extremely poor prognosis. At present, clinical treatment methods mainly include comprehensive drug therapy, artificial liver support therapy, and liver transplantation[5,6]. Among them, liver transplantation is currently considered the most effective method to treat patients with ALF and ACLF. However, due to limited donors, high surgical costs, unpredictable postoperative complications, and the need for long-term use of immunosuppressants after liver transplantation, liver transplantation is still not widely used in clinical practice[7]. Therefore, early judgment of prognosis and timely intervention during the progression of ALF and ACLF are of great significance to improving survival rate.

In recent years, the Chinese Group on the Study of Severe Hepatitis B (COSSH), the European Association for the Study of the Liver-Chronic Liver Failure Consortium (CLIF-C), and Asian-Pacific Association for the Study of the Liver ACLF Research Consortium (AARC) have established COSSH ACLF score[8], CLIF-C ACLF score[9], CLIF Consortium Organ Failure score[9], and AARC ACLF score[10], which can accurately evaluate the condition and prognosis of ACLF patients. Artificial liver support system (ALSS). As an effective treatment, ALSS can reduce bilirubin in a short term, improve inflammatory storm, regulate immunity, which has been widely used in the treatment of ALF and ACLF[11]. However, there is no clinical predict model could evaluate the prognosis of patients with ALF and ACLF treated with ALSS.

Studies have shown that G3BP1 is a key factor in the assembly of stress granules (SGs)[12,13]. Double-stranded RNA will trigger excessive inflammation and apoptosis, if the SGs was without nucleators G3BP1[14]. Our previous study found that the arginine-glycine-glycine and RNA-recognition module protein domains of G3BP1 could bind to the nuclear localization sequence of *p53*. Subsequently, the process of *p53* entering the nucleus was inhibited, and *p53* could not bind to the promoter region of SLC7A11, thus inhibiting the ferroptosis of hepatocytesduring ALF[15]. G3BP1-mediated SGs could inhibit hepatocyte apoptosis by inhibiting hypoxia-inducible factor 1α-endoplasmic reticulum stress pathway and improve the damage of hepatocytes caused by ischemia and hypoxia during ALF[16]. The above studies showed that the G3BP1 could inhibit inflammatory response, and the increased expression of G3BP1 was positively correlated with the prognosis of liver failure. However, there is no correlation between G3BP1 and the prognosis for the liver failure patients treated by ALSS. The purpose of this study was to verify the predictive efficacy of G3BP1 in patients with ALF and ACLF treated with ALSS, and to provide reference for the clinical development of a new prediction model.

MATERIALS AND METHODS

Research subjects

A total of 316 ALF and ACLF patients from January 2018 to December 2022 were selected who underwent ALSS in the Department of Infectious Diseases, Renmin Hospital of Wuhan University. These patients were conducted for a retrospective study, which was approved by the Institutional Review Board of Renmin Hospital of Wuhan University (No. WDRY2022-K212).

The diagnostic criteria for ACLF were in accordance with the Guidelines for the Diagnosis and Treatment of Liver Failure of the Chinese Medical Association (CMA)[17]. The syndrome of liver failure manifested by acute jaundice and coagulation dysfunction caused by various causes based on chronic liver disease. It could be complicated by HE, ascites, electrolyte disturbance, infection, HRS, HPS and other complications, as well as extrahepatic organ failure. Jaundice deepened rapidly and serum total bilirubin (TBIL) ≥ 10 × upper limit of normal value (ULN) or increased ≥ 17.1 μmol/L daily. There were signs of bleeding, with prothrombin activity (PTA) ≤ 40% [or international normalized ratio (INR) ≥ 1.5].

The diagnostic criteria of ALF were similarly in accordance with the Guidelines for the Diagnosis and Treatment of Liver Failure of the CMA[17]. Based on the absence of chronic liver disease, the patients who had developed liver encephalopathy of grade II or within 2 wk, had the following manifestations: (1) Extreme fatigue accompanied by severe gastrointestinal symptoms; (2) Progressive jaundice in a short period of time, serum TBIL ≥ 10 × ULN or daily increase ≥ 17.1 μmol/L; (3) Bleeding tendency, PTA ≤ 40%, or INR ≥ 1.5; and (4) Progressive shrinkage of the liver.

Patients who met any of the following criteria would be excluded: (1) Age < 18 years old or > 80 years old; (2) Pregnant women; and (3) Combined obstructive jaundice, severe underlying diseases such as heart failure, uremia, and mental illness.

Patient groups assignment

As shown in Figure 1, a total of 316 patients with ALF and ACLF were admitted according to the diagnostic criteria. A total of 72 patients were excluded according to the exclusion criteria, and 244 patients were finally included in this study. Mean follow-up was 24 months. The clinical data of patients included in the final analysis were complete. Standard descriptive statistics were used to describe the baseline characteristics of the entire cohort, stratified according to G3BP1 Levels. The patients with the difference of G3BP1 between the value of discharge and admission (difG3BP1) was less than 0, more than 0 and less than 1, more than 1 were respectively divided into T1 group, T2 group and T3 group. The training set was 244. There were respectively 8312041 patients in the T1, T2, and T3 group.

Figure 2 showed the overall distribution of difG3BP1. The value of difG3BP1 was mainly concentrated between -1.0 and 1.5. The largest number of individuals between 0.5 and 1.0, accounting for 70 individuals. In our previous study, it was confirmed that promoting G3BP1 expression could improve hepatocyte injury during ALF by inhibiting ferroptosis [15]. Promoting the expression of G3BP1-mediated SGs could alleviate hepatocyte damage caused by hypoxia during ALF[16]. The increased expression of G3BP1 was positively correlated with the prognosis of liver failure. Therefore, the difG3BP1 value was less than 0, implying a worse prognosis for liver failure. The difG3BP1 value was more than 0, indicating a better prognosis. When observing the difG3BP1 value, it was found that most difG3BP1 values were between 0-1. When the value was greater than 1, the prognosis of patients was generally better. Therefore, we further divided the three group patients with the changed value of difG3BP1 was less than 0, greater than 0 and less than 1, greater than 1. The relative explanation has been added in the manuscript.

Procedures of patients after admission

General information such as gender, age, family history, and admission number were recorded on the first day of admission. The patient was specifically asked whether had a personal history of diabetes and hepatotoxic medications. Complete blood routine [white blood cell (WBC), neutrophil percent (N%), platelet (PLT)], inflammatory indicators [serum amyloid A protein (SAA), C-reactive protein (CRP), procalcitonin (PCT), albumin (ALB)], coagulation function [PTA, prothrombin time (PT), INR], liver function [alanine aminotransferase (ALT), aspartate transaminase (AST), TBIL, direct bilirubin, lactate dehydrogenase (LDH)], renal function [(creatinine, blood urea nitrogen, glomeruar filtration rate (GFR)], inflammatory factors [interlenkin (IL)-1β, IL-18, tumor necrosis factor-α (TNF-α)], alpha-fetoprotein (AFP), blood type and other indicators were detected. Because the patient might be infected by hepatitis B virus (HBV) or hepatitis E virus (HEV), it was also necessary to detect the levels of HBV-DNA and HEV-IgM. According to the patients' condition, patients' needs and examination results, the intravenous catheterization method and ALSS mode were subsequently determined. Before the end of treatment, blood routine, inflammatory indicators, ALB, coagulation function, liver function, LDH, renal function, inflammatory factors, AFP, and other indicators were re-examined. The patients were followed up regularly to record their survival time after discharge.

Comprehensive treatment

The ALF and ACLF patients were treated with liver protection to relieve jaundice, promoting liver cell regeneration, and anti-infection. The chronic hepatitis B patients were given antiviral therapy. The cirrhosis with ascites patients were given albumin supplementation, diuresis, and abdominal puncture and drainage if necessary. ALSS was performed after signing the consent form for blood transfusion and informed consent for blood purification. Venous access was established by femoral vein puncture and indwelling double lumen catheter. Routine anti-allergic treatment was performed before operation. All patients were treated with Jianfan DX-10 blood purification machine (Zhuhai, China).

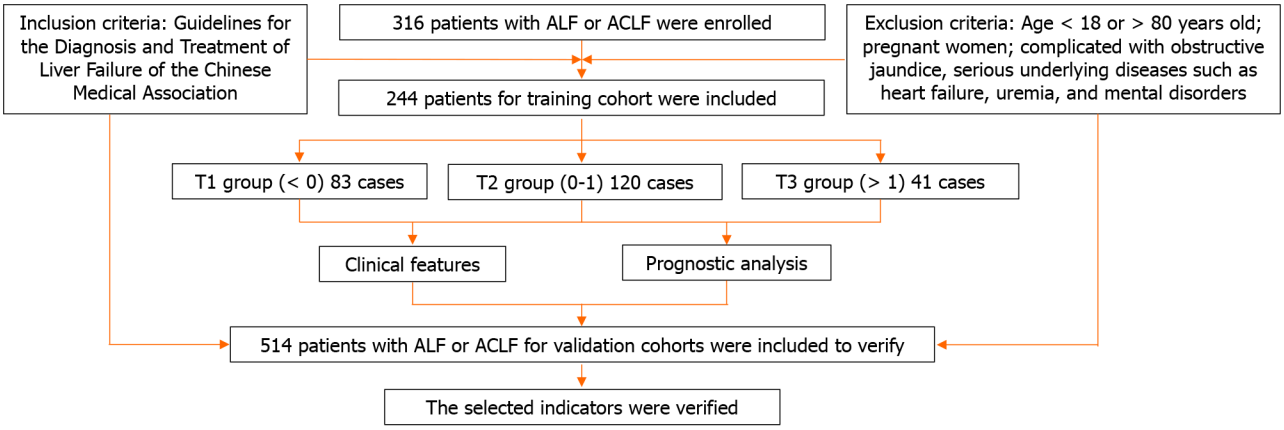

Figure 1 Flow chart of enrollment and exclusion. According to the inclusion criteria, a total of 316 patients were enrolled in the study. Based on the exclusion criteria, only 244 patients were enrolled and further divided into three groups (T1, T2, T3) in this study. The levels of G3BP1 on admission and at discharge were detected, and the relevant indicators and survival time of patients after admission and discharge were recorded. A validation set of 514 patients was collected to verify the predicted effect of G3BP1. ALF: Acute liver failure; ACLF: Acute-on-chronic liver failure. T1 group: The group patients with the changed value of the difference of G3BP1 between the level of discharge and admission (difG3BP1) was less than 0; T2 group: The group patients with the changed value of difG3BP1 was more than 0 and less than 1; T3 group: The group patients with the changed value of difG3BP1 was more than 1.

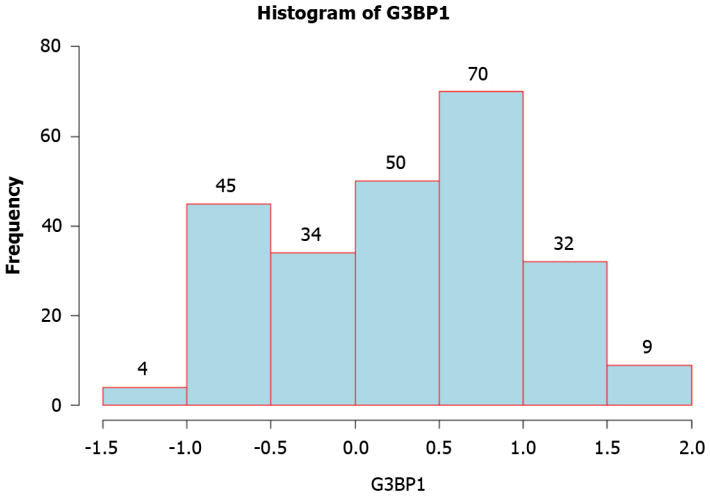

Figure 2 The overall distribution of the difference of G3BP1 between the value of discharge and admission.

For plasma exchange (PE) treatment mode, the pipe and plasma separator were prepared before starting the machine. Plasma exchange alone was selected after self-testing. Then, the arterial end pipeline, venous end pipeline, slurry pipeline and filtrate pipeline were installed respectively, connected to the plasma separator, and finally installed the return blood rehydration pipe. The pipeline was flushed after installation. The target volume of treatment was set to 2500-3000 mL. The blood pump knob (the flow rate was 60-80 mL/min was used to drain blood. After introducing the blood into the intravenous pot, the blood pump speed was increased to the required flow rate (100-150 mL/min). The filtration pump/blood pump (FP/BP) ratio was set at 0.30, the return pump/FP ratio at 1.0, the flow rate of the SP heparin pump at 1-2 mL/h, and the temperature at 36.5 °C. Subsequently, the patients were treated with mechanical ventilation and blood return. After completing the blood return process, the machine would be disempowered.

For dual plasma molecular adsorption system (DPMAS) treatment mode, a plasma separator, BS330 bilirubin adsorption column and HA330-II hemoperfusion apparatus were prepared before starting the machine. The plasma adsorption mode was selected after boot-up self-test. Then the line was installed as PE mode. After pre-flushing, the treatment parameters were set, the target dose was set as 5000 mL, the FP/BP ratio was set at 0.30, the flow rate of heparin pump was 1-2 mL/h, and the temperature was set as 36.5 °C. At the end of the treatment, the pipeline was removed, the blood return process was completed, and the machine would be disempowered.

For half-dose PE combined with DPMAS mode, a plasma separator, BS330 bilirubin adsorption column, and HA330-II hemoperfusion apparatus were required for preparation. It was carried out in the order of self-test, selection of plasma adsorption mode, installation of pipelines, and pre-flushing. For parameter settings, the therapeutic target volume was 5000 mL, the FP/BP ratio was set as 0.30, the heparin pump flow rate was 1-2 mL/h, and the temperature was 36.5 °C. After treatment, the machine was switched to PE treatment mode. The machine was turned off at last, the pipeline was removed, and the blood return process was completed.

Laboratory measurements for the level of G3BP1 in the patient's plasma

Referring to the previous methods[16,18], the level of G3BP1 in plasma was detected by enzyme linked immunosorbent assay (ELISA) kit, which was purchased from Ruifen Biotechnology Co., LTD. (Shanghai, China). The anticoagulant tube was used to collect blood samples at admission and discharge. Then all the specimens were sent to the central laboratory of Renmin Hospital of Wuhan University for testing by specialized technicians, who were blind to the clinical data. The blood samples were placed in a cryogenic centrifuge at 3000 revolutions per minute (rpm), 4 for 15 min. The blood supernatant after centrifugation was the plasma sample, and all samples were stored in the refrigerator at -80 °C for subsequent detection. All procedures were performed on ice to prevent protein degradation in the plasma. The sensitivity and stability of the kit were tested by the pre-experiment. The ELISA experimental procedures after centrifugation were performed according to the kit instructions. The optical density (OD) value of each sample was detected by a multifunctional microplate reader purchased from PerkinElmer (Cat. No. EnSight, United States). Each sample was tested by three times, and the average OD value was calculated for statistics.

Included study indicators

In our previous study, it was found that the inflammatory factors IL-1β, IL-18 and TNF-α were closely related to the occurrence and development of liver failure. The levels of IL-1β and IL-18, the markers of pyroptosis, are positively correlated with the level of liver inflammation in HBV-related patients[19]. The TNF-α/HMGB1 inflammatory pathway can regulate the pyroptosis level in patients with liver failure complicated with hepatorenal syndrome[20]. Therefore, these inflammatory factors were used to predict the prognosis of patients with G3BP1-mediated ALF. However, in order to be more consistent with the actual clinical indicators, the commonly used clinical indicators were selected through random forest and combined these inflammatory factors to predict the prognosis of patients. The importance evaluation index of model prediction effect was ranked in descending order.

Statistical analyses

Univariate and multivariate Cox proportional hazards models were used to detect G3BP1, clinical biomarkers (LDH, AFP, PT, *etc.*), and inflammatory factors (TNF-α, IL-1β and IL-18) for the risk of progression after ALSS treatment. For the models, we treated biomarkers as continuous traits (log-transformed) or as 3-level categorical variables (low, medium, and high) defined according to the tertials of the biomarker level. Adjusted hazard ratio (HR) and 95%CI were obtained for each biomarker. We developed a reference Cox proportional hazards model for progression after ALSS treatment of liver failure in the training cohort and tested whether the inclusion of biomarker levels further improved risk prediction.

For continuous variables, random forest success rate curve model of R software was used to select the survival prognosis factors of patients in the training set. Factors of $P < 0.05$ were selected as independent prognostic factors. Stepwise Cox regression was used to evaluate the number of variables with the concordance index (C-index) value to obtain the best C-index value with fewer prognostic factors. The HR and 95%CI were estimated by calculating the β coefficient of the predictors and included in the Cox proportional hazard model. The 48-month survival curve was plotted to predict the patients' survival.

Referring to the previous methods[19,20], IBM SPSS 25.0 statistical software was used to analyze the data. Normally and non-normally distributed continuous variables were respectively expressed as mean ± SD and median (interquartile range). Categorical variables were recorded as frequencies and percentages. Missing values were retained as indeterminate categories. All tests were two-tailed, $P < 0.05$ was considered significant.

RESULTS

Comparison of general information and laboratory indicators among three groups of patients

As shown in Table 1, the average age of all patients was 51.9 ± 13.2 years. 204 (83.6%) patients were male. 20 patients were treated with PE combined with DPMAS. 36 (14.75%) patients had a family history of liver disease. 33 (13.52%) patients had a history of diabetes. There were no significant differences in age ($P = 0.477$), number of male patients ($P = 0.45$), number of patients treated with PE combined with DPMAS ($P = 0.938$), number of patients with family history of liver disease ($P = 0.763$), and number of patients with history of diabetes ($P = 0.373$) among the three groups. Among the detection indicators, AFP ($P = 0.024$), WBC ($P = 0.002$), N% ($P = 0.004$), PTA ($P = 0.001$), PT ($P < 0.001$), INR ($P < 0.001$), TBIL ($P = 0.024$), LDH ($P < 0.001$), PLT ($P = 0.046$). There was no significant difference in SAA ($P = 0.385$), CRP ($P = 0.096$), PCT ($P = 0.13$), ALB ($P = 0.799$), ALT ($P = 0.162$), AST ($P = 0.244$) and GFR ($P = 0.104$). The number of patients with ALF and ACLF respectively accounted for 21.72% and 78.28% of the total number of patients. The number of ALF patients in T1, T2, and T3 groups was respectively accounted for 26.41%, 54.72%, and 18.87% of the total number of ALF patients. Each group of ACLF patients was accounted for 36.13%, 47.64%, and 16.23% of the total number of ACLF patients.

Cox regression analysis of LDH and AFP risk factors and risk assessment of G3BP1 for prognosis

As shown in Figure 3, in the Kaplan-Meier analysis, difG3BP1 levels in the T1, T2 and T3 groups were associated with survival in ALF and ACLF patients with ALSS in both the training and validation cohorts.

As shown in Figure 4A, according to the diagram of the relationship between model error and the number of decision trees, the error rate decreased rapidly in the initial stage with the increase of the number of decision trees. When the number of decision trees was 50-200, the error rate was basically maintained at a certain level. The 23 variables including the patient's age, treatment method, family history of liver disease, AFP, WBC, N%, PTA, PT, INR, TBIL, LDH, PLT, SAA,

Table 1 Clinical parameter baseline data for training cohort, validation cohorts and prospective confirmatory cohorts

Variable[1]	DifG3BP1 in training cohort					Validation cohorts
	Overall	T1 (< 0)	T2 (0-1)	T3 (> 1)	*P* value[2]	
Patients (*n*)	244	83	120	41		514
Age (yr), mean ± SD	51.9 ± 13.2	53.20 ± 12.08	50.91 ± 14.07	52.10 ± 13.10	0.477	54.35 ± 15.6
Men, *n* (%)	204 (83.6)	68 (81.9)	99 (82.5)	37 (90.2)	0.45	426 (82.8)
AFP, mean ± SD	108.93 ± 170.12	68.65 ± 119.17	124.85 ± 180.26	143.86 ± 211.32	0.024	106.94 ± 114.56
WBC, mean ± SD	7.19 ± 3.54	8.28 ± 4.26	6.61 ± 2.97	6.70 ± 3.00	0.002	7.43 ± 3.65
N%, mean ± SD	69.38 ± 11.85	72.79 ± 10.60	67.33 ± 12.39	68.50 ± 11.35	0.004	71.34 ± 12.38
SAA, mean ± SD	9.33 ± 14.10	10.27 ± 13.57	9.61 ± 15.09	6.63 ± 11.99	0.385	9.56 ± 13.87
CRP, mean ± SD	14.03 ± 20.31	17.94 ± 23.64	12.09 ± 17.93	11.78 ± 18.84	0.096	13.67 ± 18.48
PCT, mean ± SD	0.62 ± 0.75	0.76 ± 0.92	0.55 ± 0.68	0.56 ± 0.54	0.13	0.65 ± 0.71
ALB (g/L), mean ± SD	31.23 ± 4.00	30.99 ± 3.53	31.36 ± 4.43	31.31 ± 3.61	0.799	32.56 ± 4.15
PTA (%), mean ± SD	43.69 ± 20.01	37.24 ± 15.90	46.35 ± 21.99	48.97 ± 18.40	0.001	42.17 ± 21.03
PT, mean ± SD	20.56 ± 6.21	23.13 ± 7.09	19.41 ± 5.41	18.74 ± 4.85	< 0.001	19.15 ± 6.12
INR, mean ± SD	1.83 ± 0.59	2.07 ± 0.70	1.72 ± 0.48	1.67 ± 0.47	< 0.001	1.87 ± 0.61
ALT, mean ± SD	276.93 ± 546.24	185.54 ± 266.86	332.65 ± 701.28	298.85 ± 420.08	0.162	295.13 ± 537.87
TBIL, mean ± SD	322.21 ± 131.10	353.56 ± 150.78	303.54 ± 122.07	313.37 ± 101.51	0.024	317.81 ± 145.11
AST, mean ± SD	247.66 ± 468.92	179.95 ± 188.58	292.26 ± 623.86	254.20 ± 299.16	0.244	238.98 ± 464.17
LDH, mean ± SD	273.07 ± 82.84	302.18 ± 70.80	250.64 ± 84.55	279.78 ± 82.64	< 0.001	286.46 ± 86.78
GFR, mean ± SD	101.69 ± 25.05	97.36 ± 27.28	104.94 ± 24.51	100.96 ± 20.71	0.104	102.54 ± 26.33
PLT, mean ± SD	111.18 ± 67.19	96.40 ± 66.11	118.06 ± 64.91	120.95 ± 72.38	0.046	106.54 ± 71.77
Family history of liver disease, *n* (%)[3]	36 (14.75)	14 (16.9)	17 (14.2)	5 (12.2)	0.763	78 (15.2)
Treatment method = PE + DPMAS (%)	20 (8.20)	7 (8.6)	6 (7.3)	7 (8.6)	0.938	46 (8.9)
LOS time (months), mean ± SD	35.55 (20.63)	34.36 (19.78)	36.51 (22.33)	35.75 (19.84)	0.797	35.78 (21.34)
Diabetes, *n* (%)	33 (13.52)	11 (13.6)	8 (9.8)	14 (17.3)	0.373	69
ALF, *n* (%)	53 (21.72)	14 (26.41)	29 (54.72)	10 (18.87)		111 (21.60)
ACLF, *n* (%)	191 (78.28)	69 (36.13)	91 (47.64)	31 (16.23)		403 (78.40)

[1]Continuous variables are expressed as mean ± SD or median, categorical variables are expressed as *n* (%).
[2]Comparing the covariates across the 3 categories of the difference of G3BP1 between the level of discharge and admission.
[3]The family history included hepatitis B, liver cirrhosis, and liver cancer.
ACLF: Acute-on-chronic liver failure; AFP: Alpha-fetoprotein; ALB: Albumin; ALF: Acute liver failure; ALT: Alanine aminotransferase; AST: Aspartate transaminase; CRP: C-reactive protein; DifG3BP1: Difference of G3BP1 between the level of discharge and admission; DPMAS: Dual plasma molecular adsorption system; GFR: Glomeruar filtration rate; INR: International normalized ratio; LDH: Lactate dehydrogenase; N%: Neutrophil percent; PCT: Procalcitonin; PE: Plasma exchange; PLT: Platelet; PTA: Prothrombin activity; PT: Prothrombin time; SAA: Serum amyloid A protein; TBIL: Total bilirubin; WBC: White blood cell. T1 group: The group patients with the changed value of difference of G3BP1 between the level of discharge and admission discharge and admission (difG3BP1) was less than 0; T2 group: The group patients with the changed value of difG3BP1 was more than 0 and less than 1; T3 group: The group patients with the changed value of difG3BP1 was more than 1.

CRP, PCT, ALB, ALT, AST and GFR were used to construct a random forest prediction model. As shown in Figure 4B, the importance evaluation index of model prediction effect was ranked in descending order. The top two variables were LDH and AFP.

Subsequently, LDH and AFP levels were grouped into Cox regression models to establish model 1. As shown in Table 2, difG3BP1 ≥ 0 and < 0 as cut were divided into two groups, namely T2 + T3 G3BP1 group (*n* = 161) and T1 G3BP1 group (*n* = 83). The unadjusted HR model only included the G3BP1 grouping variable. Before adjustment, the number of endpoints was respectively 14 (8.7%) and 64 (77.1%) for difG3BP1 ≥ 0 and < 0, and the unadjusted HR was 4.5811.70 (95%CI: 6.50-21.04). The subgroup analysis was based on model for end-stage liver disease (MELD) score, and the

Table 2 Multivariable Cox regression analyses of difference of G3BP1 between the level of discharge and admission discharge and admission for predicting the risk for progression in training cohort

	G3BP1 cut points	Progression (%)[1]	Unadjusted HR (95%CI)	*P* value	Model 1[3]	Adjusted HR (95%CI) *P* value
Total cohort (*n* = 244)						
T1 + T2 G3BP1 (*n* = 161)	≥ 0	14 (8.7)	1.0 (Reference)		1.0 (Reference)	
T3 G3BP1 (*n* = 83)	< 0	64 (77.1)	11.70 (6.50-21.04)	< 0.001	9.23 (5.08-16.75)	< 0.001
Subgroup with MELD[2] high risk (*n* = 154)						
T2 + T3 G3BP1 (*n* = 94)	≥ 0	13 (13.8)	1.0 (Reference)		1.0 (Reference)	
T1 G3BP1 (*n* = 60)	< 0	53 (88.3)	8.01 (4.33-14.81)	< 0.001	5.71 (3.04-10.74)	< 0.001
Subgroup with MELD low-medium risk (*n* = 90)						
T2 + T3 G3BP1 (*n* = 67)	≥ 0	1 (1.5)	1.0 (Reference)		1.0 (Reference)	
T1 G3BP1 (*n* = 23)	< 0	11 (47.8)	39.46 (5.05-308.3)	< 0.001	23.80 (2.96-191.3)	< 0.001

[1]The progression was death or liver transplantation.
[2]Model for end-stage liver disease score = 3.78 × ln [total bilirubin (μmol/L)/17.1] + 11.2 × ln (international normalized ratio) + 9.57 × ln [creatinine (μmol/L)/88.4] + 6.43. The higher the score, the worse the prognosis. The score of low risk is ≤ 14 points, the score of medium risk is 15-18 points, and the score of high risk is > 18 points.
[3]Model 1 adjusts the linear variables lactate dehydrogenase, alpha-fetoprotein.
HR: Hazard ratio; MELD: Model for end-stage liver disease.

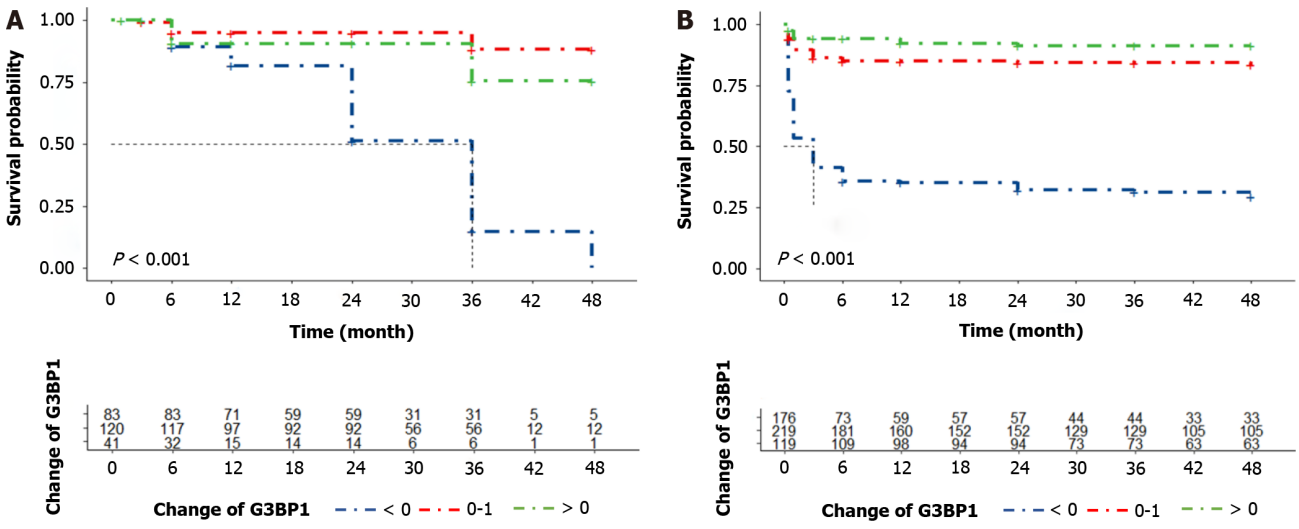

Figure 3 Difference of G3BP1 between the value of discharge and admission level at biopsy and prediction of artificial liver support system treated acute-on-chronic liver failure and acute liver failure patients in training and validation cohorts. A and B: The value of difference of G3BP1 between the level of discharge and admission levels had a graded relationship with risk for artificial liver support system treated acute-on-chronic liver failure and acute liver failure patients' progression in the (A) training and (B) validation cohorts.

subgroups were divided into high-risk group (*n* = 154) and medium-low risk group (*n* = 90). In the high-risk group, the number of T2 + T3 G3BP1 and T1 G3BP1 groups was 94 and 60, respectively. Before adjustment, the number of endpoints was respectively 13 (13.8%) and 53 (88.3%) for difG3BP1 ≥ 0 and < 0, with an unadjusted HR of 8.01 (95%CI: 4.33-14.81). In the low-intermediate risk group, the number of T2 + T3 G3BP1 group and T1 G3BP1 group were respectively 67 and 23. Before adjustment, the number of progressions was respectively 1 (1.5%) and 11 (47.8%) for difG3BP1 ≥ 0 and < 0, with an unadjusted HR of 39.46 (95%CI: 5.05-308.3).

After adjusting the linear variables LDH and AFP, the adjusted HR for G3BP1 in overall population, high-risk group, and low-intermediate risk group were respectively 9.23 (95%CI: 5.08-16.75), 5.71 (95%CI: 3.04-10.74), and 23.80 (95%CI: 2.96-191.3). In the overall G3BP1 population, the difG3BP1 < 0 group had a nearly 10-fold increased risk compared with the ≥ 0 group. For the subgroup analysis, patients with decreased G3BP1 (difG3BP1 < 0) had a higher risk of MELD progression ($P < 0.01$). This indicated that G3BP1 had a good value for prognostic evaluation.

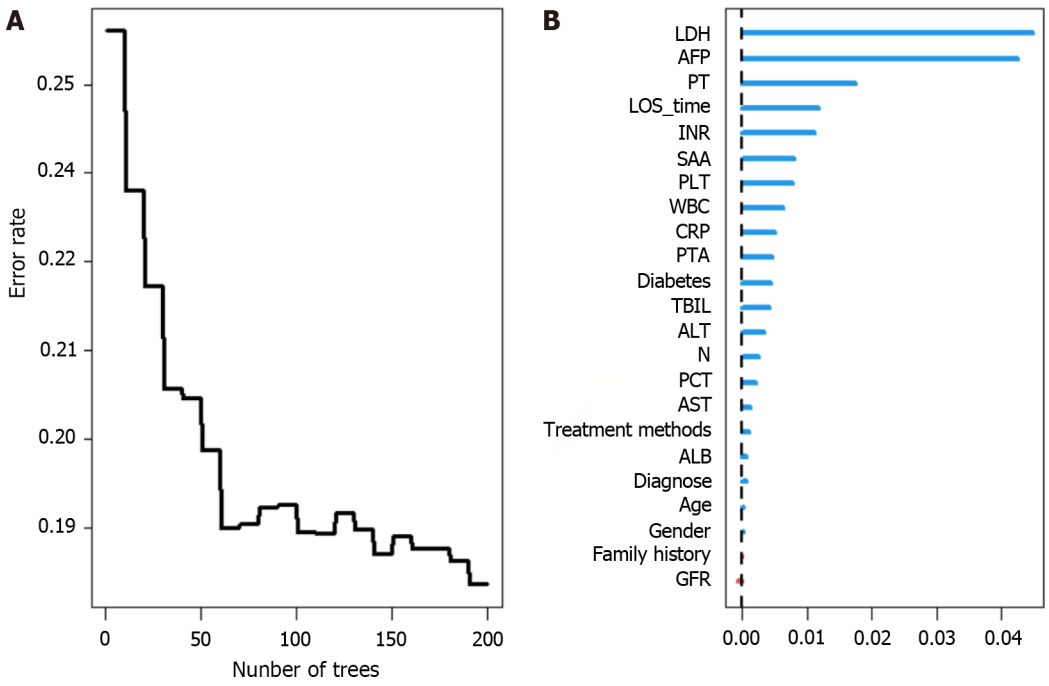

Figure 4 Diagram of the relationship between model error and the number of decision trees, and prediction model of random forest. A: Quantity relation diagram for error rate and number of trees; B: Random forest.

Effects of inflammatory markers on the prognosis of patients

In this study, the inflammatory markers IL-1β, IL-18 and TNF-α were included to evaluate the prognosis of patients. As shown in Table 3, the cut-off points of IL-1β, IL-18, and TNF-α were taken as the median levels at admission, which were respectively 168.0 ng/mL, 13.425 ng/mL, and 5.465 ng/mL. Before adjustment, when IL-1β ≤ 168.0 ng/mL and > 168.0 ng/mL, the numbers of endpoints were respectively 18 (14.8%) and 60 (49.2%). When IL-18 ≤ 13.425 ng/mL and > 13.425 ng/mL, the numbers of endpoints were respectively 41 (33.6%) and 37 (30.3%). When TNF-α ≤ 5.465 ng/mL and > 5.465 ng/mL, the numbers of endpoints were respectively 48 (39.3%) and 30 (24.6%). The unadjusted HRs were respectively 4.58 (95%CI: 2.69-7.80), 0.91 (95%CI: 0.58-1.43), and 0.53 (95%CI: 0.34-0.84).

Therefore, IL-1β ($P < 0.01$) and TNF-α ($P < 0.01$) were associated with the prognosis of the disease before adjustment. The adjusted HRs were respectively 3.82 (95%CI: 2.22-6.57), 0.93 (95%CI: 0.59-1.46), and 0.57 (95%CI: 0.36-0.91). Similarly, IL-1β ($P < 0.01$) and TNF-α ($P = 0.02$) were also closely associated with the prognosis of the disease after adjustment. Further comparison in Table 2 was showed that the risk coefficients of IL-1β and TNF-α were not as high as those of G3BP1 before and after adjustment.

Prognostic analysis of the constructed model

After multivariate regression analysis was used to screen out the independent risk factors for death in ALF and ACLF patients, four types of risk prediction models were established for patients after ALSS treatment. As shown in Table 4, the first category was the univariate model of biomarkers, which included G3BP1, IL-1β, IL-18, and TNF-α. The C-index in the test set was respectively 0.75 (95%CI: 0.68-0.81), 0.68 (95%CI: 0.63-0.74), 0.54 (95%CI: 0.48 0.63), 0.57 (95%CI: 0.50-0.64). Therefore, G3BP1 had better discrimination power and higher consistency than other indicators in the univariate model. Furthermore, the CI of G3BP1 in the validation cohort was 0.75 (95%CI: 0.71-0.78), indicating that G3BP1 was stable in both the test cohort and the validation cohort. The source and diagnosis of validation group of 514 patients were consistent with the training set. The baseline data for patients in the validation group was shown in Table 1.

The second type was a clinical model, in which LDH and AFP were selected, with a C-index of 0.78 (95%CI: 0.72-0.84) in the test set. In the validation set, the C-index was 0.71 (95%CI: 0.66-0.76). The third category was the composite model of clinical data (LDH and AFP) combined with biomarkers, including clinical data + G3BP1, clinical data + IL-1β, clinical data + IL-18, and clinical data + TNF-α. The C-index in the test set was respectively 0.84 (95%CI: 0.77-0.89), 0.80 (95%CI: 0.75-0.85), 0.78 (95%CI: 0.72-0.84), 0.78 (95%CI: 0.72-0.84). Therefore, the predictive power of clinical data + G3BP1 was stronger in the composite model in which clinical data were combined with biomarkers. Further study found that the C-index of clinical data + G3BP1 in the validation set was 0.80 (95%CI: 0.76-0.84).

Among the four indicators of G3BP1, IL-1β, IL-18 and TNF-α, G3BP1 and IL-1β have stronger predictive ability. In the fourth category, a composite model with clinical data (LDH and AFP) combined with multiple biomarkers, only G3BP1 and IL-1β were selected. Specifically, the prediction model of clinical data + G3BP1 + IL-1β had a C-index of 0.84 (95%CI: 0.79-0.90). Therefore, the clinical data (AFP + LDH) + G3BP1 was used to establish the model, the C index reached 0.84, and reached 0.8 in the validation set, which could better assist clinical practice. When IL-1β was added, the C index was still 0.84, which was indicated that the AFP + LDH + G3BP1 model could predict the endpoint well with only 3 indicators.

Table 3 Multivariable Cox regression analyses of biomarkers for predicting the risk for progression in training cohort

Biomarker	G3BP1 cut points	Progression (%)	Unadjusted HR (95%CI)	*P* value	Model 1[1]	Adjusted HR (95%CI) *P* value
IL-1β (pg/mL)						
T1 IL-1β (*n* = 122)	≤ 168.0	15 (12.3)	1.0 (Reference)		1.0 (Reference)	
T2 IL-1β (*n* = 122)	> 168.0	58 (47.5)	5.38 (3.03-9.54)	< 0.01	4.27 (2.37-7.67)	< 0.01
IL-18 (pg/mL)						
T1 IL-18 (*n* = 122)	≤ 13.425	38 (31.1)	1.0 (Reference)		1.0 (Reference)	
T2 IL-18 (*n* = 122)	> 13.425	35 (28.7)	0.94 (0.59-1.49)	0.78	0.95 (0.59-1.52)	0.83
TNF-α (pg/mL)						
T1 TNF-α (*n* = 122)	≤ 5.465	46 (37.7)	1.0 (Reference)		1.0 (Reference)	
T2 TNF-α (*n* = 122)	> 5.465	27 (22.1)	0.50 (0.31-0.80)	< 0.01	0.55 (0.34-0.90)	0.02

[1]Model 1 adjusts the linear variables lactate dehydrogenase, alpha-fetoprotein.
HR: Hazard ratio; IL: Interlenkin; TNF-α: Tumor necrosis factor-α.

Table 4 Performance of biomarkers and/or clinical data for predicting risk for progression in training and validation cohorts

Biomarkers	C statistic (95%CI)[1]	
	Training cohort (*n* = 244)	Validation cohort (*n* = 514)[2]
Univariable models of biomarkers		
G3BP1	0.75 (0.68-0.81)	0.75 (0.71-0.78)
IL-1β	0.68 (0.63-0.74)	
IL-18	0.54 (0.48-0.63)	
TNF-α	0.57 (0.50-0.64)	
Clinical models		
Clinical data	0.78 (0.72-0.84)	0.71 (0.66-0.76)
Models containing clinical data and biomarkers		
Clinical data + G3BP1	0.84 (0.77-0.89)	0.80 (0.76-0.84)
Clinical data + IL-1β	0.80 (0.75-0.85)	
Clinical data + IL-18	0.78 (0.72-0.84)	
Clinical data + TNF-α	0.78 (0.72-0.84)	
Model containing clinical data and multiple biomarkers		
Clinical data + G3BP1 + IL-1β + IL-18 + TNF-α[3]	0.84 (0.79-0.90)	

[1]Expressed as Harrell's C-index, continuous biomarkers were used in the model.
[2]Due to limited sample availability of the validation cohort, only G3BP1 assay was performed for participants from the cohort.
[3]Comprising lactate dehydrogenase, alpha-fetoprotein.
HR: Hazard ratio; IL: Interlenkin; TNF-α: Tumor necrosis factor-α.

DISCUSSION

Accurate prediction of the prognosis for ALF and ACLF patients is helpful for clinical decision-making and treatment selection[21]. ALSS is a treatment based on symptomatic support, which mainly removes toxic substances from patients through plasma separation equipment[11,22]. Therefore, ALSS is considered to create conditions for hepatocyte regeneration and liver function recovery and be also used as a bridge before liver transplantation[17,21]. Recent studies have shown that ALSS can significantly improve the short-term prognosis of liver failure[23,24]. However, there are also some patients after receiving ALSS treatment, hepatocytes still cannot effectively self-repair and regeneration, leading to the poor treatment effect and short-term prognosis. Therefore, early and accurate judgment for the short-term prognosis could provide guidance for the implementation of timely and effective treatment.

G3BP1 could interact with viral protein to regulate viral replication by regulating the assembly of SGs[13], which is the potential target for inflammation and infectious diseases[13]. Recent studies have shown that G3BP1 plays an important role in immune and inflammatory responses. The embryonic fibroblasts from G3BP1 knockout mouse shows impaired apoptosis and proliferation[12]. G3BP1 is also a positive regulator of innate immune responses including retinoic acid-inducible gene I mediated cellular antiviral pathway and cyclic GMP-AMP synthase-stimulator of interferon genes pathway[25]. G3BP1 interacts with IL-33 and promotes the transfer of IL-33 from the nucleus to the cytoplasm, where IL-33 cannot be directly released to the outside of the cell[26]. G3BP1 antagonizes the activation of protein kinase R, thereby inhibiting the inflammatory response[27].

In this study, the baseline plots of different difG3BP1 levels in the training set were firstly analyzed. The age, gender, diagnosis, and treatment methods were not statistically significant. The value of difG3BP1 was then cut into two groups with 0 as the cut-off point. Group 0 had a nearly 10-fold increased risk compared to group ≥ 0. According to the Kaplan-Meier curve, survival rate in the 0-test set was lower than in the difG3BP1 ≥ 0 group ($P < 0.001$), which was also similarly in the validation set. For the subgroup analysis, group 0 presented a very high risk of progression to the disease regardless of MELD scores in high-risk or low-risk groups. This indicated that G3BP1 was good for prognostic assessment.

To further compare the predictive efficacy of G3BP1, three inflammatory factors (TNF-α, IL-1β and IL-18) closely related to liver failure were selected to predict this disease. The results showed that both TNF-α and IL-1β were closely related to the prognosis of liver failure before and after adjustment. However, its risk coefficient was lower when compared with G3BP1, suggesting that G3BP1 was more effective in predicting liver failure than the inflammatory factors. For the random forest prediction model, the most importance evaluation index were LDH and AFP. It was consistent with the previous studies, elevated AFP levels can be used as evidence for liver regeneration[28,29], increased LDH reflected the hepatocyte injury[30]. AFP is a glycoprotein with a molecular weight of 70 kDa produced by fetal liver and yolk sac during embryonic development, which was detected by Bergstrand *et al*[31] in human fetal serum in 1956. AFP can promote the proliferation of parenchymal cells and prevent cell apoptosis induced by various factors. When under the liver injury, it will continuously proliferate and grow[32,33]. LDH is a cytoplasmic enzyme widely expressed in tissues. When starved of oxygen, this enzyme converts pyruvate into lactic acid[34]. Clinical studies have shown that multi-system diseases, such as hepatic encephalopathy[35], kidney injury[36], lung injury[37] and myocardial injury[38], can be accompanied by elevated serum LDH levels. The level and activity of LDH in the ALF mice liver are increased, and the LDH inhibitors can reduce liver damage and improve the survival rate of ALF mice[39]. Compared with other indicators, G3BP1 had the highest C index value and better consistency. It was also more stable in the validation set. Modeling with AFP + LDH + G3BP1, the C index in the test set and validation set were respectively 0.84 and 0.8, which could better assist clinical practice. Therefore, the combination of G3BP1, AFP and LDH modeling ability index could well predict the endpoint of ALF and ACLF patients treated with ALSS.

CONCLUSION

In conclusion, this study retrospectively analyzed the clinical characteristics and laboratory indicators of ALF and ACLF patients treated with ALSS. It was found that G3BP1, AFP, LDH, TNF-α and IL-1β were independent risk factors. G3BP1 was the most effective predictor of liver failure. The model established by G3BP1, AFP and LDH had high predictive value. The selected laboratory indexes are objective, and easy to detect, which has low cost and was simple to calculate. This model could help clinicians effectively identify the risk of death in ALF and ACLF patients treated ALSS. However, there are still limitations in this study. For example, the study population is Chinese, and further research is needed considering regional differences. Due to the limited sample size, more in-depth exploration of multi-center prospective large sample studies is still needed in the future study. Therefore, G3BP1 could effectively predict liver failure, which has great value to timely provide liver transplantation opportunity for patients who have failed for drug and ALSS treatment. It can effectively reduce the fatality rate and improve the prognosis of patients.

ARTICLE HIGHLIGHTS

Research background

Acute liver failure (ALF) and acute on chronic liver failure (ACLF) are the most common causes of liver disease death. As an effective treatment, Artificial liver support system (ALSS) can reduce bilirubin in a short term, improve inflammatory storm, regulate immunity, which has been widely used in the treatment of ALF and ACLF. However, there is no clinical predict model could evaluate the prognosis of patients with ALF and ACLF treated with ALSS. G3BP1 could inhibit inflammatory response, and the increased expression of G3BP1 was positively correlated with the prognosis of liver failure. However, there is no correlation between G3BP1 and the prognosis for the liver failure patients treated by ALSS.

Research motivation

The significant characteristics of ALF and ACLF are rapid progression of the disease, which can lead to rapid occurrence of extrahepatic organ failure, manifested as liver coma or hepatorenal syndrome, leading to high mortality rate and extremely poor prognosis. Therefore, early judgment of prognosis and timely intervention during the progression of ALF and ACLF are of great significance to improving survival rate.

Research objectives

The purpose of this study was to verify the predictive efficacy of G3BP1 in patients with ALF and ACLF treated with ALSS, and to provide reference for the clinical development of a new prediction model. Based on the above studies, we investigated whether G3BP1 can predict the prognosis of patients with ALF and ACLF, and provide a basis for clinical decision-making programs and timing.

Research methods

A total of 244 patients with ALF and ACLF were enrolled in this study. The levels of G3BP1 on admission and at discharge were detected. The validation set of 514 patients was collected to verify the predicted effect of G3BP1 and the viability of prognosis. Univariate and multivariate Cox proportional hazards models were used to detect G3BP1, clinical biomarkers [lactate dehydrogenase (LDH), alpha-fetoprotein (AFP) and prothrombin time (PT), *etc.*], and inflammatory factors [tumor necrosis factor-α (TNF-α), interlenkin (IL)-1β and IL-18] for the risk of progression after ALSS treatment. We treated biomarkers as continuous traits (log-transformed) or as 3-level categorical variables (low, medium, and high) defined according to the tertials of the biomarker level. We developed a reference Cox proportional hazards model for progression after ALSS treatment of liver failure in the training cohort and tested whether the inclusion of biomarker levels further improved risk prediction. The random forest success rate curve model of R software was used to select the survival prognosis factors of patients in the training set. Stepwise Cox regression was used to evaluate the number of variables with the concordance index (C-index) value to obtain the best C-index value with fewer prognostic factors.

Research results

The value of difference of G3BP1 between the value of discharge and admission (difG3BP1) was then cut into two groups with 0 as the cut-off point. Group 0 had a nearly 10-fold increased risk compared to group ≥ 0. According to the Kaplan-Meier curve, survival rate in the 0-test set was lower than in the difG3BP1 ≥ 0 group ($P < 0.001$), which was also similarly in the validation set. For the subgroup analysis, group 0 presented a very high risk of progression to the disease regardless of model for end-stage liver disease scores in high-risk or low-risk groups. This indicated that G3BP1 was good for prognostic assessment. Moreover, TNF-α and IL-1β were closely related to the prognosis of liver failure before and after adjustment. However, its risk coefficient was lower when compared with G3BP1, suggesting that G3BP1 was more effective in predicting liver failure than the inflammatory factors. For the random forest prediction model, the most importance evaluation index were LDH and AFP. Compared with other indicators, G3BP1 had the highest C index value and better consistency. It was also more stable in the validation set. Modeling with AFP + LDH + G3BP1, the C index in the test set and validation set were respectively 0.84 and 0.8, which could better assist clinical practice. Therefore, the combination of G3BP1, AFP and LDH modeling ability index could well predict the endpoint of ALF and ACLF patients treated with ALSS.

Research conclusions

For the clinical characteristics and laboratory indicators of ALF and ACLF patients treated with ALSS, G3BP1, AFP, LDH, TNF-α and IL-1β were independent risk factors. G3BP1 was the most effective predictor of liver failure. The model established by G3BP1, AFP and LDH had high predictive value. The selected laboratory indexes are objective, and easy to detect, which has low cost and was simple to calculate. This model could help clinicians effectively identify the risk of death in ALF and ACLF patients treated ALSS.

Research perspectives

G3BP1 could effectively predict liver failure, which has great value to timely provide liver transplantation opportunity for patients who have failed for drug and ALSS treatment. The combination of G3BP1, AFP and LDH could accurately evaluate the disease condition and predict the clinical endpoint of patients. Based on the predicted role of G3BP1, it can effectively reduce the fatality rate and improve the prognosis of patients.

FOOTNOTES

Author contributions: Wang Y take responsibility for the integrity of the work as a whole, from inception to published article; Li WY, Wang LW, Dong J and Wang Y conceived and designed the study; Li WY and Wang LW collected and analyzed the data; Li WY and Wang LW wrote the paper; Wang Y edited the article; Li WY and Wang LW contributed equally to this article; All authors approved the final version of the manuscript.

Supported by the National Natural Science Foundation of China, No. 82100630 and No. 82100894; and the Beijing iGandan Foundation, No. iGandanF-1082022-RGG031.

Institutional review board statement: This study was approved by the Institutional Ethics Committee of Renmin Hospital of Wuhan University, No. 2022K-K215(C01).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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

Data sharing statement: All datasets generated for this study are included in the article.

STROBE statement: The authors have read the STROBE statement, and the manuscript was prepared and revised according to the STROBE statement.

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S-Editor: Zhang H
L-Editor: A
P-Editor: Yuan YY

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 264-278

DOI: 10.4254/wjh.v16.i2.264

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

Yinhuang granule alleviates carbon tetrachloride-induced liver fibrosis in mice and its mechanism

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Selamoglu Z, Turkey

Received: October 18, 2023
Peer-review started: October 18, 2023
First decision: December 26, 2023
Revised: January 9, 2024
Accepted: February 1, 2024
Article in press: February 1, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

Liver fibrosis is a formidable global medical challenge, with no effective clinical treatment currently available. Yinhuang granule (YHG) is a proprietary Chinese medicine comprising *Scutellariae* Radix and *Lonicerae Japonicae* Flos. It is frequently used for upper respiratory tract infections, pharyngitis, as well as acute and chronic tonsillitis.

AIM

To investigate the potential of YHG in alleviating carbon tetrachloride (CCl_4)-induced liver fibrosis in mice.

METHODS

To induce a hepatic fibrosis model in mice, this study involved intraperitoneal injections of 2 mL/kg of CCl_4 twice a week for 4 wk. Meanwhile, liver fibrosis mice in the low dose of YHG (0.4 g/kg) and high dose of YHG (0.8 g/kg) groups were orally administered YHG once a day for 4 wk. Serum alanine/aspartate aminotransferase (ALT/AST) activity and liver hydroxyproline content were detected. Sirius red and Masson's trichrome staining assay were conducted. Real-time polymerase chain reaction, western-blot and enzyme-linked immunosorbent assay were conducted. Liver glutathione content, superoxide dismutase activity level, reactive oxygen species and protein carbonylation amount were detected.

RESULTS

The administration of YHG ameliorated hepatocellular injury in CCl_4-treated mice, as reflected by decreased serum ALT/AST activity and improved liver histological evaluation. YHG also attenuated liver fibrosis, evident through reduced liver hydroxyproline content, improvements in Sirius red and Masson's trichrome staining, and lowered serum hyaluronic acid levels. Furthermore, YHG hindered the activation of hepatic stellate cells (HSCs) and ameliorated oxidative stress injury and inflammation in liver from CCl_4-treated mice. YHG prompted the nuclear accumulation of nuclear factor erythroid 2-related factor 2 (Nrf2) and upregulated the expression of Nrf2-dependent downstream antioxidant genes. In addition, YHG promoted mitochondrial biogenesis in liver from CCl_4-treated mice, as demonstrated by increased liver adenosine triphosphate content, mitochondrial DNA levels, and the expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha and nuclear respiratory factor 1.

CONCLUSION

YHG effectively attenuates CCl_4-induced liver fibrosis in mice by inhibiting the activation of HSCs, reducing inflammation, alleviating liver oxidative stress damage through Nrf2 activation, and promoting liver mitochondrial biogenesis.

Key Words: Yinhuang granule; Liver fibrosis; Hepatic stellate cells; Oxidative injury; Nuclear factor erythroid 2-related factor 2; Inflammation

Core Tip: Yinhuang granule (YHG), a Chinese patent medicine comprising *Scutellariae* Radix and *Lonicerae Japonicae* Flos, is traditionally employed for the management of tonsillitis, pharyngitis, as well as upper respiratory tract infections in clinical practice. Here, our study found that YHG effectively alleviated liver fibrosis in carbon tetrachloride-treated mice through various mechanisms, including the inhibition of hepatic stellate cells activation, reduction of inflammation, alleviation of liver oxidative stress damage by prompting nuclear factor erythroid 2-related factor 2 activation, and promotion of liver mitochondrial biogenesis. These findings substantiate the potential clinical use of YHG as a therapy for liver fibrosis.

Citation: Ouyang H, Miao H, Li Z, Wu D, Gao SC, Dai YY, Gao XD, Chai HS, Hu WY, Zhu JF. Yinhuang granule alleviates carbon tetrachloride-induced liver fibrosis in mice and its mechanism. *World J Hepatol* 2024; 16(2): 264-278
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/264.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.264

INTRODUCTION

Liver fibrosis is a complex process of continuous hepatic injury and subsequent tissue repair in response to various types of chronic liver insults, resulting in the pathological accumulation of extracellular matrix (ECM) components within the hepatic microenvironment[1,2]. In the absence of timely intervention, the relentless cycle of liver injury and futile regeneration persists, ultimately leading to the gradual progression of liver fibrosis into advanced cirrhosis and the potential development of hepatocellular carcinoma[1,2]. Notably, liver fibrosis can arise from diverse etiologies, encompassing viral hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis, cholestasis, autoimmune hepatitis, *etc* [3,4]. Epidemiological data suggest that liver fibrosis affects approximately 18.0%-27.0% of individuals afflicted by various chronic liver diseases[5]. Indeed, liver fibrosis represents a significant global health concern, underscored by the current absence of an efficacious pharmaceutical intervention in clinical practice.

A plethora of studies have underscored the pivotal role of hepatic stellate cells (HSCs) activation in the progression of liver fibrosis[1,6,7]. Activated HSCs manifest an exuberant production of diverse ECMs including fibronectin, proteoglycan, collagen I, and laminin, culminating in the formation of scar in liver tissue[1,7]. Furthermore, activated HSCs secrete pro-inflammatory cytokines and chemokines, thereby recruiting immune cells from the periphery into the liver, thus exacerbating hepatic inflammatory injury[8,9]. Aside from inflammation, the significance of oxidative stress-induced liver injury in the relentless progression of liver fibrosis has been underscored for decades, fostering the notion that enhancing cellular antioxidant capacity may present a promising therapeutic avenue for liver fibrosis management[10,11].

With the continuous deepening of research, there is increasing evidence that numerous traditional Chinese patent medicines, natural products and ingredients have demonstrated efficacy in effectively ameliorating liver injury and treating liver dieaseas[12-19]. Yinhuang granule (YHG) is a Chinese patent medicine comprising *Scutellariae* Radix and *Lonicerae Japonicae* Flos. Previous study has demonstrated the potential hepatoprotective effects of the individual components of YHG, with the water extract of *Lonicerae Japonicae* Flos ameliorating liver fibrosis in CCl_4-treated mice, and the methanol extract of *Scutellariae* Radix inhibiting liver fibrosis induced by bile duct ligation or CCl_4 in rats[17,18]. Additionally, baicalin and chlorogenic acid, the primary bioactive compounds within YHG, have also exhibited pro-

mising hepatoprotective effects against liver fibrosis[19-23]. Although YHG is traditionally employed for the management of chronic and acute tonsillitis or pharyngitis, as well as upper respiratory tract infections in clinical practice in China, its potential application for the therapy of liver fibrosis remains unexplored. The study aims to investigate the hepatoprotective effects of YHG against liver fibrosis induced by CCl_4 in mice and to uncover the underlying mechanisms through which YHG exerts its protective actions.

MATERIALS AND METHODS

Reagents

YHG was provided by Prof. Lili Ji, Institute of Chinese Medicine, Shanghai University of Traditional Chinese Medicine. The reagents used in this study are listed in Table 1.

Experimental animals

SPF male C57BL/6 mice (20 ± 2 g), obtained from the Shanghai Experimental Animal Center of Chinese Academy of Sciences, were kept at a controlled environment, and received humane care following the institutional animal care guidelines approved by the Experimental Animal Ethical Committee of Shanghai University of Traditional Chinese Medicine (Approval No. PZSHUTCM190912010).

Mice were divided into 5 groups (n = 6 per group) including control group, CCl_4 model group, CCl_4+YHG (0.4 g/kg) group, CCl_4+YHG (0.8 g/kg) group, YHG (0.8 g/kg) group. YHG (dissolved in 0.5% CMC-Na solution) was orally administered to mice every day, and CCl_4 (mixed 1:3 in olive oil, 2 mL/kg) was i.p. injected into mice twice a week for a total of 4 wk. The selection of the CCl_4 dose followed a previous study[24]. Following the treatment period, the mice were euthanized, and samples were collected for subsequent analysis.

Liver histological observation

Liver samples were sectioned and stained with H&E, Sirius red and Masson's trichrome for histological evaluation of liver injury and hepatic collagen deposition.

Measurement of Serum alanine/aspartate aminotransferase activity, liver hydroxyproline content, glutathione, adenosine triphosphate, superoxide dismutase, activity protein carbonylation amounts and Enzyme-linked immunosorbent assay

We performed these experiments following the manufacturer's instructions.

Hepatic reactive oxygen species amount analysis

Hepatic reactive oxygen species (ROS) level was measured previously described[25].

Mitochondrial DNA extraction

Mitochondrial DNA was extracted following the manufacturer's instruction.

Real-time polymerase chain reaction analysis

Real-time polymerase chain reaction was performed as previously described[25]. The primer sequences are shown in Table 2.

Western-blot analysis

Western-blot was detected as previously described[25]. The quantification of protein bands was standardized by calculating the average ratio of integrated optical density. Internal controls such as β-actin or Lamin B1 expression were used for normalization, and further standardized to the control group.

Statistical analysis

The data is presented as the mean ± SEM. Group differences were assessed using non-parametric one-way The Analysis of Variance (ANOVA), followed by the least significant difference post hoc test when ANOVA indicated a significant F-value and homogeneity of variance. In cases where homogeneity of variance was not met, the Mann-Whitney U non-parametric ANOVA was employed. Statistical significance was set at $P < 0.05$.

RESULTS

YHG reduced liver injury induced by CCl_4 in mice.

As depicted in Figure 1A, YHG (0.4, 0.8 g/kg) effectively decreased the elevated serum alanine aminotransferase (ALT) activity in CCl_4-treated mice. Furthermore, YHG at a dosage of 0.8 g/kg also effectively decreased the elevated serum aspartate aminotransferase (AST) activity in CCl_4-treated mice (Figure 1B). Notably, YHG (0.8 g/kg) did not exert any impact on ALT or AST activity alone (Figure 1A and B). Evaluation of liver histology unveiled that CCl_4 administration

Table 1 List of Reagents

Reagents	Company
Anti-Lamin B1	Hangzhou Hua-An Biotechnology Co., Ltd. (Hangzhou, China)
Anti-α-SMA	Cell Signaling Technology (Danvers, MA, United States)
Anti-NRF1	Cell Signaling Technology (Danvers, MA, United States)
Anti-β-actin	Cell Signaling Technology (Danvers, MA, United States)
Anti-Nrf2	Gene Tex Inc. (Alton Parkway lrvine, CA, United States)
Anti-GCLC	Santa Cruz (Santa Cruz, CA, United States)
Anti-NQO1	Abways Technology, Inc. (Shanghai, China)
Anti-GCLM	Abways Technology, Inc. (Shanghai, China)
Anti-PGC1	Abcam (Shanghai, China)
Kits for detecting ALT/AST activity	Nanjing Jiancheng Bioengineering Institute (Nanjing, China)
Kits for detecting liver hydroxyproline content	Nanjing Jiancheng Bioengineering Institute (Nanjing, China)
Kits for detecting hepatic GSH	Nanjing Jiancheng Bioengineering Institute (Nanjing, China)
Kits for detecting protein carbonylation amount	Nanjing Jiancheng Bioengineering Institute (Nanjing, China)
Kits for detecting SOD activity	Nanjing Jiancheng Bioengineering Institute (Nanjing, China)
ATP content	Beyotime Biotech (Shanghai, China)
ELISA kits	RapidBio (West Hills, CA, United States)
mitochondrial DNA extraction	Sangon Biotech (Shanghai, China)
NE-PER nuclear and cytoplasmic extraction reagents	Thermo Fisher Scientific (Waltham, MA, United States)
BCA Protein Assay Kits	Thermo Fisher Scientific (Waltham, MA, United States)
PrimeScript Master Mix	Takara (Shiga, Japan)
SYBR Premix Ex Taq	Takara (Shiga, Japan)
Trizol reagent	Life Technology (Carlsbad, CA, United States)
2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA)	Life Technology (Carlsbad, CA, United States)

Other reagents unless indicated were obtained from Sigma Chemical Co. (St. Louis, MO, United States).

induced obvious liver injury in mice, which was characterized by immune cell infiltration, as well as hepatocyte swelling and necrosis (Figure 1C). However, YHG (0.4, 0.8 g/kg) effectively alleviated these pathological changes.

YHG reduced hepatic collagen deposition and the increased serum hyaluronic acid content in CCl_4-treated mice

As shown in Figure 2A, YHG (0.8 g/kg) decreased the increased hydroxyproline content in liver of CCl_4-treated mice. Additionally, YHG (0.4, 0.8 g/kg) significantly reduced the increased serum hyaluronic acid levels induced by CCl_4 (Figure 2B). YHG (0.8 g/kg) alone did not affect liver hydroxyproline content or serum hyaluronic acid levels (Figure 2A and B). Furthermore, as depicted in Figure 2C and D, the treatment with YHG (0.4, 0.8 g/kg) effectively decreased hepatic collagen deposition in CCl_4-treated mice. It's worth noting that YHG (0.8 g/kg) alone did not induce any significant changes in the staining patterns, as demonstrated by Masson's trichrome staining and Sirius red staining.

YHG reduced HSCs activation in CCl_4-treated mice

Figure 3A illustrated that YHG (0.4, 0.8 g/kg) significantly reduced the enhanced hepatic of Col1a1, Col3a1, and fibronectin (Fn1) mRNA expression in CCl_4-treated mice. Additionally, YHG (0.4, 0.8 g/kg) significantly attenuated the increased hepatic mRNA expression of transforming growth factor (TGF)-β in CCl_4-induced mice (Figure 3B). The typical biomarker for HSCs activation, alpha-smooth muscle actin (α-SMA), showed reduced hepatic mRNA and protein expression upon treatment with YHG (0.4, 0.8 g/kg) in CCl_4-treated mice (Figure 3B-D).

YHG ameliorated hepatic oxidative stress damage and inflammation induced by CCl_4 in mice.

As demonstrated in Figure 4A, CCl_4 caused a decline in hepatic glutathione (GSH) content in mice, which was reversed by YHG (0.4, 0.8 g/kg). Furthermore, as depicted in Figure 4B and C, YHG (0.4, 0.8 g/kg) effectively reduced the increased levels of hepatic ROS and liver protein carbonylation in CCl_4-induced mice. Moreover, CCl_4 decreased hepatic superoxide dismutase (SOD) activity in mice, which was restored by YHG (0.8 g/kg) (Figure 4D). Additionally, Figure 4E

Table 2 List of Primers for real-time polymerase chain reaction

Target	Primer	Sequence (5'-3')
Col1a1	FP	TGACTGGAAGAGCGGAGAGT
	RP	GACGGCTGAGTAGGGAACAC
Col3a1	FP	ATGGGTTTCCCTGGTCCTAA
	RP	TGCCTTGTAATCCTTGTGGA
Fn1	FP	AGAACCAGAGGAGGCACAAG
	RP	CCGTGTAAGGGTCAAAGCAT
Tgfb1	FP	TGCCCTCTACAACCAACACA
	RP	GTTGGACAACTGCTCCACCT
Acta2	FP	GGGAGTAATGGTTGGAATGG
	RP	GGTGATGATGCCGTGTTCTA
TNFα	FP	AGGCACTCCCCCAAAAGAT
	RP	CAGTAGACAGAAGAGCGTGGTG
IL-1β	FP	AGTTGACGGACCCCAAAAG
	RP	CTTCTCCACAGCCACAATGA
IL-6	FP	ACAAAGCCAGAGTCCTTCAGAGAG
	RP	TTGGATGGTCTTGGTCCTTAGCC
iNOS	FP	CAGGCGGTGCCTATGTCTC
	RP	CAGCTGGGCTGTACAAACCTT
Nqo1	FP	CTCGTGGAGACGCTTTACAT
	RP	CGTTTCTTCCATCCTTCCAG
Gclc	FP	CGGAGGAACGATGTCTGAGT
	RP	CTGGGGAATGAAGTGATGGT
Gclm	FP	CAATGACCCGAAAGAACTGC
	RP	CAATGACCCGAAAGAACTGC
Actin	FP	TTCGTTGCCGGTCCACACCC
	RP	GCTTTGCACATGCCGGAGCC
18s	FP	CGCGGTTCTATTTTGTTGGT
	RP	AGTCGGCATCGTTTATGGTC
ND1	FP	CTAGCAGAAACAAACCGGGC
	RP	CCGGCTGCGTATTCTACGTT

FP: Forward primer; RP: Reverse primer; TNF: Tumour necrosis factor; IL: Interleukin; iNOS: Inducible nitric oxide synthase.

shows that YHG (0.4, 0.8 g/kg) suppressed the hepatic mRNA expression of tumour necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, and inducible nitric oxide synthase (iNOS) in mice treated with CCl_4.

YHG induced the activation of nuclear factor erythroid 2-related factor 2 antioxidant signaling pathway in CCl_4-treated mice

As demonstrated in Figure 5A and B, YHG (0.8 g/kg) promoted the nuclear accumulation of nuclear factor erythroid 2-related factor 2 (Nrf2) in livers from mice exposed to CCl_4. Additionally, YHG (0.8 g/kg) increased hepatic mRNA expression of glutamate-cysteine ligase (GCLC), modifier subunit of glutamate-cysteine ligase (GCLM) and NAD(P)H:quinone oxidoreductase-1 (NQO1). Furthermore, YHG (0.4 g/kg) also elevated mRNA expression of GCLM in livers of mice exposed to CCl_4 (Figure 5C). Notably, YHG (0.8 g/kg) increased the hepatic protein expression of GCLC, GCLM, and NQO1 in livers of mice exposed to CCl_4 (Figure 5D and E).

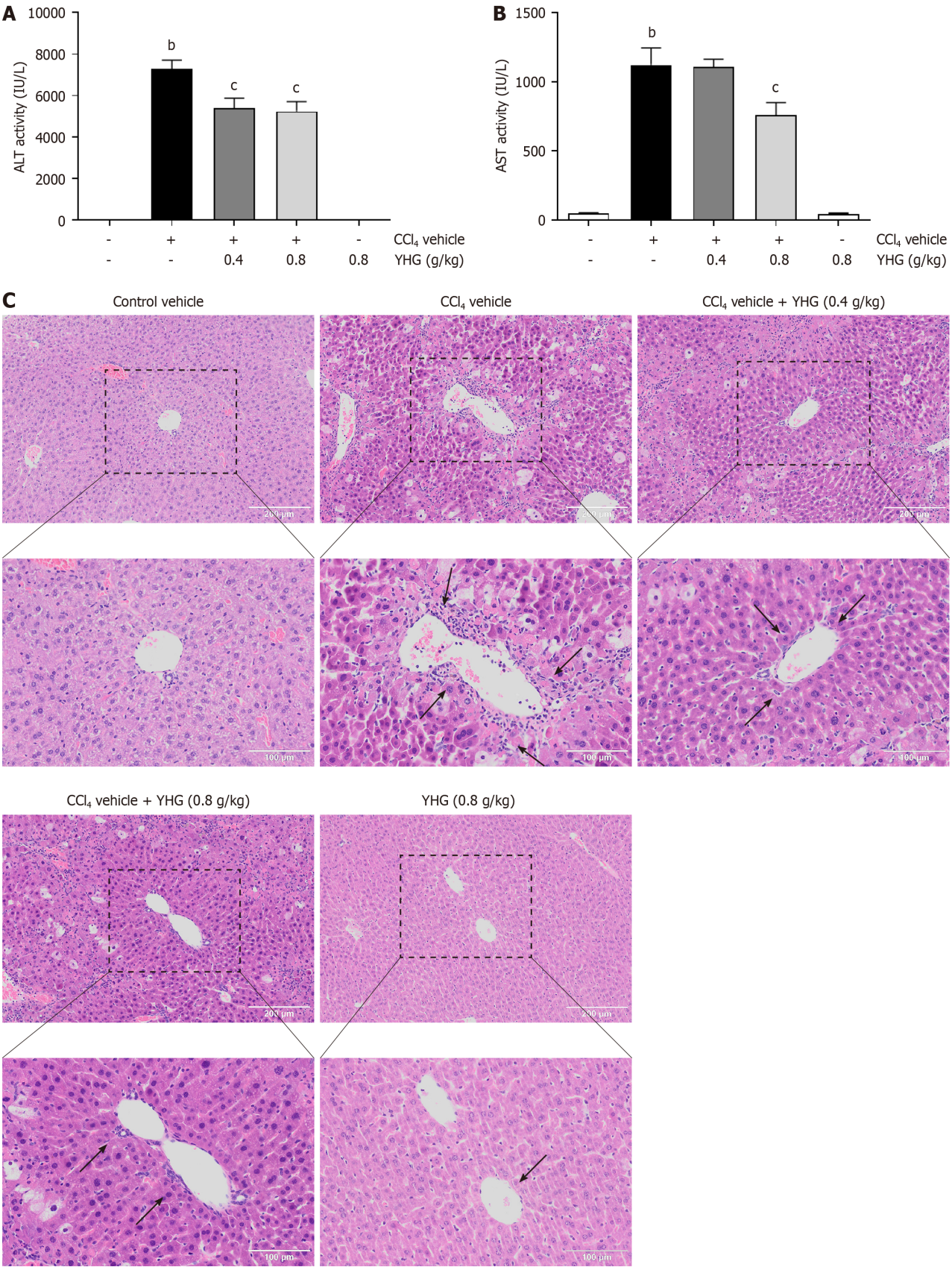
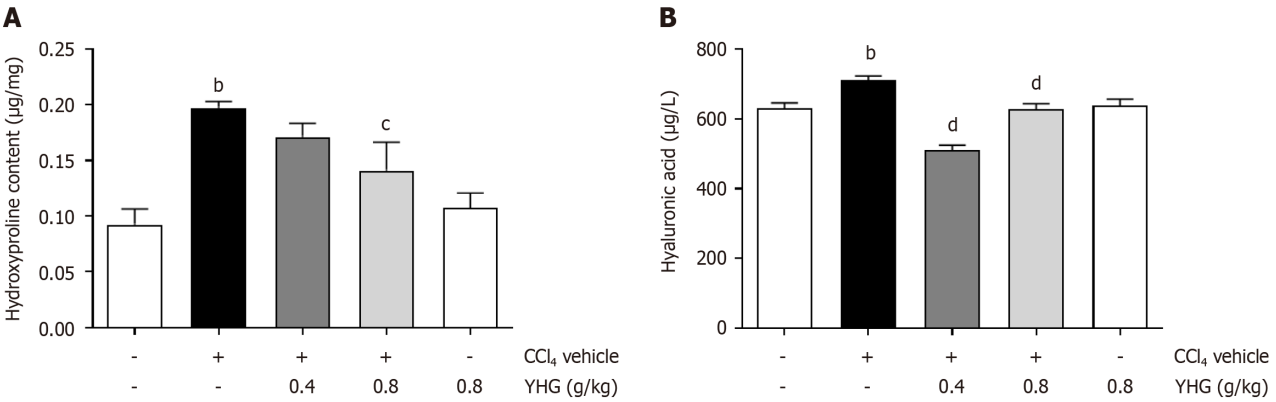
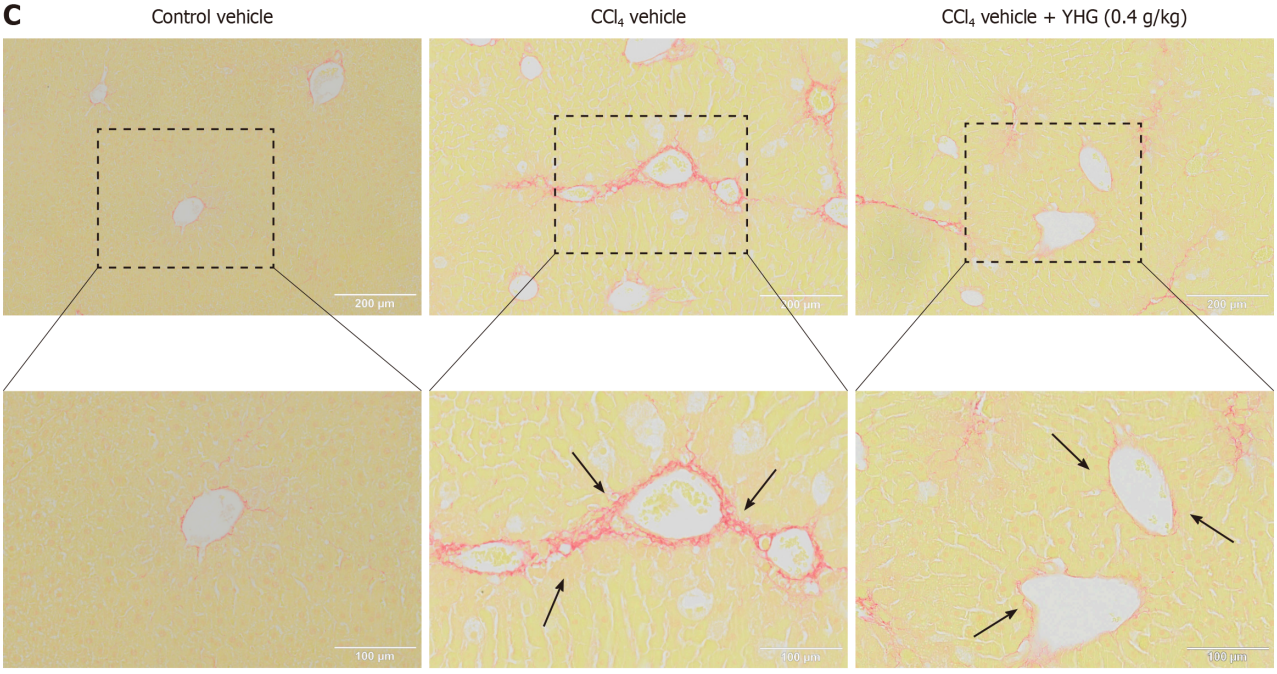
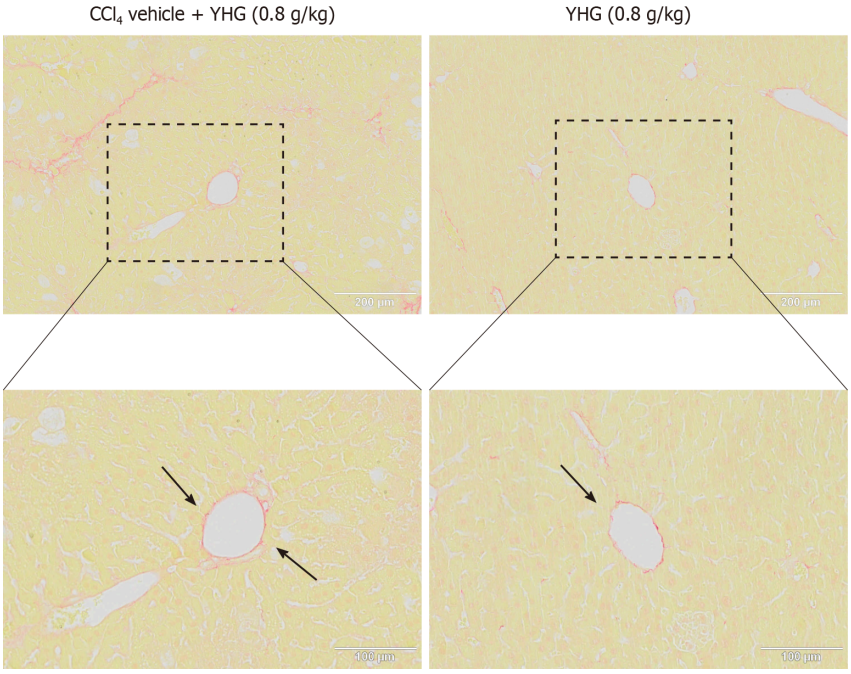


Figure 1 Effects of Yinhuang granule on serum activities of alanine/aspartate aminotransferase and liver histological evaluation. A: Serum alanine aminotransferase activity; B: Serum aspartate aminotransferase activity; C: Liver H&E staining. Arrows indicate hepatic infiltration of immune cells, swelling and necrosis of hepatocytes. Typical images were chosen from each experimental group. (Original magnification ×100, upper images; partial enlarged pictures, down images). Data are expressed as mean ± SEM (n = 5). $^{b}P < 0.01$ *vs* control vehicle; $^{c}P < 0.05$ *vs* CCl_4 vehicle. CCl_4: Carbon tetrachloride. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.


A
Hydroxyproline content (μg/mg)
0.25
0.20
0.15
0.10
0.05
0.00
b
c
- + + + - CCl4 vehicle
- - 0.4 0.8 0.8 YHG (g/kg)
B
Hyaluronic acid (μg/L)
800
600
400
200
0
b
d
d
- + + + - CCl4 vehicle
- - 0.4 0.8 0.8 YHG (g/kg)


C
Control vehicle
CCl4 vehicle
CCl4 vehicle + YHG (0.4 g/kg)
200 μm
100 μm


CCl4 vehicle + YHG (0.8 g/kg)
YHG (0.8 g/kg)
200 μm
100 μm

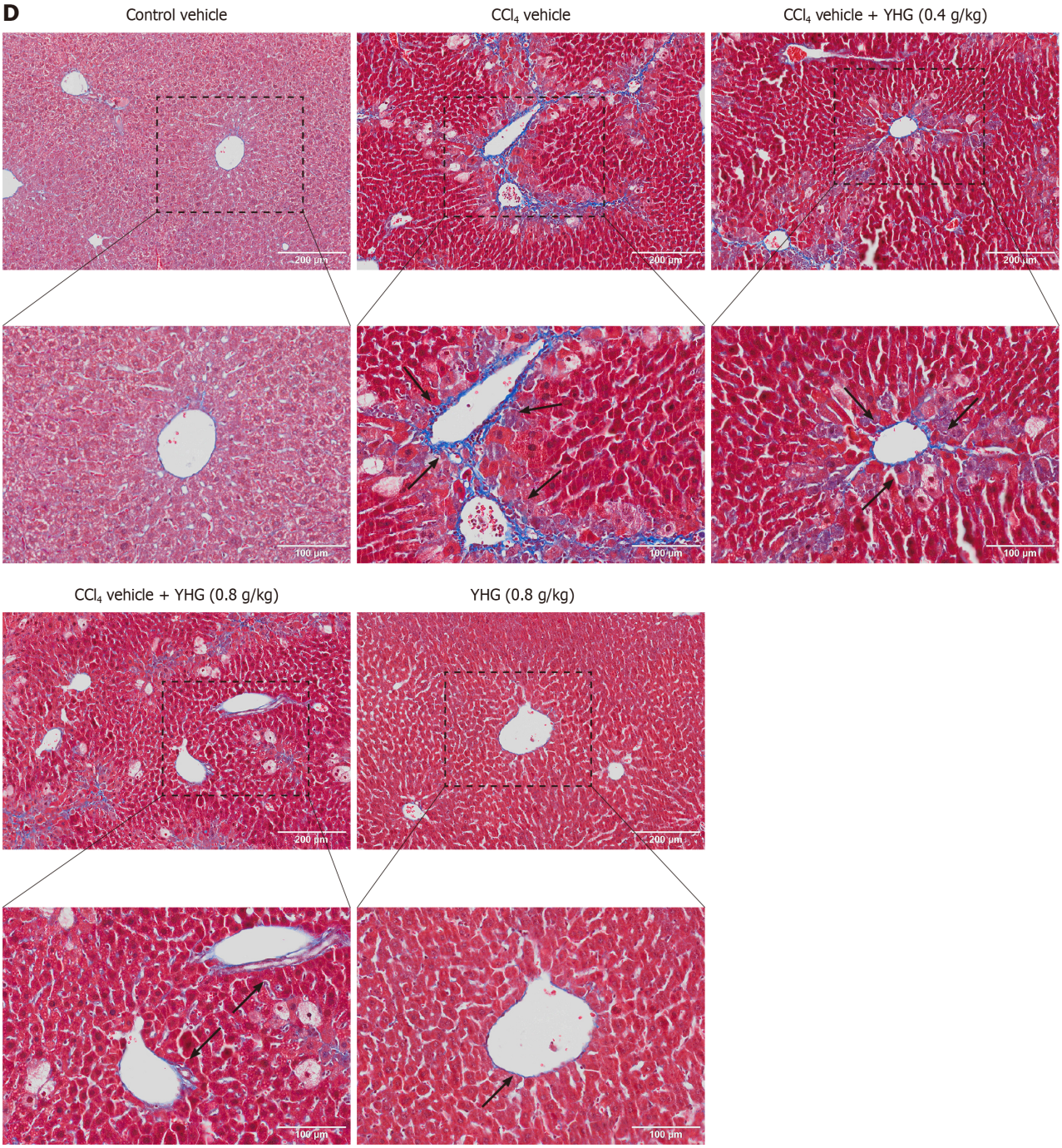


Figure 2 Yinhuang granule decreased liver hydroxyproline content and hepatic collagen expression in carbon tetrachloride-treated mice.
A: Liver hydroxyproline content (n = 6); B: Serum content of hyaluronic acid (n = 6); C: Liver Masson's trichrome staining. Arrows indicate collagen disposition; D: Liver Sirius red staining. Arrows indicate collagen disposition. (Original magnification ×100, upper images; partial enlarged pictures, down images). Data were expressed as mean ± SEM. $^{b}P < 0.01$ *vs* control vehicle; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* carbon tetrachloride vehicle. CCl_4: Carbon tetrachloride; YHG: Yinhuang granule.

YHG induced mitochondrial biogenesis in livers from CCl_4-treated mice

As depicted in Figure 6A, YHG (0.4, 0.8 g/kg) obviously elevated the decreased expression of hepatic mitochondrial DNA (mtDNA) copy in liver from mice exposed to CCl_4. Additionally, YHG (0.4, 0.8 g/kg) significantly increased adenosine triphosphate (ATP) content in liver from CCl_4-treated mice (Figure 6B). Furthermore, YHG (0.4, 0.8 g/kg) elevated the reduced hepatic expression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1α) protein, while YHG (0.8 g/kg) enhanced the decreased expression of nuclear respiratory factor1 (NRF1) protein in livers from CCl_4-induced mice (Figure 6C and D).

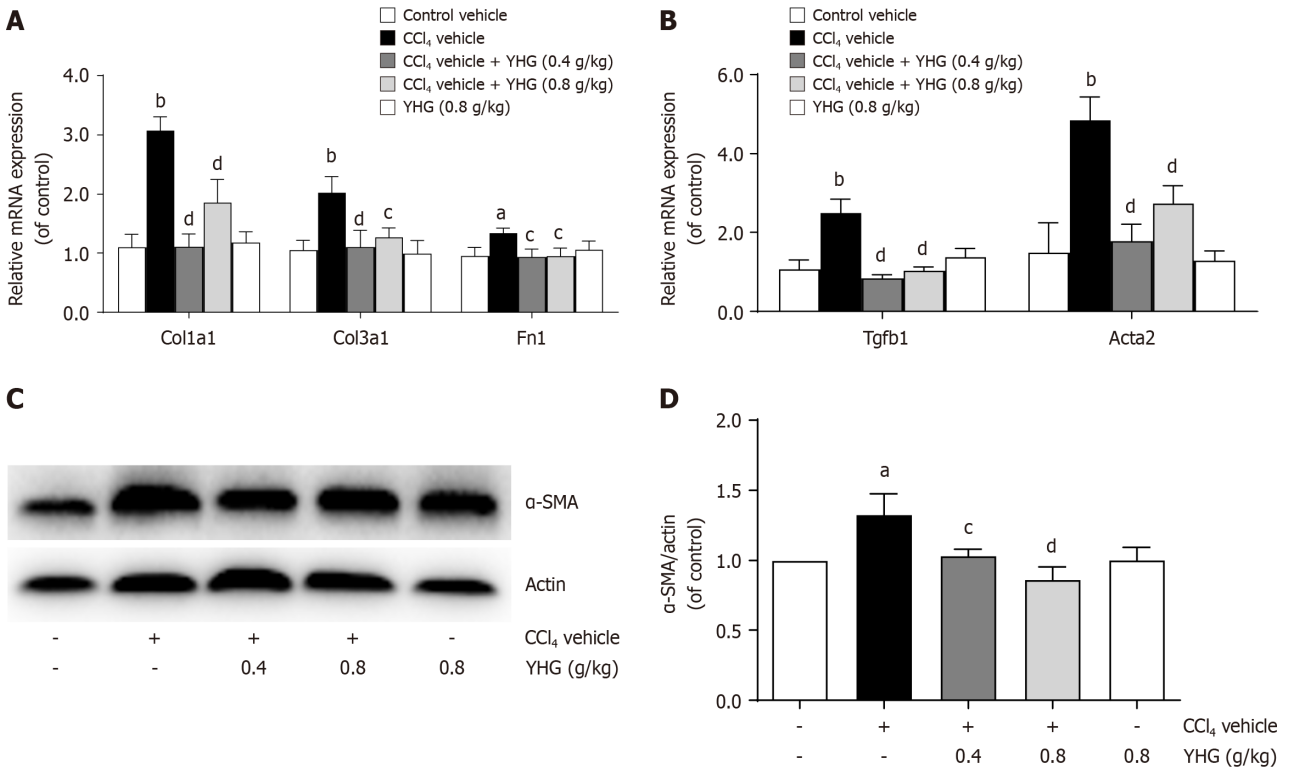


Figure 3 Yinhuang granule decreased hepatic stellate cells activation in carbon tetrachloride-treated mice. A: Hepatic mRNA expression of Col1a1, Col3a1 and Fn1 (n = 5-6); B: Hepatic mRNA expression of Tgfb1 [transforming growth factor (TGF)-β] and Acta2 [α-smooth muscle actin (α-SMA)] (n = 6); C: The expression of liver α-SMA protein was detected by Western blot, and β-actin was used as a loading control. The results represent four independent experiments; D: The protein bands of α-SMA were normalized to basal β-actin expression (n = 4). Data were expressed as mean ± SEM. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control vehicle; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* carbon tetrachloride vehicle. CCl_4: Carbon tetrachloride; YHG: Yinhuang granule.

DISCUSSION

YHG has excellent anti-inflammatory capacity and is generally used in clinic for clearing hotness and wind, and pharyngeal detoxification. In this study, YHG was demonstrated to alleviate hepatocellular injury, hepatic collagen deposition, and inflammation in CCl_4-treated mice. It also showed inhibitory effects on HSCs, as evidenced by the reduction in the elevated hepatic expression of α-SMA, a key indicating HSCs transdifferentiation and activation[26]. The enhanced expression of ECM components including Col1a1, Col3a1, and Fn1 in the livers of CCl_4-treated mice was decreased by YHG. Furthermore, YHG reduced the elevated expression of TGFβ, a predominant pro-fibrogenic molecule [27], in the livers of CCl_4-treated mice. These findings collectively highlight the immense potential of YHG in the clinical treatment of liver fibrosis.

Recent studies have discovered novel pathways and signals that play significant roles in regulating the activation of HSCs during the progression of liver fibrosis, including oxidative stress and inflammatory responses[28]. Oxidative stress is characterized by an imbalance between the production of ROS and the antioxidant system's ability to scavenge these harmful molecules. Free radicals generated during oxidative stress have been shown to induce the activation and proliferation of HSCs[29,30]. In this study, YHG was found to reduce the elevated hepatic levels of ROS and protein carbonylation, as well as restore the diminished hepatic GSH content and SOD activity in mice treated with CCl_4. Furthermore, YHG was found to reduce the elevated hepatic expression of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6, and iNOS. These findings collectively suggest that YHG has the ability to alleviate hepatic oxidative stress injury and inflammatory response in CCl_4-treated mice, which may contribute to its potential in alleviating CCl_4-induced liver fibrosis in mice.

Nrf2 serves as the principal transcription factor that plays a crucial role in regulating the expression of various downstream antioxidant enzymes and cytoprotective genes[31]. Numerous studies have demonstrated that enhancing Nrf2 activation to combat liver oxidative stress injury is crucial for alleviating liver fibrosis, as observed with various natural compounds such as schisandrin B, asiatic acid, Xiaochaihutang, stevia, tanshinol, and hyperoside[32-37]. In CCl_4-treated mouse livers, the nuclear accumulation of Nrf2 was decreased, but YHG was able to rescue this reduction. GCLC, GCLM, and NQO1 are known as downstream antioxidant enzymes regulated by Nrf2[38]. The elevated hepatic expression of GCLC, GCLM, and NQO1 in CCl_4-treated mice following YHG administration indicates that YHG activates the transcription of Nrf2. The activation of Nrf2 is likely responsible for the protection against CCl_4-induced oxidative stress damage in the livers in these mice. Nrf2-regulated genes, such as those involved in the synthesis of GCLC, GCLM and NQO1, are crucial for combating oxidative stress and maintaining liver health.

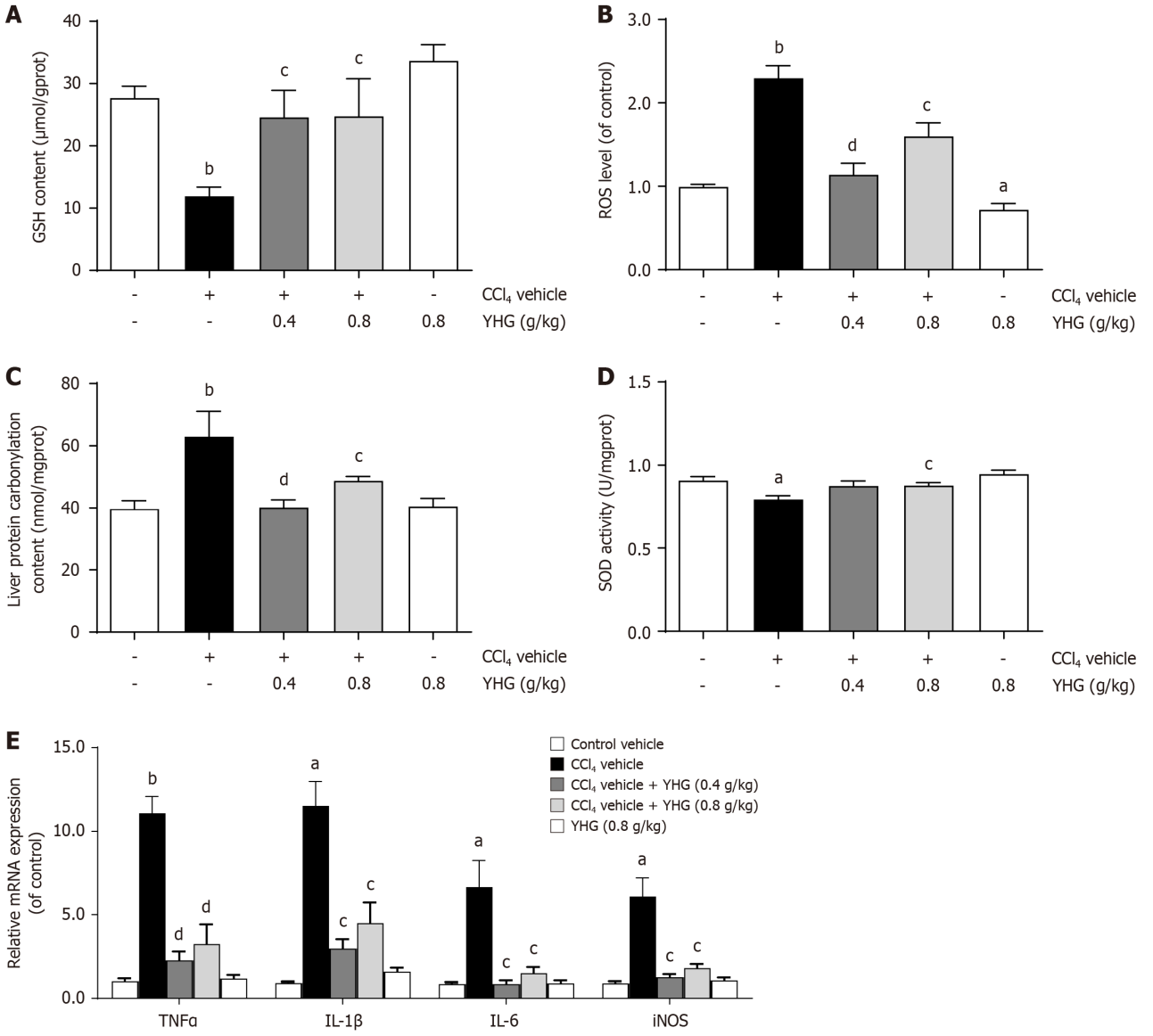


Figure 4 Yinhuang granule ameliorated hepatic oxidative stress damage and inflammation induced by carbon tetrachloride in mice. A: Liver glutathione content (n = 6); B: Liver reactive oxygen species level (n = 6); C: Liver protein carbonylation content (n = 6); D: Liver superoxide dismutase activity (n = 5); E: Hepatic mRNA expression of tumour necrosis factor alpha, interleukin (IL)-1b, IL-6 and inducible nitric oxide synthase (n = 4-5). Data were expressed as mean ± SEM. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control vehicle; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* carbon tetrachloride vehicle. CCl_4: Carbon tetrachloride; YHG: Yinhuang granule; GSH: Liver glutathione; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TNF: Tumour necrosis factor; IL: Interleukin; iNOS: Inducible nitric oxide synthase.

Mitochondria play a core role in the production of energy and cellular metabolism, and their dysfunction can lead to a range of health issues. To maintain mitochondrial health and overall cellular function, a balance between mitochondrial turnover, fission and fusion processes, and the promotion of mitochondrial biogenesis is indeed crucial. Mitochondrial biogenesis involves the generation of the new mitochondria to replace damaged ones and maintain cellular energy production. This process helps ensure that cells have a healthy population of mitochondria and can effectively meet their energy demands[39]. Recent studies have shown that inducing mitochondrial biogenesis is beneficial in alleviating liver fibrosis in rats with secondary biliary cirrhosis or treated with carbon tetrachloride[40,41], as well as in mice with diet-induced obesity and non-alcoholic steatohepatitis[42]. Additionally, resveratrol has been reported to induce HSCs death through apoptosis, autophagy/mitophagy, and mitochondrial biogenesis[43]. The transcription of mtDNA holds a pivotal role in the process of mitochondrial biogenesis, and PGC1α and NRF1 tightly regulate this mechanism[39,44]. Furthermore, Nrf2 not only assumes a central role in protecting against oxidative stress injury but also enhances the structural and functional integrity of mitochondria under stress conditions[45]. It has been reported that Nrf2 enhances the expression of NRF1 by binding to its promoter sites[46]. In this study, YHG was found to enhance hepatic ATP levels, increase the reduced mtDNA content, and improve the decreased expression of PGC1α and NRF1 in CCl_4-treated mice. These findings imply that YHG promotes mitochondrial biogenesis in CCl_4-induced liver fibrosis in mice, which contributes to its protective effects against liver fibrosis.

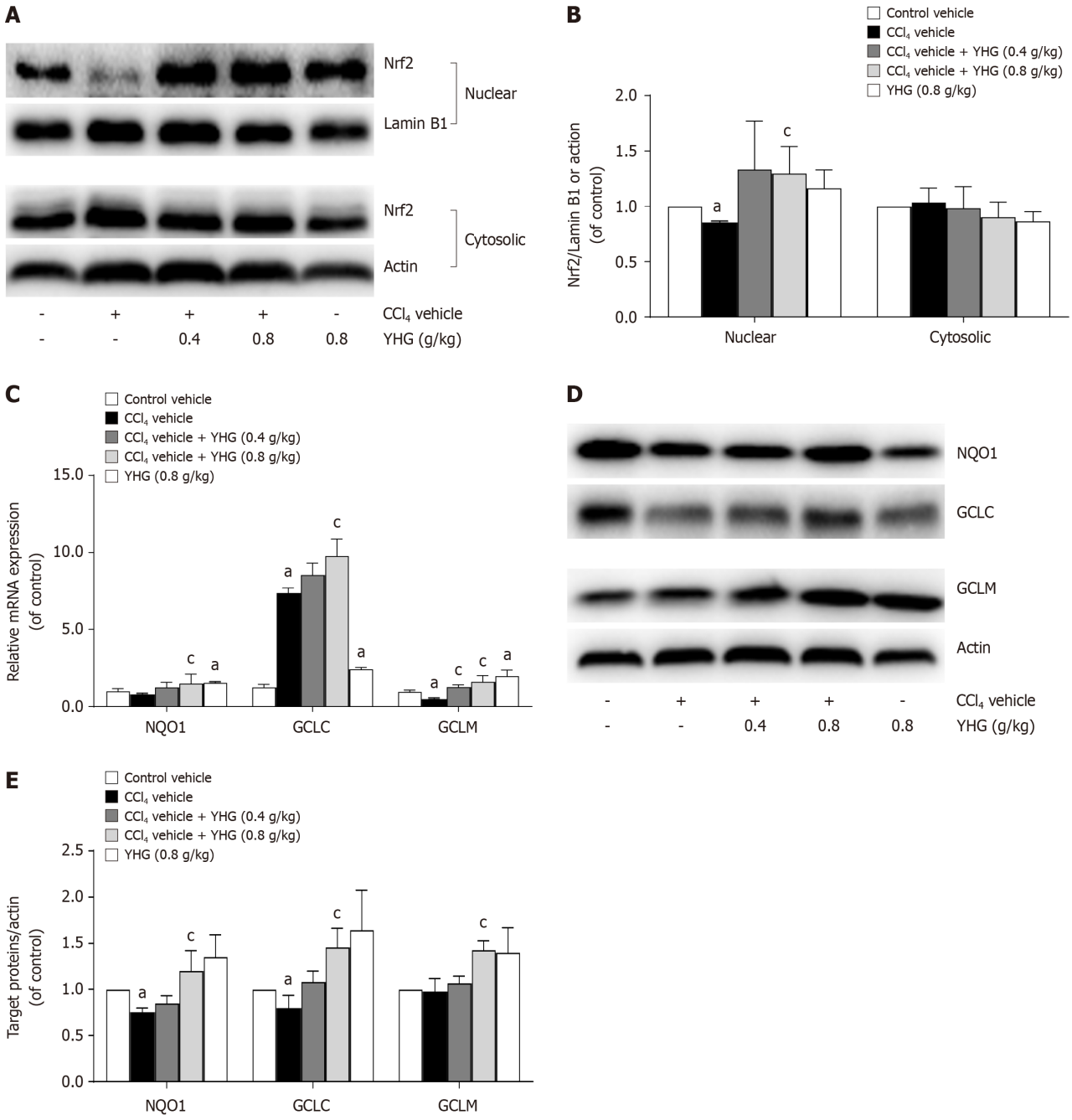


Figure 5 Yinhuang granule induced the activation of hepatic nuclear factor erythroid 2-related factor 2 antioxidant signaling pathway in carbon tetrachloride-treated mice. A: The expression of liver hepatic nuclear factor erythroid 2-related factor 2 (Nrf2) was detected by Western blot, and b-actin and Lamin B1 were used as loading controls. The results represent at least three independent experiments; B: The protein bands of Nrf2 were normalized to basal b-actin or Lamin B1 expression (n = 3-4); C: Hepatic mRNA expression of NAD(P)H:quinone oxidoreductase-1 (NQO1), glutamate-cysteine ligase (GCLC) and modifier subunit of glutamate-cysteine ligase (GCLM) (n = 3); D: The expression of liver NQO1, GCLC and GCLM was detected by Western blot, and b-actin was used as a loading control. The results represent at least three independent experiments; E: The protein bands of NQO1, GCLC and GCLM were normalized to basal b-actin expression (n = 3-4). Data were expressed as mean ± SEM. $^{a}P < 0.05$ *vs* control vehicle; $^{c}P < 0.05$ *vs* carbon tetrachloride vehicle. CCl_4: Carbon tetrachloride; YHG: Yinhuang granule; Nrf2: Nuclear factor erythroid 2-related factor 2; NQO1: NAD(P)H:quinone oxidoreductase 1; GCLC: Glutamate-cysteine ligase; GCLM: Glutamate-cysteine ligase.

CONCLUSION

YHG effectively alleviated liver fibrosis induced by CCl_4 in mice *via* various mechanisms, including the inhibition of HSCs activation, reduction of inflammation, alleviation of liver oxidative stress damage by promoting Nrf2 activation, and promotion of liver mitochondrial biogenesis. These findings suggest that YHG has immense promise for clinical utilization in the management of liver fibrosis.

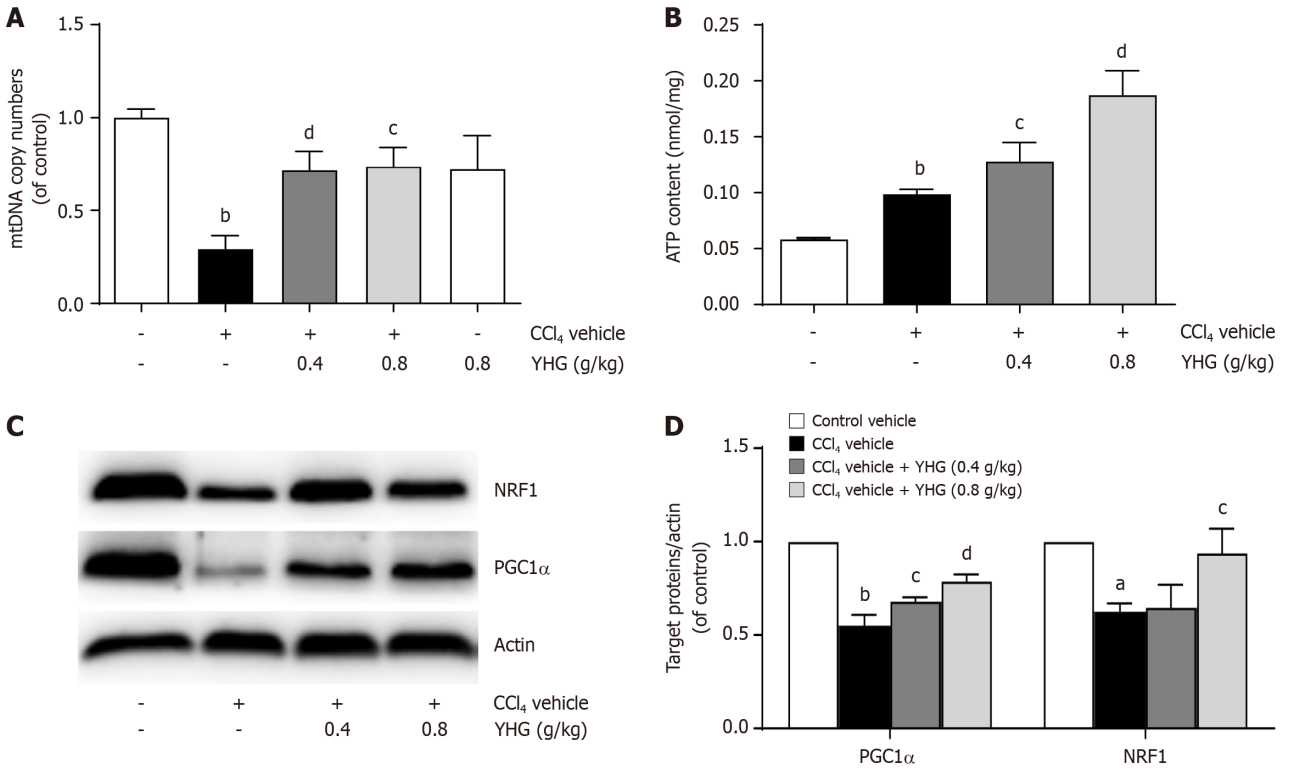


Figure 6 Yinhuang granule induced mitochondrial biogenesis in carbon tetrachloride-treated mice. A: Liver mitochondrial DNA (mtDNA) copy numbers (n = 5); B: Liver adenosine triphosphate level (n = 5); C: The expression of liver peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) and nuclear respiratory factor 1 (NRF1) was detected by Western blot, and b-actin was used as a loading control. The results represent three independent experiments; D: The protein bands of PGC1a and NRF1 were normalized to basal b-actin expression (n = 3). Data were expressed as mean ± SEM. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control vehicle; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* carbon tetrachloride vehicle. ATP: Adenosine triphosphate; CCl_4: Carbon tetrachloride; YHG: Yinhuang granule; PGC1α: Proliferator-activated receptor gamma coactivator 1 alpha; NRF1: Nuclear respiratory factor 1.

ARTICLE HIGHLIGHTS

Research background

Liver fibrosis is a formidable global medical challenge, with no effective clinical treatment currently available. Yinhuang granule (YHG) is a proprietary Chinese medicine comprising *Scutellariae* Radix and *Lonicerae Japonicae* Flos. However, its pharmacological mechanism is still unclear.

Research motivation

To investigate the potential of YHG in alleviating liver fibrosis in mice.

Research objectives

To investigate the potential of YHG against liver fibrosis in mice through *in vivo* and *in vitro* experiments.

Research methods

Liver fibrosis model mice were generated by intraperitoneal injections of 2 mL/kg of carbon tetrachloride (CCl_4) twice a week for 4 wk. Liver fibrosis mice in the low dose of YHG (0.4 g/kg) and high dose of YHG (0.8 g/kg) groups were orally administered YHG once a day for 4 wk. Serum alanine/aspartate aminotransferase activity and liver hydroxyproline content were detected. Sirius red and Masson's trichrome staining assay were conducted. Real-time polymerase chain reaction, western-blot and enzyme-linked immunosorbent assay were conducted. Liver glutathione content, superoxide dismutase activity level, reactive oxygen species and protein carbonylation amount were detected.

Research results

YHG ameliorated hepatocellular injury and liver fibrosis in CCl_4-treated mice. YHG inhibited hepatic stellate cells (HSCs) activation, alleviated oxidative stress, inhibited inflammation, and promoted mitochondrial biogenesis.

Research conclusions

YHG effectively attenuates CCl_4-induced liver fibrosis in mice by inhibiting the activation of HSCs, reducing inflammation, alleviating liver oxidative stress damage through Nrf2 activation, and promoting liver mitochondrial biogenesis.

Research perspectives

Further investigation into the mechanism of YHG against liver fibrosis is necessary.

FOOTNOTES

Author contributions: Ouyang H, Miao H, Li Z, Wu D, Gao SC, Dai YY, Gao XD, Chai HS, Hu WY, Zhu JF designed and coordinated the study; Ouyang H, Miao H performed the experiments, acquired and analyzed data; Ouyang H, Miao H, Li Z, Wu D, Gao SC, Dai YY, Gao XD, Chai HS, Hu WY, interpreted the data and discussed the results; Ouyang H and Zhu JF wrote the manuscript.

Supported by Preclinical Study of A New Chinese Herbal Medicine for the Treatment of Ascites of Liver Cirrhosis (Spleen and Kidney Yang Deficiency Type) with the Clinical Formula of Qigui Xiaogu Cataplasm, No. 23S21900100; Traditional Chinese Medicine/Chinese and Western Medicine Advantage Specialty Construction Specialty for Department of Hepatology, No. YW(2023-2024)-01-03; National Natural Science Foundation of China, No 82074386; Construction of Special Disease Alliance of Traditional Chinese Medicine in East China Area and Municipal Level, Shanghai Special Disease Alliance of Traditional Chinese Medicine for Liver Cirrhosis Ascites (Water sickness), and Clinical Research Plan of SHDC, No. SHDC2020CR3095B; and National Funded Postdoctoral Researcher Program, No. GZB20230448.

Institutional animal care and use committee statement: The study was reviewed and approved by the institutional animal care guidelines approved by the Experimental Animal Ethical Committee of Shanghai University of Traditional Chinese Medicine (Approval No. PZSHUTCM190912010).

Informed consent statement: Consent was not needed as the study without exposure to the patients' data.

Conflict-of-interest statement: The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country/Territory of origin: China

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S-Editor: Liu JH
L-Editor: A
P-Editor: Zheng XM

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 279-285

DOI: 10.4254/wjh.v16.i2.279

ISSN 1948-5182 (online)

CASE REPORT

Coinfection with hepatic cystic and alveolar echinococcosis with abdominal wall abscess and sinus tract formation: A case report

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Oprea V, Romania

Received: November 16, 2023
Peer-review started: November 16, 2023
First decision: December 25, 2023
Revised: December 29, 2023
Accepted: January 25, 2024
Article in press: January 25, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

Hepatic cystic and alveolar echinococcosis coinfections, particularly with concurrent abscesses and sinus tract formation, are extremely rare. This article presents a case of a patient diagnosed with this unique presentation, discussing the typical imaging manifestations of both echinococcosis types and detailing the diagnosis and surgical treatment experience thereof.

CASE SUMMARY

A 39-year-old Tibetan woman presented with concurrent hepatic cystic and alveolar echinococcosis, accompanied by abdominal wall abscesses and sinus tract formation. Initial conventional imaging examinations suggested only hepatic cystic echinococcosis, but intraoperative and postoperative pathological examination revealed the coinfection. Following radical resection of the lesions, the patient's condition improved, and she was discharged soon thereafter. Subsequent outpatient follow-ups confirmed no recurrence of the hydatid lesion and normal surgical wound healing. Though mixed hepatic cystic and alveolar echinococcosis with abdominal wall abscesses and sinus tract formations are rare, the general treatment approach remains consistent with that of simpler infections of alveolar echinococcosis.

CONCLUSION
Lesions involving the abdominal wall and sinus tract formation, may require radical resection. Long-term prognosis includes albendazole and follow-up examinations.

Key Words: Cystic echinococcosis; Alveolar echinococcosis; Abdominal wall abscess; Surgical treatment; Sinus tract; Case report

Core Tip: Echinococcosis, also known as hydatid disease, is a zoonotic parasitic disease caused by echinococcus infection, mostly parasitic in the liver. There are two common pathogenic types of hepatic echinococcosis, *Echinococcus granulosus* and *Echinococcus multilocularis*. Infection with a single species of echinococcus was common, while co-infection with two species of echinococcus was rare, accounting for only 0.92% of the patients with hepatic echinococcosis. This article introduces the diagnosis and treatment of a patient with co-infection of two types of hepatic echinococcosis, abdominal wall abscess and sinus formation.

Citation: Wang MM, An XQ, Chai JP, Yang JY, A JD, A XR. Coinfection with hepatic cystic and alveolar echinococcosis with abdominal wall abscess and sinus tract formation: A case report. *World J Hepatol* 2024; 16(2): 279-285
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/279.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.279

INTRODUCTION

Echinococcosis is prevalent in pastoral areas like northwest and southwest China, with an average prevalence of approximately 1.08% in western China[1]. Two types of hepatic echinococcosis exist: Cystic echinococcosis (CE) caused by *Echinococcus granulosus* (*E. granulosus*), and alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* (*E. multilocularis*). Clinical diagnosis relies primarily on imaging and immunological tests. Imaging typically involves abdominal ultrasonography (US) and computed tomography (CT), while immunological detection employs enzyme-linked immunosorbent assay (ELISA)[2-4]. Definitive diagnosis requires pathological examination.

Currently, no established guidelines or general consensus exists for treating coinfections with both echinococcosis types, though radical resection is widely considered optimal[5-7]. Such coinfections are rare, representing only 0.92% of all hepatic echinococcosis cases, and can be difficult to diagnose and manage[4]. To date, only five such cases have been documented at Qinghai Provincial People's Hospital, China (Table 1). Notably, only one case involved concurrent abdominal wall invasion and sinus tract formation.

This report details the diagnosis and treatment of a patient with a mixed hepatic echinococcosis infection, presenting with abdominal wall invasion and sinus tract formation, managed at the General Surgery Department of Qinghai Provincial People's Hospital.

CASE PRESENTATION

Chief complaints

A 39-year-old Tibetan woman presented to our hospital with intermittent upper abdominal pain and discomfort for over a month, worsening in the past week.

History of present illness

The patient had intermittent epigastric distension and pain in January without any obvious cause, not accompanied by nausea, vomiting, fever and other discomforts, and did not undergo formal diagnosis and treatment, and the above symptoms worsened a week ago, so the patient came to our outpatient clinic, and the outpatient clinic was admitted to the department of our department with "abdominal pain to be investigated", and the patient was in a clear state of mind since the onset of the disease, his mental state was clear, and the spirit was fine, and he had normal urination and defecation, and he did not see any significant reduction of his body weight in recent days. Since the onset of the disease, he has been in a clear state of mind, with a normal spirit, normal bowel movements and no significant weight loss.

Physical examination

Physical examination revealed normal skin, sclerae, palpebral conjunctiva, heart, and lungs. However, an abdominal microbulge, skin rupture with pus outflow, and tenderness were found 5.0 cm above the umbilicus (Figure 1A). No abdominal varices, gastrointestinal peristalsis, or swelling were observed, but umbilical secretions and decreased

Table 1 Clinical data of five patients

No.	Sex	Age (yr)	Lesion (location)	Child-Pugh score	Surgery	Postoperative complication	Case characteristics
1	Female	42	S5 and S8	A	Anatomic right hemihepatectomy	No	Surgical treatment of patients with preoperative misdiagnosis of echinococcosis and mixed infections of both types of echinococcosis should be based on ensuring the patient's surgical safety, and radical surgery should be performed to remove all lesions whenever possible
2	Female	26	S4, S5 and S8	A	Removal of the internal capsule, with subtotal excision of the external capsule the in Echinococcus granulosus lesions	Bile leakage, ascites, and bilateral pleural effusions	The patient traveled to an out-of-state hospital for autologous liver transplantation. Staged surgical treatment may be considered in end-stage patients with insufficient residual liver volume or in advanced patients who cannot be completely resected at one time, and only palliative surgery or conservative treatment is required for patients who have lost the chance of radical surgical treatment
3	Female	49	S2, S3, S7 and S8	A	Multiple segmental hepatectomy	No	Surgical treatment of patients with preoperative misdiagnosis of echinococcosis and mixed infections of both types of echinococcosis should be based on ensuring the patient's surgical safety, and radical surgery should be performed to remove all lesions whenever possible
4	Male	56	S4, S5, S6, S7 and S8	A	Extended right hemicolectomy	No	Ultrasound-guided PTCD for jaundice reduction was performed on day 2 of admission, and jaundice was reduced until surgery on day 33 after admission
5	Female	39	S6 and S7	A	Multiple hepatic segment resection with abdominal wall sinus resection	No	Misdiagnosed preoperatively as echinococcosis, intraoperative and postoperative pathology confirmed mixed infection of the two types of echinococcosis in the liver with abdominal wall abscesses and sinus tracts, and the surgery needed to be considered as radical resection of the lesions, sinus tracts, and abdominal wall abscesses, which in turn followed the principle of individualized treatment of echinococcosis

PTCD: Percutaneous transhepatic biliary drainage; S: Segment (Couinaud's segmentation method divides the liver into eight segments according to anatomical position).

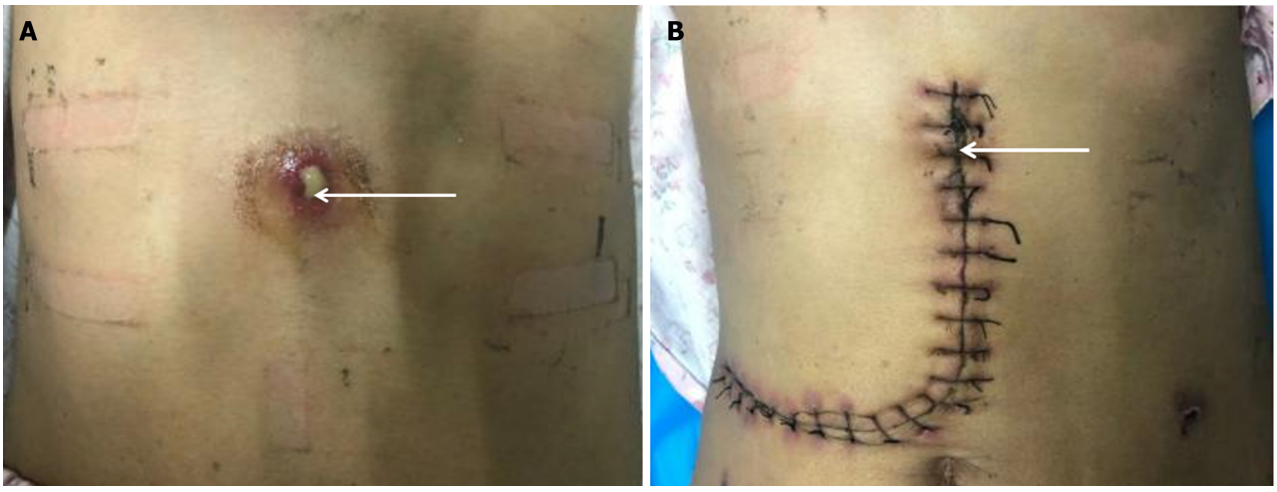

Figure 1 Preoperative lesions and postoperative incisions. A: Preoperative site of the patient's abdominal wall abscess; B: Postoperative abdominal wall abscess site. Note: The white arrows indicates the site of the abdominal wall abscess sinus tract.

abdominal breathing were present. Upper abdominal and periumbical tenderness, rebound pain, and muscle tension were noted, while the rest of the abdomen was unremarkable.

Laboratory examinations

Initial laboratory tests showed: Red blood cells, 5.98 × 10^{12} cells/L; white blood cells, 5.54 × 10^{12} cells/L; hemoglobin, 167 g/L; and platelet count, 240 × 10^{9} cells/L. Liver function tests revealed: Alanine aminotransferase, 9 U/L; aspartate aminotransferase, 13 U/L; total bilirubin, 7.2 mol/L; direct bilirubin, 1.4 mol/L; indirect bilirubin, 5.8 mol/L; albumin,

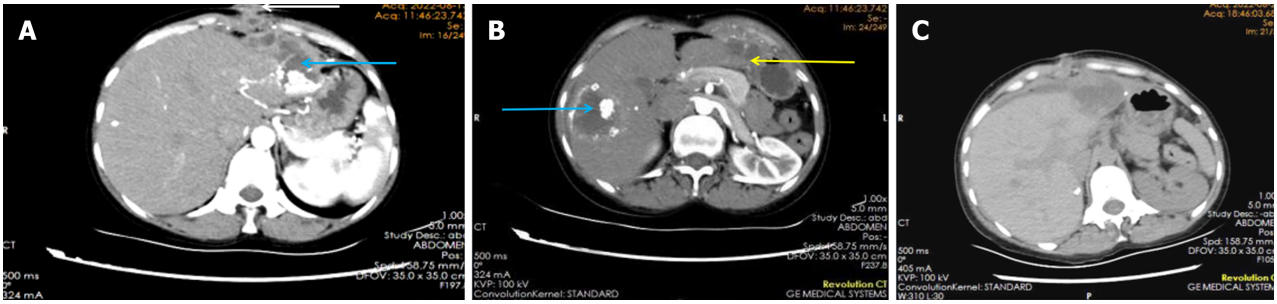

Figure 2 Pre- and postoperative imaging. A: Preoperative computed tomography (CT) images; B: Preoperative CT images; C: Postoperative CT images. Note: The white arrows indicate the site of the abdominal wall abscess sinus tract, the blue arrows indicate the site of the hepatic alveolar echinococcosis lesion, and the yellow arrows indicates the site of the hepatic cystic echinococcosis lesion.

32.2 g/L; and cholinesterase, 6140 U/L. A hydatid ELISA test yielded a positive result.

Imaging examinations

Abdominal color Doppler US revealed a 51 mm × 40 mm solid mass with a hyperechoic rim in the right hepatic lobe, suggestive of CE consolidation. Abdominal CT scan confirmed echinococcosis in the left lobe and anterior liver space, adhesion to the diaphragm, and a subxiphoid abscess with adjacent abdominal wall swelling. Scattered calcifications were identified within the right lobe CE (Figure 2A and B).

Further diagnostic work-up

Based on these findings, a diagnosis of hepatic CE with abdominal wall abscesses was made. The preoperative evaluation showed normal cardiopulmonary function and Child–Pugh grade A (5 points).

Intraoperatively (Figure 3), an oval cystic mass, measuring approximately 10.0 cm × 10.0 cm × 10.0 cm, was visualized in the right anterior hepatic lobe, exhibiting characteristics consistent with hepatic unilocular *E. granulosus*. Moreover, an irregular solid mass, measuring about 5.0 cm × 5.0 cm × 5.0 cm, in the left outer lobe was identified, presenting features compatible with hepatic *E. multilocularis*. Lastly, dense adhesions connected both masses to the anterior abdominal wall, characteristic of hepatic multilocular Echinococcus larvae.

FINAL DIAGNOSIS

Based on these findings, the diagnosis was revised to mixed-type encapsulated hepatic echinococcosis (cystic and alveolar), with abdominal wall abscess and sinus tract formation.

TREATMENT

A combined multisegmental hepatectomy with abdominal wall sinus tract resection was performed.

OUTCOME AND FOLLOW-UP

Postoperatively, the patient was encouraged to wake up and eat early to promote organ function recovery[8]. A follow-up CT scan 7 days postoperatively revealed a blurred fat space in the surgical area, with effusion, gas accumulation, and slight swelling of the right lower abdominal wall, with minimal exudate and pneumatosis; and unchanged scattered calcifications in the liver (Figure 2C).

Pathological examination of the liver hydatid tissue and fibrous cyst wall tissue revealed fibrous hyperplasia with hyalinization, necrosis, calcification, inflammatory cell infiltration, and minimal lamellar structures, consistent with echinococcosis (Figure 4). Nine days after surgery, the abdominal incision had healed well (Figure 1B), and the patient was discharged.

Regular oral albendazole therapy was initiated, as per the 2019 diagnostic criteria and expert guidelines for hepatic echinococcosis.

DISCUSSION

While relying on the aforementioned criteria, this patient's CT scan only revealed CE lesions and could not pinpoint the AE focus. Two factors might explain this. Initially, AE lesions often develop complete internal necrosis after infection,

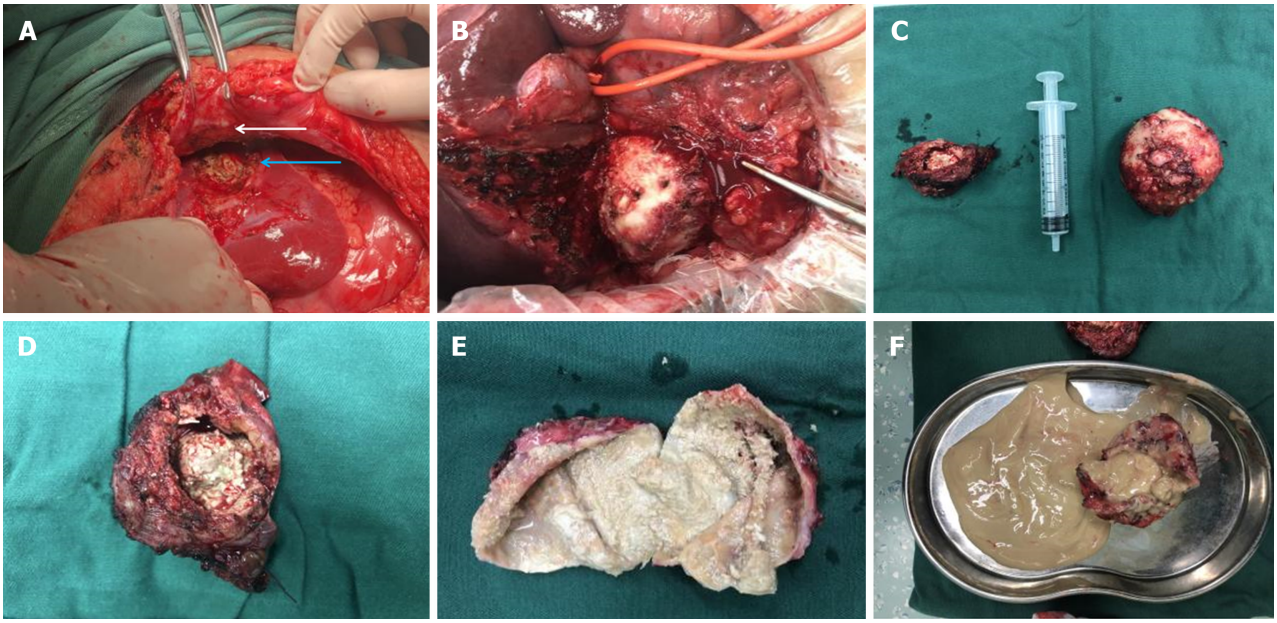

Figure 3 Intraoperative pathology specimens. A and B: Intraoperative visible lesions; C: Intraoperative excision of pathologic specimens (hepatic cystic echinococcosis and hepatic alveolar echinococcosis); D: Intraoperative excision of pathologic specimens (hepatic alveolar echinococcosis); E: Intraoperative excision of pathologic specimens (hepatic cystic echinococcosis); F: Intraoperative excision of pathologic specimens. Note: The white arrows indicate the site of the abdominal wall abscess sinus tract, and the blue arrows indicate the site of the hepatic alveolar echinococcosis lesion.

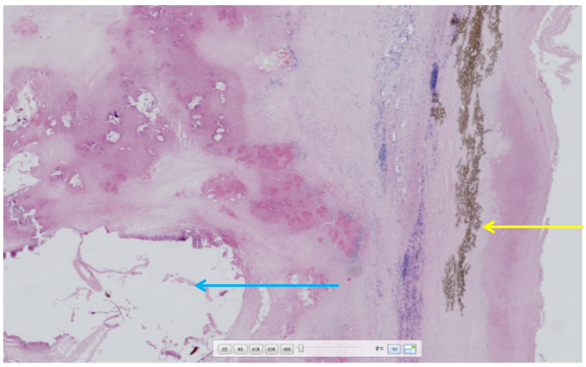

Figure 4 Postoperative pathology slides Histopathological examination by hemotoxylin-eosin staining (200 ×) fibrous connective tissue proliferation and inflammatory cell infiltration are seen around the blue arrow vesicles, forming nodules of varying sizes (alveolar echinococcosis) yellow arrow laminar-like structures are clearly visible (cystic echinococcosis).

forming a thin and uniform abscess wall indistinguishable from CE on CT. Additionally, mixed CE and AE infections are uncommon and rarely appear clearly on scans. Even with CT, the optimal view for diagnosis is not always achieved, leading to potential bias in this report.

Surgical treatment for patients with combined CE and AE infections prioritizes surgical safety. Aim for radical surgery to remove all lesions comprehensively, while still employing individualized approaches for each patient. Postoperatively, all coinfected patients should adhere to the standard AE diagnosis and treatment regimen, involving ongoing benzimidazole therapy[8].

Echinococcosis primarily targets organs like the liver, lungs, and spleen, with abdominal wall invasion is highly unusual. This patient presented with a long-standing abdominal wall abscess and sinus tract, but had no other symptoms of discomfort. Despite no major liver vessel involvement, the complications were significant. For such cases, successful surgery hinges on radical resection of the abdominal wall abscess and sinus tract. Insufficient resection risks AE recurrence, while exceeding necessary bounds can compromise remaining liver volume and functionality, leaving a large abdominal wall defect. Therefore, surgeons must ensure a safe 1.0-cm resection margin while preserving enough normal abdominal tissue (transverse diameter < 3 cm) and adequate blood supply[9,10]. By adhering to these principles, supported by thorough preoperative evaluation, accurate surgical planning, and optimal postoperative care, our patient achieved complete recovery and was discharged without complications.

CONCLUSION

This report summarized our experience in diagnosing and treating this rare condition: A mixed infection of both *Echinococcus* species with abdominal wall invasion and sinus tract formation. While the general treatment principles remain consistent with those of a simple infection by either *E. granulosus* or *E. multilocularis*, the presence of these additional complications necessitates additional considerations. For patients with long-standing lesions and established sinus tracts, radical resection of the affected tissue, including the sinus tracts and any abdominal wall abscesses, should be considered during surgery. This aligns with the principle of individualized treatment of echinococcosis. However, the long-term prognosis for such patients require postoperative albendazole treatment and regular follow-up protocols.

FOOTNOTES

Co-first authors: Miao-Miao Wang and Xiu-Qing An.

Co-corresponding authors: Ji-De A and Xiang-Ren A.

Author contributions: Wang MM, An XQ and Chai JP conceptualized and designed the research; Yang JY, A JD and A XR screened patients and acquired clinical data; Wang MM, An XQ and Chai JP collected blood specimen and performed laboratory analysis; Yang JY, A JD and A XR performed Data analysis; Wang MM, An XQ and Chai JP wrote the paper. All the authors have read and approved the final manuscript. Wang MM and An XQ prepared the first draft of the manuscript. Both authors have made crucial and indispensable contributions towards the completion of the project and thus qualified as the co-first authors of the paper. Both A JD and A XR have played important and indispensable roles in the experimental design, data interpretation and manuscript preparation as the co-corresponding authors. A XR conceptualized, designed, and supervised the whole process of the project. He searched the literature, revised and submitted the early version of the manuscript with the focus on the diagnosing and treating this rare condition: A mixed infection of both *Echinococcus* species with abdominal wall invasion and sinus tract formation. This collaboration between A JD and A XR is crucial for the publication of this manuscript and other manuscripts still in preparation.

Supported by National Natural Science Foundation of China, No. 82260412.

Informed consent statement: This published consent was obtained from the patients or their representatives.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

CARE Checklist (2016) statement: The authors have read CARE Checklist (2016), and the manuscript was prepared and revised according to CARE Checklist (2016).

Country/Territory of origin: China

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S-Editor: Li L
L-Editor: A
P-Editor: Cai YX

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 286-293

DOI: 10.4254/wjh.v16.i2.286

ISSN 1948-5182 (online)

CASE REPORT

Autoimmune hepatitis and primary sclerosing cholangitis after direct-acting antiviral treatment for hepatitis C virus: A case report

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Mahmoud MZ, Saudi Arabia

Received: November 30, 2023
Peer-review started: November 30, 2023
First decision: December 8, 2023
Revised: December 22, 2023
Accepted: January 16, 2024
Article in press: January 16, 2024
Published online: February 27, 2024

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Abstract

BACKGROUND

Chronic hepatitis C virus (HCV) infection is a major global health concern that leads to liver fibrosis, cirrhosis, and cancer. Regimens containing direct-acting antivirals (DAAs) have become the mainstay of HCV treatment, achieving a high sustained virological response (SVR) with minimal adverse events.

CASE SUMMARY

A 74-year-old woman with chronic HCV infection was treated with the DAAs ledipasvir, and sofosbuvir for 12 wk and achieved SVR. Twenty-four weeks after treatment completion, the liver enzyme and serum IgG levels increased, and antinuclear antibody became positive without HCV viremia, suggesting the development of autoimmune hepatitis (AIH). After liver biopsy indicated AIH, a definite AIH diagnosis was made and prednisolone was initiated. The treatment was effective, and the liver enzyme and serum IgG levels normalized. However, multiple strictures of the intrahepatic and extrahepatic bile ducts with dilatation of the peripheral bile ducts appeared on magnetic resonance cholangiopancreatography after 3 years of achieving SVR, which were consistent with primary sclerosing cholangitis.

CONCLUSION

The potential risk of developing autoimmune liver diseases after DAA treatment should be considered.

Key Words: Liver; Hepatitis C virus; Autoimmune hepatitis; Primary sclerosing cholangitis; Immune system; Case report

Core Tip: Direct-acting antivirals (DAAs) for chronic hepatitis C virus (HCV) infection are widely used as a safe and effective treatment intervention. Chronic HCV infection alters the innate and adaptive immune responses, both functionally and phenotypically. Rapid viral clearance following DAAs treatment restores adaptive immune function. Herein, we report a rare case of autoimmune hepatitis and primary sclerosing cholangitis that developed after DAAs treatment for HCV. The potential risk of developing autoimmune liver diseases after DAAs treatment owing to the restoration of host immunity following rapid viral clearance should be considered.

Citation: Morihisa Y, Chung H, Towatari S, Yamashita D, Inokuma T. Autoimmune hepatitis and primary sclerosing cholangitis after direct-acting antiviral treatment for hepatitis C virus: A case report. *World J Hepatol* 2024; 16(2): 286-293
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/286.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.286

INTRODUCTION

Hepatitis C virus (HCV) infection is a growing international concern because of its substantial morbidity and mortality. HCV has a worldwide prevalence of 0.7%, infecting over 56.8 million people, with approximately 1.5 million new infections every year[1]. Most patients (50%-90%) develop chronic infections and chronic liver diseases, such as cirrhosis, liver failure, and hepatocellular carcinoma[2]. Pegylated interferon alpha and ribavirin administration was the basis of the antiviral therapy for HCV; however, the frequent side effects and poor treatment outcomes were problematic[3-5]. In 2013, the first interferon-free treatment regimen was approved for the treatment of chronic hepatitis C (CHC) and several other direct-acting antivirals (DAAs) have been developed since. Such DAA regimens present excellent safety profiles and high response rates, which exceed 97% not only in clinical trials, but also in real-world clinical settings. As a result, most patients with CHC have achieved sustained virological response (SVR)[6,7].

Recently, various studies have focused on the functional changes of the immune system induced by the rapid viral clearance of DAAs after chronic HCV infections, and some reports have demonstrated the recovery of innate and adaptive immune responses after the SVR[8]. Interestingly, some case reports have described patients who developed autoimmune hepatitis (AIH) after DAA treatment for HCV, suggesting that the recovery of host immunity is associated with the development of autoimmune liver disorders.

Herein, we report a rare case of a woman with CHC who developed AIH and primary sclerosing cholangitis (PSC) after antiviral therapy with DAA.

CASE PRESENTATION

Chief complaints

A 74-year-old woman visited our department for the treatment of HCV infection.

History of present illness

She was administered a 12-wk combination regimen of sofosbuvir (SOF) and ledipasvir (LDV) and achieved SVR. Twenty-four weeks after treatment completion, the liver enzyme levels increased.

History of past illness

She had a history of ectopic pregnancy and had received a blood transfusion 50 years before.

Personal and family history

She had no remarkable family history or history of autoimmune diseases. She had a history of smoking and consumed 350 mL of beer once a week.

Physical examination

She denied having fever or chills, malaise, or fatigue but presented discrete weight loss.

Laboratory examinations

Laboratory examinations showed an undetectable serum HCV RNA; however, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were elevated at 335 U/L and 329 U/L, respectively (Table 1). The total bilirubin level was not increased, and prothrombin time was not prolonged. The serum IgG level was increased at 3481 mg/dL. The antinuclear antibody (ANA) and antismooth muscle antibody (ASMA) titers were 1:80 in a homogeneous

Table 1 Blood test

Blood test	Value	Unit
Hematology		
WBC	59.00	$\times 10^2/\mu L$
RBC	400.00	$\times 10^4/\mu L$
Hb	12.30	g/dL
Ht	37.30	%
PLT	13.30	$\times 10^4/\mu L$
PT (%)	81.90	%
Serum chemistry		
TP	9.70	g/dL
ALB	3.60	g/dL
T-Bil	1.00	mg/dL
AST	418.00	U/L
ALT	366.00	U/L
ALP	371.00	U/L
γ-GT	102.00	U/L
LD	388.00	U/L
BUN	13.40	mg/dL
Cre	0.65	mg/dL
CRP	0.02	mg/dL
IgA	605.00	mg/dL
IgM	795.00	mg/dL
IgG	3481.00	mg/dL
ANA	80	
ASMA	640	
AMA	< 20	
HAV-IgM	Negative	
HEV-IgA	Negative	
HBs-Ag	Negative	
HBs-Ab	Negative	
HBc-Ab	Positive	
HBV-DNA	< 2.1	Logcopy/mL
HCV-Ab	Positive	
HCV-RNA	< 1.2	LogIU/mL
CMV-IgM	Negative	
CMV-IgG	Positive	
VCA-IgM	Negative	
VCA-IgG	Positive	
EBNA	Positive	
HLA	DR4	

WBC: White blood cell; RBC: Red blood cell; Hb: Hemoglobin; Ht: Hematocrit; PLT: Platelet; PT: Prothrombin time; TP: Total protein; ALB: Albumin; T-Bil:

Total-bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; γ-GT: γ-Glutamyl transpeptidase; LD: Lactate dehydrogenase; BUN: Blood urea nitrogen; CRP: C reactive protein; ANA: Antinuclear antibody; ASMA: Antismooth muscle antibody; AMA: Antimitochondrial antibody; HAV: Hepatitis A virus; HEV: Hepatitis E virus; VCA: Viral capsid antigen; HCV: Hepatitis C virus; EBNA: Epstein-Barr virus nuclear antigen; HLA: Human leukocyte antigen.

pattern and 1:640, respectively, whereas the antimitochondrial antibody (AMA) was negative. Notably, ANA and ASMA were negative before the start of the DAA regimen, yet the titers of both antibodies gradually increased during and after treatment (Table 2).

Imaging examinations

Contrast-enhanced computed tomography revealed no abnormal findings at the time of exacerbation. Liver biopsy examination showed inflammatory cell infiltration mainly composed of lymphocytes and plasma cells in the portal and lobular areas and severe interface hepatitis with rosette formation (Figure 1A and B).

FINAL DIAGNOSIS

The definite diagnosis of AIH was made according to the International Diagnostic Criteria and Simplified Criteria for AIH, based on laboratory tests and liver biopsy findings, with scores of 18 and 8 points, respectively.

TREATMENT

After the AIH diagnosis, a daily 20 mg (0.5 mg/kg) dose of prednisolone was started. Serum AST, ALT, and IgG levels decreased significantly and reached normal limits (Figure 2). Daily 600 mg ursodeoxycholic acid (UDCA) doses were administered along with tapering of the prednisolone dose. Remission of AIH and normalization of serum AST, ALT, and IgG levels were maintained with daily doses of prednisolone (2.5 mg) and UDCA (600 mg).

OUTCOME AND FOLLOW-UP

The patient was still receiving treatment with 2.5 mg prednisolone and 600 mg UDCA three years after achieving SVR. Although laboratory examinations showed no elevation of serum liver enzymes, IgG, or IgG 4, magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography revealed diffuse stenosis extending from the intrahepatic bile duct to the common hepatic duct, and dilation of the peripheral bile duct (Figure 3). A second liver biopsy was performed, which showed improvement of the interface hepatitis, yet massive infiltration of lymphocytes and plasma cells and proliferation of interlobular bile ducts in the portal triad area (Figure 1C and D). She was diagnosed with PSC based on the 2016 diagnostic criteria.

DISCUSSION

Most cases of HCV infection do not present spontaneous remission and evolve to chronic viral hepatitis, which can lead to cirrhosis and hepatocellular carcinoma. Persistent HCV infection alters innate and adaptive immune responses functionally and phenotypically, including natural killer (NK) cell dysfunction and reduced NK cell diversity[9], viral escape mutation[10], HCV-specific CD8 T cell exhaustion, increased regulatory CD4 T cells (Treg), and deletion of HCV-specific CD4 T cells[11-13]. T cell exhaustion occurs due to ongoing antigen stimulation and is characterized by the loss of effector functions and increased expression of inhibitory markers[9,14,15]. Recently, DAAs have enabled almost all patients to completely eliminate HCV and achieve SVR. The effect of DAAs on rapid viral clearance is being investigated worldwide. Several reports have revealed that DAA therapy rapidly restores some adaptive immune functions, such as relative Treg reduction and HCV-specific T-cell function recovery[16,17]. In patients with AIH, a low number of functional CD4+ Tregs has been reported[18]. The restoration of the immune function may disrupt immune tolerance and cause autoimmune diseases.

Mucosal-associated invariant T (MAIT) cells have recently gathered attention as possible factors associated with autoimmune disease[19]. MAIT cells are an innate-like T cell subset that comprises 5%-10% peripheral T cells and approximately 12%-50% of T cells in the liver and gastrointestinal tract[20,21]. The dominance of MAIT cells in the liver indicates their potential essential role in the pathogenesis of chronic HCV infection[22]. Among patients infected with HCV, the number and function of intrahepatic and peripheral MAIT cells were significantly reduced compared to those in healthy controls[23]. Additionally, impaired peripheral MAIT cells do not recover after successful antiviral therapy; in contrast, the number of MAIT cells in the liver increased after therapy[22]. In multiple sclerosis, an autoimmune disease, MAIT cells are reportedly reduced in the peripheral blood and can be detected in most of the cerebrospinal fluid[24]. Therefore,

Table 2 Blood test results (antinuclear antibody and antismooth muscle antibody)

Blood test	ANA	ASMA
Before DAAs (SOF/LDV) therapy	< 20	< 20
After DAAs (SOF/LDV) therapy	40	20
SVR 12	40	160
SVR 24	80	640

ANA: Antinuclear antibody; ASMA: Antismooth muscle antibody; DAA: Direct antiviral agent; SOF: Sofosbuvir; LDV: Ledipasvir; SVR: Sustained virological response.

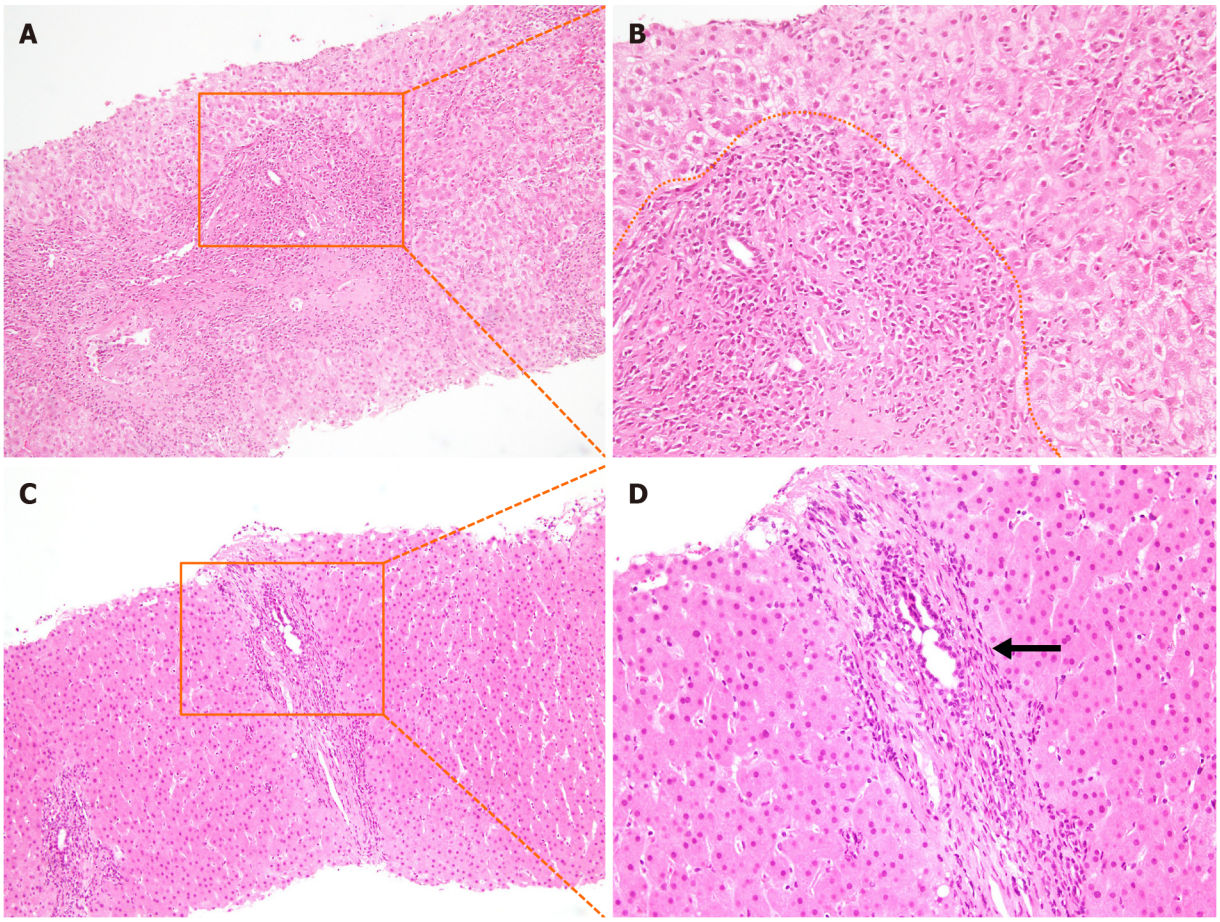

Figure 1 Initial and second liver biopsy. A and B: The initial liver biopsy showing inflammatory cell infiltration mainly composed of lymphocytes and plasma cells in the portal and lobular areas, and severe interface hepatitis with rosette formation. Hematoxylin and eosin staining (HE: A, × 100; B, × 200); C and D: A second liver biopsy shows improvement of the interface hepatitis, yet a massive infiltration of lymphocytes and plasma cells is noted. B and D are magnified images of the yellow square in A and C, the black arrow shows the proliferation of interlobular bile ducts in the portal triad area (HE: C, × 100; D, × 200).

activation of MAIT cells in lesions may facilitate inflammation and fibrosis in autoimmune diseases.

Notably, human leukocyte antigen (HLA)-DR4 was positive in our case. Classical (type 1) AIH is strongly associated with the HLA-DR3 (HLA-DRB1*03) and HLA-DR4 (HLA-DRB1*04), whereas the type 2 disease is associated with the HLA-DRB1*07 and HLA-DRB1*03[25,26]. In Japan, where HLA-DR3 is rare, AIH is primarily associated with the HLA-DR4 serotype[27]. Genetic and environmental factors are involved in the development of AIH[28]. In our case, HLA-DR4 as a genetic factor and various levels of immune activation associated with the elimination of HCV due to DAA treatment as an environmental factor likely induced an immune response to liver autoantigens, leading to AIH onset. Further studies are required to elucidate these underlying mechanisms.

Only four cases of AIH that developed after DAA treatment have been reported in the English literature, including our case[29-31] (Table 3). All identified patients were females, with a median age of 76 years. HLA-DR4 was positive in our case; however, such was not identified in the other cases. Three cases had HCV genotype Ib and one had serotype I. Only one case had a coexisting autoimmune disease, namely, idiopathic thrombocytopenic purpura with CHC infection[31]. Although reports of HCV and AIH overlap exist, the four cases have no findings that suggested AIH before DAA

Table 3 Four cases of autoimmune hepatitis development following direct antiviral agent treatment

Ref.	Age	Sex	HLA-DR4	Autoimmune disease	Genotype	DAA	Onset	Treatment	Follow up
Matsumoto *et al* [29], 2018	81	Female	Negative	None	1 (serotype)	EBR/GZR	2 months	PSL	Improvement
Covini *et al*[31], 2018	72	Female	NA	ITP	1b	SOF/LDV	2 wk	PSL	Improvement
Montón *et al*[30], 2020	72	Female	NA	None	1b	SOF/LDV	3 yr	PSL	Improvement
Our case	80	Female	Positive	None	1b	SOF/LDV	9 months	PSL + UDCA	Development of PSC

DAA: Direct antiviral agent; EBR/GZR: Elbasvir/Grazoprevir; HLA: Human leukocyte antigen; ITP: Idiopathic thrombocytopenic purpura; PSC: Primary sclerosing cholangitis; PSL: Prednisolone; SOF/LDV: Sofosbuvir/Ledipasvir; UDCA: Ursodeoxycholic acid.

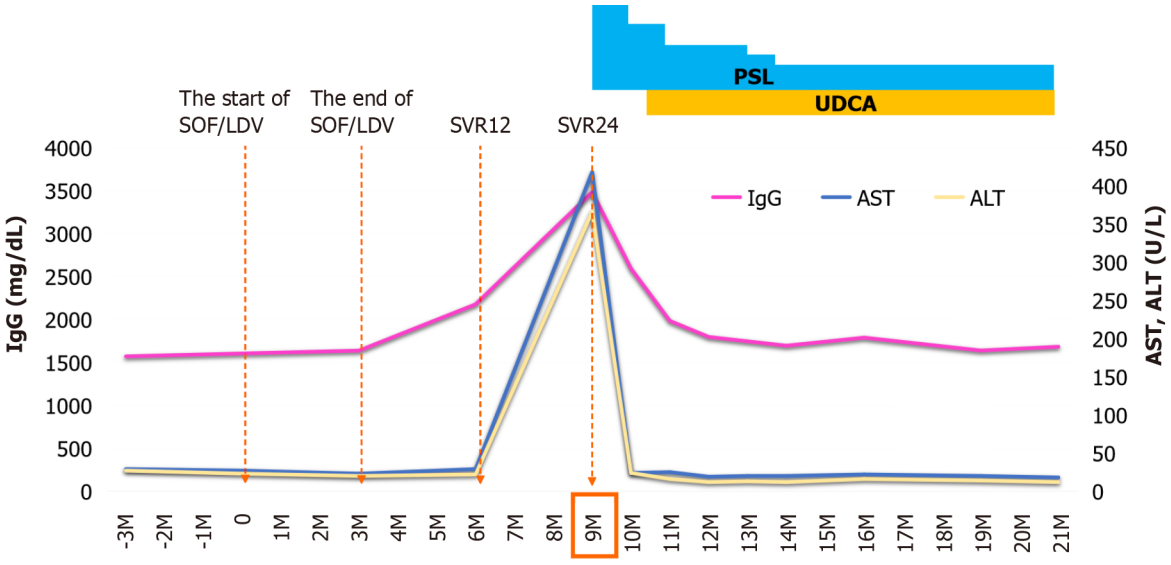

Figure 2 Aspartate aminotransferase, alanine aminotransferase, and IgG levels over time. The figure shows that aspartate aminotransferase (AST), alanine aminotransferase (ALT), and IgG are elevated after direct acting antiviral agents therapy. After the start of prednisolone, AST, ALT, and IgG decrease significantly and approach the normal limits. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDV: Ledipasvir; PSL: Prednisolone; SOF: Sofosbuvir; SVR: Sustained virological response; UDCA: Ursodeoxycholic acid.

treatment initiation. In our case, ANA and ASMA were negative before the start of the DAA regimen, and the titers of both antibodies gradually increased during and after treatment. Therefore, the DAA treatment is thought to have triggered the development of AIH. Three cases were treated with SOF/LDV[30,31], and one case was treated with elbasvir and grazoprevir[29]. Laboratory examinations showed an increase in ANA in all cases and an increase in ASMA in two cases[30]. IgG levels were elevated in three cases[29,30]. All patients underwent liver biopsy, which revealed interface hepatitis and infiltration of various inflammatory cells, including plasma cells, in the portal zonal areas. Serological and histological findings suggested the development of AIH. All patients received prednisolone, which led to improvements in serum AST, ALT, and IgG levels. Furthermore, serum HCV RNA was continuously undetectable in all cases. Only in our case, PSC was diagnosed 3 years after prednisolone treatment. Despite the small number of cases, immunosuppressive therapy is likely to be effective when AIH develops after HCV treatment.

Our case suggested that AIH and PSC development may be attributed to the rapid changes in immune function induced by DAA treatment. However, it was not possible to directly elucidate the mechanism underlying the association in this report. Therefore, accumulation of similar cases to clarify the mechanism is required.

CONCLUSION

Studies on antiviral therapy for HCV, highlighting the safety and effectiveness of DAA regimens, have reported sporadic episodes of adverse effects associated with immune dysregulation. We encountered a rare case of AIH with PSC that developed after DAA treatment. The potential risk of developing autoimmune liver diseases after DAA treatment owing

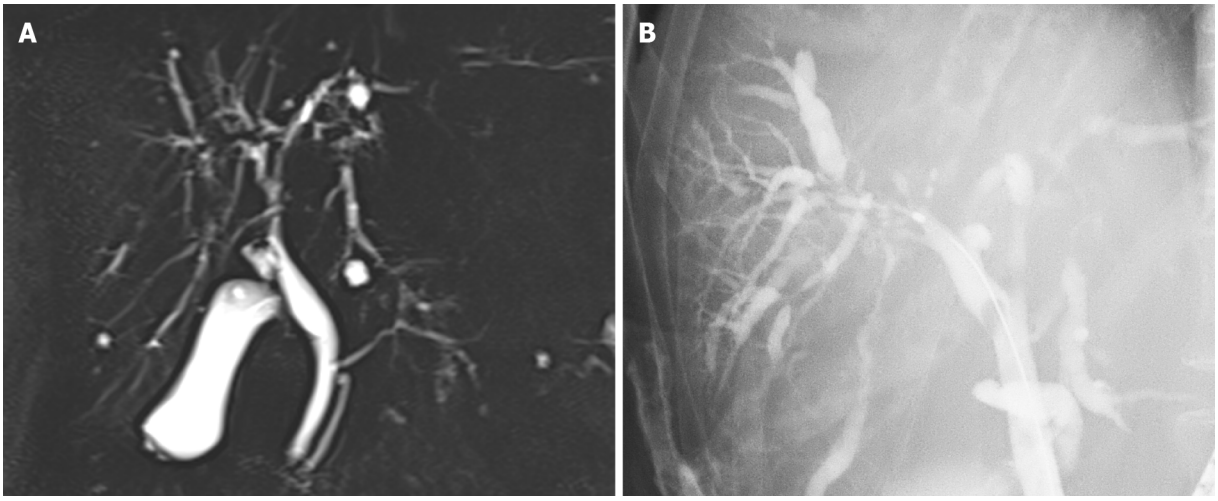

Figure 3 Magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography. A: Magnetic resonance cholangiopancreatography; B: Endoscopic retrograde cholangiopancreatography shows diffuse stenosis extending from the intrahepatic bile duct to the common hepatic duct, with dilation of the peripheral bile duct.

to the restoration of host immunity associated with rapid viral clearance should be considered. Further studies are necessary to clarify the frequency and mechanism of autoimmune liver diseases following DAA treatment.

FOOTNOTES

Author contributions: Morihisa Y drafted the manuscript; Chung H, Towatari S, Yamashita D, and Inokuma T contributed to the critical revision of the manuscript; all authors have read and approved the final manuscript.

Informed consent statement: Informed written consent was obtained from the patients for the publication of this report and any accompanying images.

Conflict-of-interest statement: All authors declare that they have no conflicts of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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S-Editor: Chen YL
L-Editor: A
P-Editor: Yuan YY

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World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2024 February 27; 16(2): 294-299

DOI: 10.4254/wjh.v16.i2.294

ISSN 1948-5182 (online)

LETTER TO THE EDITOR

Anti-oxidative stress treatment and current clinical trials

Chun-Ye Zhang, Ming Yang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ebraheim LLM, Egypt

Received: November 2, 2023
Peer-review started: November 2, 2023
First decision: December 27, 2023
Revised: January 8, 2024
Accepted: February 5, 2024
Article in press: February 5, 2024
Published online: February 27, 2024

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Abstract

Oxidative stress disturbs the balance between the production of reactive oxygen species (ROS) and the detoxification biological process. It plays an important role in the development and progression of many chronic diseases. Upon exposure to oxidative stress or the inducers of ROS, the cellular nucleus undergoes some biological processes *via* different signaling pathways, such as stress adaption through the forkhead box O signaling pathway, inflammatory response through the IκB kinase/nuclear factor-κB signaling pathway, hypoxic response *via* the hypoxia-inducible factor/prolyl hydroxylase domain proteins pathway, DNA repair or apoptosis through the p53 signaling pathway, and antioxidant response through the Kelch-like ECH-associated protein 1/nuclear factor E2-related factor 2 signaling pathway. These processes are involved in many diseases. Therefore, oxidative stress has gained more attraction as a targeting process for disease treatment. Meanwhile, anti-oxidative stress agents have been widely explored in pre-clinical trials. However, only limited clinical trials are performed to evaluate the efficacy of anti-oxidative stress agents or antioxidants in diseases. In this letter, we further discuss the current clinical trials related to anti-oxidative stress treatment in different diseases. More pre-clinical studies and clinical trials are expected to use anti-oxidative stress strategies as disease treatment or dietary supplementation to improve disease treatment outcomes.

Key Words: Anti-oxidative stress treatment; Clinical trials; Drugs; Dietary invention; Reactive oxygen species

Core Tip: Oxidative stress disturbs the balance between the production and detoxification of reactive oxygen species, which is implicated in many diseases. Therefore, anti-oxidative stress agents have been widely explored to treat chronic and metabolic diseases. In this letter, we further discuss the current clinical trials related to anti-oxidative stress treatment and summarize current medicines under investigation.

Citation: Zhang CY, Yang M. Anti-oxidative stress treatment and current clinical trials. *World J Hepatol* 2024; 16(2): 294-299
URL: https://www.wjgnet.com/1948-5182/full/v16/i2/294.htm
DOI: https://dx.doi.org/10.4254/wjh.v16.i2.294

TO THE EDITOR

With great interest, we read a recently published review paper authored by Li *et al*[1], discussing the progress of using herbal extracts from traditional Chinese medicine as a therapeutic method to treat liver fibrosis *via* inhibiting oxidative stress.

We agree with the authors that oxidative stress is a critical factor that can be targeted in the treatment of liver fibrosis. Oxidative stress is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) and the biological system to detoxify ROS products[2]. The accumulation of ROS can cause cell damage through the destruction of proteins and lipids, genetic modification, and disturbance of cellular signaling[2,3]. Therefore, oxidative stress has been recognized as a crucial factor involved in the underlying mechanisms of disease development and progression[4]. In fact, oxidative stress has gained more and more attraction recently due to its important roles in many diseases, such as heart disease[5], cancer[6], hypertension[7], cardiovascular diseases[8], aging[9], neurodegenerative disease[10,11], Alzheimer's disease[12], Parkinson's disease[13], and metabolic disorders. Moreover, oxidative stress also plays a pivotal role in organ transplantation[14] and infectious diseases[15]. Therefore, anti-oxidative stress as a therapeutic strategy has gained more attention for disease treatment.

Accumulating studies are performed to decipher the mechanism of oxidative stress in disease. Oxidative stress inducers include endogenous sources and exposomes[16]. Endogenous sources can induce the endoplasmic reticulum stress that may be caused by misfolded proteins, resulting in elevated levels of ROS[17]. The exposomes include but are not limited to toxins, irradiation exposure, air pollution, smoking, nutrients, chemicals, and infection[18]. Upon exposure to oxidant sources, the cellular nucleus undergoes several biological processes (Figure 1), such as stress adaption *via* the forkhead box signaling pathway[19], inflammatory responses through the nuclear factor (NF)-κB and inhibitor of NF-κB kinase signaling pathway[20,21], hypoxic responses controlled by hypoxia-inducible facto-prolyl hydroxylase domain proteins[22], DNA repair or apoptosis process through the p53 signaling pathway[23], and antioxidant responses through the Kelch-like ECH-associated protein 1 (KEAP1)-transcription factor NF-E2 p45-related factor 2 (NRF2) (KEAP1-NRF2) signaling pathway[24]. The mitochondrion serves as an important organelle to generate ATP as an energy source, and ROS is also produced in this process. The accumulated excessive levels of ROS can result in oxidative stress[25]. Thus, the imbalance of the production of excessive oxidants and antioxidant processes leads to disease development and progression.

Inspired by this published review article, here, we give a further discussion on the current clinical trials that are related to anti-oxidative stress in different diseases using various intervention methods. Currently, two major categories including dietary supplement and drug treatment are used in clinical trials and summarized in this letter (Table 1). The most tested drug in these clinical trials is N-acetylcysteine with application in different diseases, such as cancer (melanoma and leukemia), pulmonary disease, renal disease, liver diseases such as non-alcoholic fatty liver disease, infectious diseases including severe acute respiratory syndrome coronavirus and human immunodeficiency virus infections, obesity, Parkinson's disease, and depressive disorders. The drug melatonin has also been used in many diseases, such as necrotizing enterocolitis, multiple sclerosis, and septic shock. Curcumin is a dietary supplement, which has been tested for renal transplantation disorder, coronary artery disease, metabolic syndrome, kidney disease, and others (Table 1). The data were collected from the website https://clinicaltrials.gov (accessed on October 28, 2023) using the keywords anti-oxidative stress, disease, and treatments such as drugs and nutrients, including ongoing and completed clinical trials.

In summary, oxidative stress is involved in many diseases and functions as a promising target in disease treatment and therapeutic drug screening. More potent antioxidants are expected to be explored to improve treatment outcomes. Meanwhile, the synergistic application of anti-oxidative drugs is an option to improve the therapeutic efficacy of other drugs.

Table 1 Clinical trials on anti-oxidative stress-related treatment

NCT number	Condition(s)	Category	Intervention(s)	Phase(s)
NCT05511766	Cirrhosis, hepatic encephalopathy	Drug	Allopurinol 300 mg, Atorvastatin 20 mg	2 and 3

NCT01054768	Anemia, sickle cell	Drug	Alpha-lipoic acid and acetyl-L-carnitine	2
NCT05558878	Diabetic peripheral neuropathy	Drug	Ambroxol oral product	NA
NCT00916448	Endotoxemia, multi-organ dysfunction	Drug	Atazanavir, *E. coli* endotoxin	NA
NCT03820245	Oxidative stress, atherosclerosis	Dietary	Bixin, norbixin, lycopene	NA
NCT05957432	*Helicobacter pylori* infection	Drug	Black seed oil, vonoprazan, amoxicillin, clarithromycin	2
NCT03529396	Vivax malaria, glucose-6-phosphate dehydrogenase	Drug	Chloroquine, primaquine	2
NCT03935958	Disorder in renal transplantation	Dietary	Curcumin	NA
NCT04458116	Coronary artery disease	Dietary	Curcumin	NA
NCT03514667	Metabolic syndrome	Dietary	Nanomicielle curcumin	NA
NCT04413266	Kidney diseases, peritoneal dialysis	Dietary	Curcumin supplementation	NA
NCT05966441	Chemotherapy peripheral neuropathy	Dietary	Curcumin, paclitaxel	2
NCT06083480	Osteoarthritis, knee arthroplasty	Drug	GlyNAC (combined glycine and N-acetylcysteine)	4
NCT01854294	Amyotrophic lateral sclerosis	Drug	GM604	2
NCT01891500	Persistent fetal circulation syndrome	Drug	Inhaled nitric oxide, nitrogen Gas	4
NCT05033639	Necrotizing enterocolitis	Drug	Melatonin 6 mg	1 and 2
NCT02463318	Multiple sclerosis	Drug	Melatonin, hydrogen peroxide	NA
NCT03557229	Septic shock	Drug	Melatonin, vitamins C and E, N-acetyl cysteine	3
NCT02587741	Diabetic retinopathy	Drug	Metformin, lantus, Novomix30	1
NCT01501929	Hypertension	Drug	Metoprolol succinate, nebivolol	4
NCT05742698	Frontotemporal dementia	Drug	Nabilone	2
NCT02294591	Bipolar disorder	Drug	N-acetyl cysteine	2
NCT02972398	Major depressive disorders	Drug	N-acetyl cysteine	NA
NCT01612221	Risk for melanoma	Drug	N-acetyl cysteine	2
NCT05611086	Lymphoblastic leukemia	Drug	N-acetyl cysteine	4
NCT01501110	Ischemic heart disease	Drug	N-acetyl cysteine	4
NCT05460858	Female infertility, endometrioma	Drug	N-acetyl cysteine	3
NCT03956888	Chronic obstructive pulmonary disease	Drug	N-acetyl cysteine	3
NCT01907061	Acute renal failure	Drug	N-acetyl cysteine	NA
NCT02124525	Tobacco smoking, inflammation	Drug	N-acetyl cysteine	3
NCT04792021	SARS-CoV-2 infection	Drug	N-acetyl cysteine	3
NCT04154982	Cardiac arrhythmia	Drug	N-acetyl cysteine	2
NCT03596125	Preterm delivery	Drug	N-acetyl cysteine	2 and 3
NCT04732000	Surgical recovery	Drug	N-acetyl cysteine	2
NCT02252341	Bipolar disorder	Dietary	N-acetyl cysteine	4
NCT01587001	Pulmonary sarcoidosis	Dietary	N-acetyl cysteine	NA
NCT01962961	HIV infection, endothelial dysfunction	Dietary	N-acetyl cysteine	1 and 2
NCT04440280	Fuchs endothelial corneal dystrophy	Drug	N-acetyl cysteine solution, visine	2
NCT02117700	Obesity, NAFLD, cardiovascular disease	Dietary	N-acetyl cysteine 600 mg	1 and 2
NCT05589584	Steatosis, non-fatty liver	Drug	N-acetyl cysteine	3
NCT04459052	Parkinson disease	Dietary	N-acetyl cysteine, F18 Fluorodopa	2
NCT01384591	Aging	Drug	N-acetyl cysteine, losartan	1 and 2
NCT03056014	Type 1 diabetes	Drug	N-acetyl cysteine, omega-6 fish oil	1
NCT04022161	Cardiovascular, endothelial dysfunction	Drug	Nitrogen gas for inhalation, nitric oxide	2

NCT03273413	Autosomal dominant polycystic kidney	Drug	Pravastatin	4
NCT02161653	Severe alcoholic hepatitis	Drug	Prednisone, metadoxine, pentoxifylline	4
NCT05770297	Endometriosis, dysmenorrhea	Dietary	Propolis	NA
NCT05753436	Diabetes, dyslipidemias, hypertension	Dietary	Puritans pride turmeric curcumin	2
NCT01663103	Renal insufficiency, chronic	Drug	Rilonacept	4
NCT01388478	Alzheimer's disease	Drug	R-pramipexole	2
NCT03738176	Oral lichen planus	Drug	Sesame oil, triamcinolone	1
NCT03402204	Ischemic stroke	Drug	Simvastatin 10 mg, simvastatin 40 mg	3
NCT05145270	Major depressive disorder	Dietary	Sulforaphane, escitalopram	4
NCT05149716	Oxidative stress	Dietary	Taurine	NA

NAFLD: Non-alcoholic fatty liver disease; NA: Not applicable.

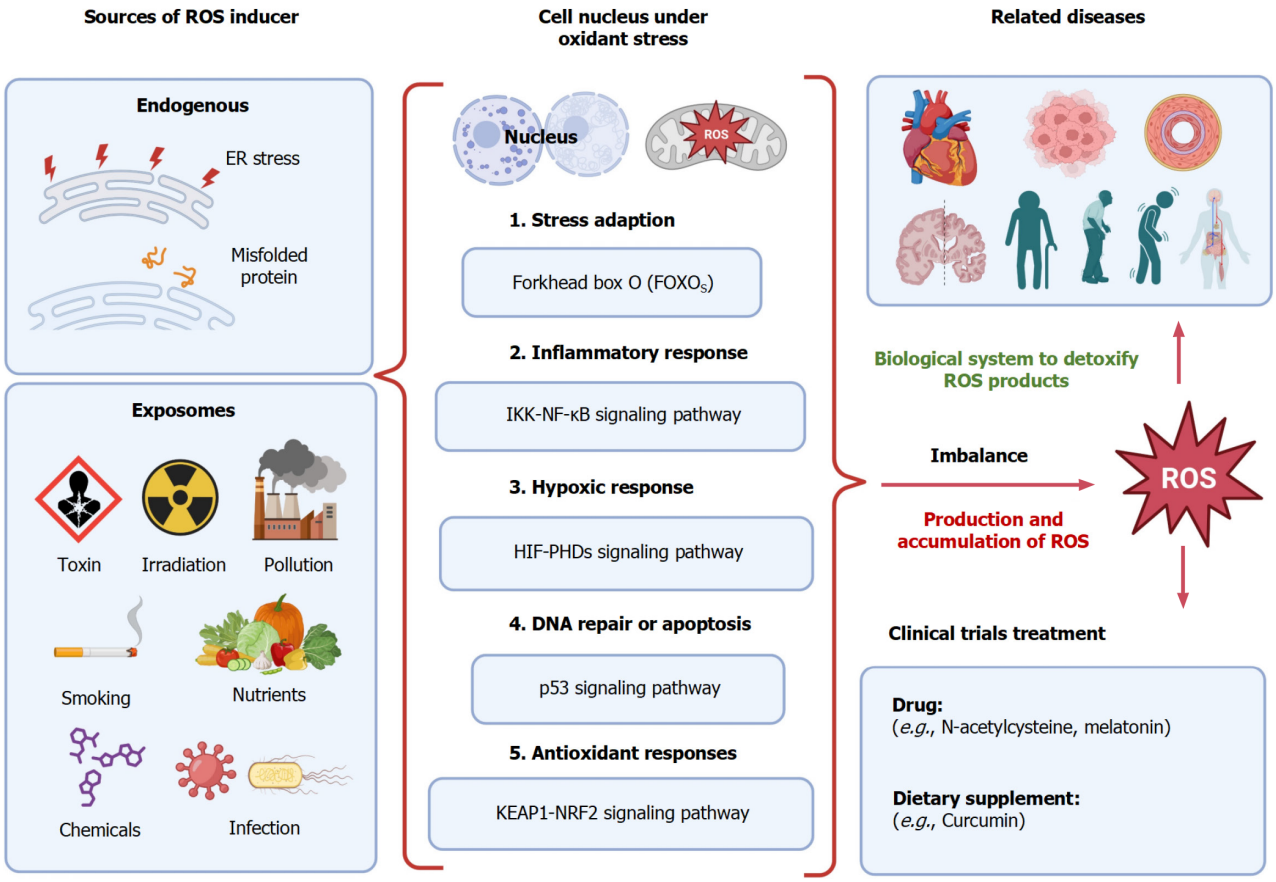

Figure 1 Diagram illustrating reactive oxygen species including inducers, mechanisms, related diseases, and clinical trial treatments. Inducers include endogenous and exposomes. The mechanism includes the cell nucleus response to exposure to reactive oxygen species (ROS) and the mitochondrial ROS response. The imbalance between the accumulation of ROS and their clearance by the biological system results in ROS-related diseases such as heart disease, cancer, hypertension, cardiovascular diseases, Alzheimer's disease, aging, neurodegenerative disease Parkinson's disease, and metabolic disorder. Current clinical trials mainly focus on the drug and dietary invention. ER stress: Endoplasmic reticulum stress; ROS: Reactive oxygen species; IKK-NF-κB: IκB kinase-nuclear factor-κB; HIF-PDHs: Hypoxia-inducible factor-prolyl hydroxylase domain proteins; p53: Tumor protein p53 or transformation-related protein 53; KEAP1-NRF2: Kelch-like ECH-associated protein 1-nuclear factor E2-related factor 2. All cartoons in this figure were prepared using Biorender (https://biorender.com, accessed on 7 January 2024).

FOOTNOTES

Author contributions: Zhang CY and Yang M designed the study, collected the data, and wrote, revised, and finalized the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country/Territory of origin: United States

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S-Editor: Qu XL
L-Editor: Wang TQ
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