ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2020 August 27; 12(8): 413-532

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 12 Number 8 August 27, 2020

OPINION REVIEW

REVIEW

MINIREVIEWS

META-ANALYSIS

CASE REPORT

LETTERS TO THE EDITOR

World Journal of Hepatology

Monthly Volume 12 Number 8 August 27, 2020

Contents

ABOUT COVER

Editorial board member of *World Journal of Hepatology*, Dr. Alberto Ferrarese is a Gastroenterologist devoted to the field of hepatology. He obtained his MD degree at Padua University Hospital, Italy, where his ongoing career research has focused mainly on decompensated cirrhosis and liver transplantation. His main research interests are complications of cirrhosis, bacterial infection in cirrhosis, organ allocation in liver transplantation, and adherence and quality of life after liver transplantation. He has authored 40 articles published in international peer-reviewed journals and he serves as a reviewer for several international journals in the field of hepatology. (L-Editor: Filipodia)

AIMS AND SCOPE

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.

INDEXING/ABSTRACTING

The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Nikolaos T Pyrsopoulos, Ke-Qin Hu, Koo Jeong Kang

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5182/editorialboard.htm

PUBLICATION DATE
August 27, 2020

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

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

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 413-422

DOI: 10.4254/wjh.v12.i8.413

ISSN 1948-5182 (online)

OPINION REVIEW

Mechanisms and consequences of COVID-19 associated liver injury: What can we affirm?

Carlos Antunes Brito, Fabio Marinho Barros, Edmundo Pessoa Lopes

ORCID number: Carlos Antunes Brito 0000-0002-5963-8178; Fabio Marinho Barros 0000-0003-1446-7739; Edmundo Pessoa Lopes 0000-0002-3470-1564.

Author contributions: All contributing authors participated in the study to the conception or design of the work or the acquisition, analysis or interpretation of the papers and subsequent revisions of the manuscript.

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

Manuscript source: Unsolicited manuscript

Carlos Antunes Brito, Edmundo Pessoa Lopes, Department of Internal Medicine, Center of Medical Sciences of Federal University of Pernambuco, Recife, Pernambuco 50740600, Brazil

Carlos Antunes Brito, Edmundo Pessoa Lopes, Clinical Hospital of Federal University of Pernambuco, Recife, Pernambuco 50740900, Brazil

Carlos Antunes Brito, Edmundo Pessoa Lopes, Post-graduation Program of Tropical Medicine of Federal University of Pernambuco, Recife, Pernambuco 50670901, Brazil

Carlos Antunes Brito, Autoimmune Research Institute, Recife, Pernambuco 52011010, Brazil

Fabio Marinho Barros, Português Hospital of Pernambuco, Recife, Pernambuco 52010075, Brazil

Corresponding author: Carlos Alexandre Brito, MD, MSc, PhD, Adjunct Professor, Internal Medicine Department, Center of Medical Sciences, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235-Cidade Universitária, Recife, Pernambuco 50740600, Brazil. carlos.brito@ufpe.br

Abstract

Since the first reports of coronavirus disease 2019 (COVID-19) cases in December 2019 in China, numerous papers have been published describing a high frequency of liver injury associated with severe acute respiratory syndrome coronavirus 2 infection, many of them proposing a link between these findings and patient outcomes. Increases in serum aminotransferase levels (ranging from 16% to 62%) and bilirubin levels (ranging from 5% to 21%) have been reported and seem to be more often observed in patients with severe forms of COVID-19. Although absolute changes in these parameters are frequently seen, other variables, such as the ratio above the upper limit of normal, the onset of liver injury as a complication in severe cases and histopathological findings, reinforce that liver changes are of dubious clinical relevance in the course of this disease. Other factors must also be considered in these analyses, such as the repercussions of hemodynamic changes, the presence of thrombotic events, and, mainly, the possible drug-induced liver injury with the current, yet off-label, treatment. This paper aimed to analyze the currently available data on liver injury in patients with COVID-19.

Key words: COVID-19; SARS-CoV-2; Liver injury; Liver enzymes; Drug induced liver

Received: May 14, 2020
Peer-review started: May 14, 2020
First decision: June 2, 2020
Revised: June 5, 2020
Accepted: August 1, 2020
Article in press: August 1, 2020
Published online: August 27, 2020

P-Reviewer: Khoury T, Scalinci SZ
S-Editor: Zhang L
L-Editor: A
P-Editor: Li JH

injury; Pandemic

Core tip: The coronavirus disease 2019 (COVID-19) pandemic has affected millions worldwide, with high lethality. Papers have been describing liver injury but with divergent results; some have suggested a positive relationship between liver involvement and severity of infection. To evaluate this matter, some aspects, such as the frequency and severity of liver enzyme abnormalities, should be analyzed according to clinical and histopathological findings; other associated factors, such as interactions with the drugs used in COVID-19 treatment, should be analyzed as well. An overview of the aspects related to liver injury during COVID-19 infection was analyzed in this study according to evidence known to date.

Citation: Brito CA, Barros FM, Lopes EP. Mechanisms and consequences of COVID-19 associated liver injury: What can we affirm? *World J Hepatol* 2020; 12(8): 413-422
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/413.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.413

INTRODUCTION

The first reports of what is now known as coronavirus disease 2019 (COVID-19) came out in December 2019 in China, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the COVID-19 etiologic agent, subsequently spread worldwide. Currently, more than 200 countries have been affected, with approximately 3 million confirmed cases and more than 200000 deaths to date (as of May 5, 2020). Severe disease is observed in up to 20% of affected patients with a lethality rate that may eventually exceed 10%[1-4].

Recently, many papers have been published reporting gastrointestinal manifestations, including acute liver injury, with increased levels of aminotransferases, in COVID-19 patients; these manifestations have been reported more frequently in patients with severe forms of this disease. However, there is a wide variation of these findings in different studies[5-18].

Despite frequent reports of liver injury in patients with COVID-19, some questions remain: What is the liver enzymes' curve and how often do they rise above the upper limit of normal (ULN) serum level? Are these abnormalities correlated with COVID-19 disease severity? Can increased serum aminotransferase levels reflect the degree of injury? What is the liver injury frequency in cases with a severe course of disease with complications and death? What do histopathological findings suggest? Are the liver parenchymal changes due to the systemic disease consequences or a direct effect of SARS-CoV-2? May drug use for COVID-19 be the cause of liver injury?

FREQUENCY OF INCREASE IN LIVER FUNCTION ENZYMES IN COVID-19 PATIENTS

Liver injury related to SARS-CoV-2 disease has been defined by increased liver enzyme serum levels, mainly aminotransferases and bilirubin, during the infection course in patients with or without previous liver disease[5-18]. Wide variability in deviations of liver enzyme serum levels from normal values is observed in infected patients, with an elevation frequency ranging from 16% to 62% for aminotransferases and from 5% to 21% for bilirubin. These abnormalities are seen mostly in severe forms of COVID-19 (Tables 1 and 2)[6,10,12,14,16].

In fact, the study by Guan *et al*. found high aminotransferase serum levels in 22% of 757 hospitalized patients, with elevated aspartate transaminase (AST) in 18.2% (112/615) of non-severe patients, in 39% (56/142) of severe patients and in 50% (26/52) in those with complicated outcomes such as intensive care unit (ICU) hospitalization, mechanical ventilation or death. In addition, bilirubin values above the ULN were present in 13.3% of non-severe patients and 20.8% of severe patients[6].

Table 1 Different studies that evaluates liver enzymes according to disease severity and treatment protocol

	n	Disease severity, *n* (%)	Death, *n* (%)	Complications, *n* (%)	Treatment (%), Antiviral therapy; Antibiotic therapy; Antimalarial	Treatment (Drugs)
Xie *et al*[12]	79	Moderate: 51 (64.5%), Severe: 28 (2.5%)	0	NR	NR	NR
Huang *et al*[5]	41	Non-severe: 28 (68.3%), Severe: 13 (31.7%)	6 (15%)	Acute respiratory distress: 12 (29%); Acute cardiac injury: 5 (12%); Acute kidney injury: 3 (7%); Secondary infection: 4 (10%); Shock: 3 (7%)	All patients: AV (93%); AB (100%) Non-ICU care: AV (93%); AB (100%) ICU care: AV (92%); AB (100%)	Antiviral: Oseltamivir Antibiotic: NR
Guan *et al*[6]	1099	Non severe: 926 (84.3%), Severe: 173 (15.7%)	15 (1.4%)	Acute respiratory distress: 37 (3.4%); Acute kidney injury: 6 (0.5%); Septic Shock: 12 (1.1%); Disseminated intravascular coagulation: 1 (0.1); Rhabdomyolysis: 2 (0.2)	All patients: AV (35.8%); AB (58%) Non-severe: AV (33.8%); AB (53.8%) Severe: AV (46.2%); AB (80.3%)	Antiviral: Oseltamivir Antibiotic: NR
Zhang *et al*[13]	115	Non severe: 84 (73%), Severe: 31 (27%)	1 (0.9%)	NR	NR	NR
Cao *et al*[31]	128	Non severe: 107 (83.6%), Severe: 21 (16.4%)	0%	NR	NR	NR
Chen *et al*[9]	99	Non severe: 76 (77%), Severe (ICU): 23 (23%)	11 (11%)	Acute respiratory injury: 8 (8%); Acute kidney injury: 3 (3%); Septic Shock: 4 (4%); Ventilator-associated pneumonia: 1 (1%)	All patients: AV (76%), AB (71%)	Antiviral: Oseltamivir, ganciclovir, lopinavir/ritonavir Antibiotic: Cephalosporins, quinolones, carbapenems, tigecycline, linezolid
Richardson *et al*[18]	5700	Non severe: 4414 (77.4%), Severe (ICU): 1286 (22.6%)	553/2634 (21%)	Acute kidney injury: 1370 (24%); Acute Hepatic injury 89 (1.6%)	NR	NR
Zhang *et al*[10]	221	Non severe: 166 (75%), Severe: 55 (25%)	12 (5.4%)	Acute respiratory injury: 48 (21.7%); Acute kidney injury: 10 (4.5%); Acute cardiac injury: 17 (7.6%); Arrhythmia: 24 (11%); Shock: 15 (6.8)	All patients: AV (88.7%) Non-severe: AV (88%), Severe: AV (90.9%)	Antiviral: NR Antibiotic: NR
Bhatraju *et al*[11]	24	Severe: 24 (100%)	12 (50%)	Shock: 17 (71%)	All patients: AV (29.2%)	Antiviral: Remdesivir
Zhou *et al*[8]	191	General: 72 (38%), Severe: 66 (35%); Critical: 53 (28%)	54 (28%)	Sepsis: 112 (59%); Respiratory failure: 103 (54%); Heart failure: 44 (23%); Septic shock: 38 (20%); acute cardiac injury: 33 (17%); Acute kidney injury: 28 (15%); Secondary infection: 28 (15%)	All patients: AV (21%), AB (95%) Survivors: AV (21%), AB (93%)Non-survivors: AV (22%), AB (98%)	Antiviral: Lopinavir/ritonavir Antibiotic: NR
Pan *et al*[15]	204	NR (total)	36 (17.6%)	NR	All patients: AV (90.2%), AB (64.7%)	Antiviral: Lopinavir/ritonavir Antibiotic: NR
Wang *et al*[7]	138	Non severe: 102 (74%), Severe (ICU): 36 (26%)	6 (4.3%)	Respiratory failure: 27 (19.6%); Arrhythmia: 23 (16.7%); Shock: 12 (8.7%); Acute cardiac injury: 10 (7.2%); Acute Kidney injury: 5 (3.6%)	All patients: AV (89.9%); AB (100%) Non-ICU care: AV (88.2%); ICU care: AV (94.4%)	Antiviral: Oseltamivir Antibiotic: Moxifloxacin, ceftriaxone, azithromycin
Fu *et al*[16]	350	Common: 211 (60.3%), Severe: 88 (25.2%); Critical ill: 51 (14.5%)	34 (9.8%)	NR	NR	NR
Chen *et al*[17]	113	NR	113 (41%)	Type I respiratory failure: 18/67 (27%), Sepsis: 179 (65%), Acute cardiac injury: 89/203 (44%), Heart failure: 43/176 (24%), Acute kidney injury: 29 (11%)	All patients: AV (86%); AB (91%); Recovered: AV (91%); AB (89%) Deaths: AV (79%); AB (93%)	Antiviral: Oseltamivir, arbidol, lopinavir/ritonavir Antibiotic: Moxifloxacin, cefoperazone, or azithromycin

NR: Not report; ICU: Intensive care unit; AV: Antiviral therapy; AB: Antibiotic therapy; AM: Antimalarial.

Moreover, among 24 hospitalized ICU patients, Bhatraju *et al*[11] found increases of 41% and 32% in AST and alanine transaminase (ALT) levels, respectively. Huang *et al*[5,7], when assessing the frequency of abnormalities among 41 patients, found AST alterations in 62% of ICU patients compared to 25% of non-ICU hospitalized patients, similar to the findings in other studies.

According to these findings, the frequency of aminotransferase elevation during COVID-19 is directly related to the disease severity; that is, the higher the COVID-19 severity, the greater the chance of liver enzyme elevation. Then, increases of aminotransferases serum levels would be a predictor factor of severity of SARS-CoV-2 infection.

SERUM LEVELS OF LIVER ENZYMES AND LIVER INJURY

It must be acknowledged, however, that in acute liver injury, hepatocyte necrosis extension is reflected by aminotransferase serum levels. Although these changes are often described in COVID-19 cases, the aminotransferase serum level abnormalities are discrete[6-8,10-16].

In a study by Cao *et al*[14] 107 non-severe COVID-19 patients had a mean AST of 30.63 U/L (30.63 ± 18.85), and even among the 21 severe cases, serum levels were lower than 100 U/L (44.13 ± 36.26)[14]. In another study involving 115 patients, 27% were categorized as severe, and among them, 85% had serum AST levels below 50 U/L, with no cases presenting an AST above 150 U/L and just one case with an ALT level above this value. For bilirubin, only seven cases presented with serum levels higher than ULN (> 21 μmol/L), and they did not exceed 31.5 μmol/L[13].

Using a stratification score for the variability in serum levels among 341 patients, Cai *et al*[19] found 25% of AST abnormalities at admission, with most of these cases (91%) having serum levels between one and two times above ULN; 8% had an elevation range of two and three times above ULN, and only 1% had an elevation above three times the ULN[19].

In the evaluation of cases that progressed to a fatal outcome, the same pattern persisted. In the study by Chen *et al*[17], 52% (59/113) of deceased patients presented an AST increase, with median serum levels of 45 U/L (IQR: 31.0-67.0). On the other hand, only 25 out of 161 (16%) patients who recovered presented AST levels higher than the ULN, with median serum levels of 25.0 (IQR: 20.0-33.3)[17].

In an analysis of 82 deaths, Zhang *et al*[10] compared the aminotransferases and bilirubin values at admission and 24 h before the fatal outcome. The alterations were higher close to the timing of death, with AST, ALT and bilirubin values above the ULN occurring in 70%, 40% and 30.6%, respectively. However, the absolute values

Table 2 Frequency and serum levels of hepatic enzymes abnormalities in different studies

	AST abnormalities %[1]	Serum levelsAST U/L[1]	ALT abnormalities %	Serum levelsALT U/L[1]	Total bilirubinabnormalities %	Serum levels, Total bilirubin mol/L[1]
Xie *et al*[12]	35.4%	[2]All patients: 30 (20-50); Moderate: 28 (22-48); Severe: 35 (22-55)	31.6%	[2]All patients: 34 (18-67); Moderate: 28 (21-43.5); Severe: 36.5 (17.5-71.5)	5.1%	[2]All patients: 13.6 (8.8-17.6); Moderate: 13.9 (8.9-18.7); Severe: 12.7 (8.1-15.4)
Huang *et al*[5]	[2]All patients: 37%, Non-ICU: 25%; ICU: 6%	[2]All patients: 34 (26-48); Non-ICU: 34 (24-40.5); ICU: 44 (30-70)	NR	[2]All patients: 32 (21-50); Non-ICU care: 27 (19.5-40); ICU care: 49 (29-115)	NR	[2]All patients: 11.7 (9.5-13.9); Non-ICU care: 10.8 (9.4-12.3); ICU care: 49 (11.9-32.9)
Guan *et al*[6]	All patients: 22.2%; Non-severe: 18.2%; Severe: 39.4%; ICU/IMV/Death: 50%	NR	All patients: 21.3%; Non-severe: 19.8%; Severe: 28.1%; ICU/IMV/Death: 40.8%	NR	All patients: 10.5%; Non-severe: 9.9%; Severe: 13.3%; ICU/IMV/Death: 20.8%	NR
Zhang *et al*[13]	17%	[2]All patients: 28.3 ± 15.6; ULN ≤ 50 U/L: 85%; 50-150 U/L: 15%; > 150: none	11%	[2]All patients: 25.71 ± 21.8; ULN: ≤ 50 U/L: 90.4%; 50-150 U/L: 8.7%; > 150: 0.9%	6.96%	[2]All patients: 11.31 ± 5.8; ULN: ≤ 21 μmol/L: 94%; 21-31.5 μmol/L: 6%
Cao *et al*[31]	NR	All patients: 30.63 ± 18.85; Non-severe: 27.98 ± 25.8; Severe: 44.13 ± 36.26	NR	All patients: 31.35 ± 20.36; Non-severe: 28.89 ± 31.83; Severe: 43.87 ± 47.8	NR	NR
Chen N *et al*[9]	35%	All patients: 34 (26-48)	28%	All patients: 39 (21-55)	18%	All patients: 15.1 ± 7.6
Richardson *et al*[10]	58.4%	All patients: 46 (31-71)	39%	All patients: 33 (21-55)	NR	NR
Zhang *et al*[10]	NR	All patients: 29 (22-49); Non-severe: 27 (20-38); Severe: 51 (29-78)	NR	All patients: 23 (16-39); Non-severe: 22 (14-33); Severe: 32 (22-57)	NR	All patients: 10 (8-14.2); Non-severe: 9.6 (7.9-13.8); Severe: 11.4 (8.6-17.4)
Bhatraju *et al*[11]	41%	NR	32%	NR	NR	0.6 (0.5-0.7)
Zhou *et al*[8]	NR	NR	All patients: 31% Survivor: 24%; Non-survivor: 48%	All patients: 30 (17-46); Survivor: 27 (15-40); Non-survivor: 40 (24-51)	NR	NR
Pan *et al*[15]	NR	All patients: 35.6 ± 59.6	NR	All patients: 35.8 ± 48.5	NR	All patients: 13.3 ± 10.2
Wang *et al*[7]	NR	All patients: 31 (24-51); Non-ICU: 29 (21-38); ICU: 52 (30-70)	NR	All patients: 24 (16-40); Non-ICU: 23 (15-36); ICU: 35 (19-57)	NR	All patients: 9.8 (8.4-14.1); Non-ICU: 9.3 (8.2-12.8); ICU: 11.55 (9.6-18.6)
Fu *et al*[16]	NR	Common: 16 (20-35); Severe: 29 (23-54); Critical ill: 49 (35-80)	NR	Common: 22 (14-35); Severe: 23 (15-36); Critical ill: 33 (19-61)	NR	[2]Common: 10.4 (7.5-14.7) Severe: 10.9 (8.0-16.2); Critical ill: 12.6 (10.5-17)
Chen *et al*[17]	All patients: 31%; Deaths: 52%;	All patients: 16 (22-46);	All patients: 22%; Deaths: 27%;	All patients: 23 (15-38);	NR	All patients: 9.6 (6.7-13.5);

Recovered: 16%	Recovered: 25 (20-33.3); Deaths: 45 (31-67)	Recovered: 19%	Recovered: 20 (14.2-32); Deaths: 28 (18-57)	Recovered: 8.4 (5.8-11.2); Deaths: 12.6 (9.4-16.7)

[1]Data is mean ± SD or median.
[2]Values on admission. ALT: Alanine transaminase; AST: Aspartate transaminase; NR: Not report; ICU: Intensive care unit; IMV: Invasive mechanical ventilation; ULN: Upper limit of normal.

were not as high as supposed, with AST, ALT and bilirubin serum levels averaging 72 U/L (IQR: 30-71), 26 U/L (IQR: 18.5-47.5) and 13.6 μmol/L (IQR: 10-22.9) on admission, respectively, and 74.5 U/L (IQR: 35.5-184), 30.5 U/L (IQR: 22-102.5) and 26 μmol/L (IQR: 18.5-47.5) 24 h before death. Moreover, the authors also compared COVID-19 patients with 119 patients with community-acquired pneumonia due to other etiologies and did not observe significant differences in aminotransferase serum levels[10].

Although uncommon, there have been published reports of significant elevation in liver enzymes, such as the elevations described among 99 COVID-19 patients in the study by Chen *et al*[9], with one case (1%) presenting an ALT of 7590 U/L and an AST of 1145 U/L[9].

According to the studies published so far, liver enzyme serum levels are not very elevated during SARS-CoV-2 infection; most often they are below twice the ULN. These findings suggest that hepatocyte necrosis on the hepatic parenchyma is discrete and that liver injury does not seem to be very relevant. Likewise, serum levels appear to increase according to the progression time of the disease COVID-19 severity. To date, rare cases of high elevations of liver enzymes have been described during COVID-19.

HISTOPATHOLOGICAL FINDINGS

Therefore, the evidence shows that liver injury has little clinical relevance in the course of COVID-19 disease. Nevertheless, liver failure is a rare complication in severe cases, even though hypoxia and shock may contribute to hepatocyte damage. On the other hand, reports of acute respiratory failure, heart failure, acute cardiac injury, acute kidney injury and shock predominate in many studies as more frequent complications and causes of death[5-8,10,13,17,18].

Little is known about how hepatocytes are damaged during SARS-CoV-2 infection. However, years ago, evaluation of three patients with SARS-CoV confirmed the presence of coronavirus in liver tissue by RT-PCR, but the virus was present in low titles because no viral inclusions were observed ultrastructurally[20,21].

Additionally, postmortem histopathological studies show discrete changes in the hepatic parenchyma, and these findings may have multifactorial causes related to the viral mode of action, inflammatory response, adjacent repercussions of systemic

hemodynamic alterations, coagulation disorders or drug induced liver injury (DILI)[22-24].

In a study developed in Milan with 48 liver biopsies from postmortem COVID-19 patients, vascular changes in the portal vein were observed, with an increased number of portal branches, terminal vessel dilations, and thrombi found in portal and sinusoidal vessels. The inflammatory alterations were discrete, with mild portal and lobular infiltrates. The authors suggested that histopathological findings in COVID-19 are suggestive of changes in the intrahepatic blood vessel network secondary to systemic alterations induced by SARS-CoV-2 that could indicate that they are a target, in addition to the lung parenchyma or cardiovascular system. However, they conclude that liver failure is not a major concern in COVID-19 cases, and this organ is not a significant inflammatory injury target[23].

Moreover, some authors suggest that liver injury in COVID-19 may be triggered by viral replication itself within hepatocytes, since SARS-CoV-2 binds cells through the angiotensin-2-converting enzyme, especially in bile epithelium cells[23]. Nevertheless, the low serum aminotransferase levels observed in COVID-19 patients do not suggest that the exacerbated inflammatory response or direct viral injury to hepatocytes is relevant. The pattern of the aminotransferase curve during SARS-CoV-2 infection is different from those observed in hepatitis associated with other epidemic viruses that induce frequent and intense LFT elevations due to diffuse parenchymal necrosis, as found, for example, in patients with dengue or yellow fever[25-28]. In fact, the liver injury found in COVID-19 looks that one observed in other viruses, such as SARS, MERS and influenza[29-31].

Lastly, the liver histopathological findings observed in most patients with COVID-19 are suggestive of vascular abnormalities possibly resulting from increased arterial flow to the liver secondary to cardiac distress and thrombotic phenomena in the portal and sinusoidal vessels[23]. Nonetheless, eventually in some patients might be the involvement of some drug, as antibiotics or antivirals, in the induction of liver injury.

OTHER CAUSES OF LIVER INJURY IN SARS-CoV-2

Other factors may be involved in hepatic enzyme alterations. Several medications used to treat COVID-19, mainly antivirals such as lopinavir/ritonavir and remdesivir, chloroquine and hydroxychloroquine antimalarials, antibiotics including azithromycin, or immune-modulators such as tocilizumab, may lead to DILI. Therefore, physicians should be aware of the LFT profile in response to drug use to help attribute liver injury to the natural history of infection[19,32-39].

Antivirals such as lopinavir/ritonavir and remdesivir that have been recently used for COVID-19 may be associated with liver injuries. DILI from lopinavir/ritonavir has been reported in 2%-10% of patients[32]. Cai *et al*[19] published a trial in which 417 patients using lopinavir/ritonavir presented a higher risk for developing liver injury [OR of 4.44 (P < 0.01)] and higher levels of bilirubin and gamaGT during hospitalization (P < 0.004)[19].

The use of antimicrobials and antibiotics, frequently prescribed for suspicious or confirmed very ill COVID-10 patients, is considered a frequent etiology of DILI[33].

In the reviewed papers, antivirals and antimicrobials were often prescribed to COVID-19 patients, ranging from 21% to 93% and 58% to 100%, respectively and many times they were used simultaneously[5-11,13,17]. Liver enzymes abnormalities were often seen, even in the trials that less frequently used antiviral treatment[6,8,11]. In Zhou *et al*[8] trial, lopinavir/ritonavir was used in around 20% of the patients either they survive or not, and ALT abnormalities was observed in 24% and 48% respectively[8]. There is also a wide variability in antivirals prescribed to patients, such as oseltamivir, remdesivir, lopinavir/ritonavir and ganciclovir. The same is also observed with the use of antimicrobials, either alone or in combination with antivirals and other drugs. This does not allow us to stablish a clear causality relationship or even the amount of importance to the use of this drugs and the liver injury. Besides it the histopathological findings do not suggest a DILI pattern[23].

Hydroxychloroquine (HCQ) has been used, though still off-label, in several countries, despite the limited number of studies published so far and divergent opinions regarding its efficacy. Although hepatotoxicity in users of HCQ is uncommon, LFTs and severe liver dysfunction have been documented[37-40].

Makin *et al*[40] reported two cases of patients with rheumatological disease who, after 2 wk of using 400 mg of HCQ daily, were admitted with fulminant hepatitis; one required a liver transplant, and both patients died[40]. Recently, Falcão *et al*[37] reported

an increase in LFTs in very sick COVID-19 patients on drug treatment, with return to normal levels once the drugs were halted[37].

The mechanisms of hepatic injury related to HCQ are poorly established, and toxicity may be due to reactive metabolites and oxidative stress induced by this drug or an idiosyncratic toxic or synergistic effect associated with inflammatory processes induced by the infection itself[41-43].

More recently, azithromycin in association with HCQ has become a therapeutic option for COVID-19 patients[44,45]. Biliary and hepatocellular injury have been associated with azithromycin use[34-36]. Another report with 18 patients presenting with azithromycin-induced DILI described a wide range of histopathological abnormalities, including hepatitis, veno-occlusive changes and/or central venulitis acute cholestasis and cholestatic hepatitis[35].

Due to the significantly increased use of HCQ and azithromycin during COVID-19 disease treatment, liver toxicity related to these drugs must be considered, and liver abnormalities should not be solely attributable to SARS-CoV-2 infection itself; the high risk of DILI seen in these scenarios should not be neglected. If DILI is suspected, COVID-19 drugs should be promptly halted.

Additionally, it is highly difficult to stablish a causality relationship between a specific drug and liver injury during COVID-19 infection, because most of the times they are used as combination of antimalarials, antivirals, antimicrobials, anticoagulants and sometimes vasoactive drugs. It is also worth remembering that the most severe cases, which do not present favorable evolution, are those where more drugs are administered in the fight against the disease.

CONCLUSION

Despite the common descriptions of liver enzyme abnormalities observed in COVID-19 patients, the frequency, intensity and impact of liver injury are discrete and of little clinical significance regarding morbidity or mortality of this disease. A better understanding of the natural history of liver involvement may be addressed in the near future with well-designed prospective studies regarding viral and immunologic research.

REFERENCES

1 **Ashour HM**, Elkhatib WF, Rahman MM, Elshabrawy HA. Insights into the Recent 2019 Novel Coronavirus (SARS-CoV-2) in Light of Past Human Coronavirus Outbreaks. *Pathogens* 2020; 9 [PMID: 32143502 DOI: 10.3390/pathogens9030186]

2 **World Health Organization**. Coronavirus disease 2019 (COVID-19) Situation Report. World Health Organization, 2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

3 **Wu Z**, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020 [PMID: 32091533 DOI: 10.1001/jama.2020.2648]

4 **Mizumoto K**, Chowell G. Estimating Risk for Death from Coronavirus Disease, China, January-February 2020. *Emerg Infect Dis* 2020; **26**: 1251-1256 [PMID: 32168464 DOI: 10.3201/eid2606.200233]

5 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

6 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

7 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

8 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]

9 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

10 **Zhang G**, Hu C, Luo L, Fang F, Chen Y, Li J, Peng Z, Pan H. Clinical features and short-term outcomes of 221 patients with COVID-19 in Wuhan, China. *J Clin Virol* 2020; **127**: 104364 [PMID: 32311650 DOI: 10.1016/j.jcv.2020.104364]

11 **Bhatraju PK**, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, Greninger AL, Pipavath S, Wurfel MM, Evans L, Kritek PA, West TE, Luks A, Gerbino A, Dale CR, Goldman JD, O'Mahony S, Mikacenic C. Covid-19 in Critically Ill Patients in the Seattle Region - Case Series. *N Engl J Med* 2020; **382**: 2012-2022 [PMID: 32227758 DOI: 10.1056/NEJMoa2004500]

12 **Xie H**, Zhao J, Lian N, Lin S, Xie Q, Zhuo H. Clinical characteristics of non-ICU hospitalized patients with coronavirus disease 2019 and liver injury: A retrospective study. *Liver Int* 2020; **40**: 1321-1326 [PMID: 32239591 DOI: 10.1111/liv.14449]

13 **Zhang Y**, Zheng L, Liu L, Zhao M, Xiao J, Zhao Q. Liver impairment in COVID-19 patients: A retrospective analysis of 115 cases from a single centre in Wuhan city, China. *Liver Int* 2020 [PMID: 32239796 DOI: 10.1111/liv.14455]

14 **Cao WL**, Shi L, Chen L, Xu XM, ZW. Clinical features and laboratory inspection of novel coronavirus pneumonia (COVID-19) in Xiangyang, Hubei. *medRxiv* 2020 [DOI: 10.1101/2020.02.23.20026963]

15 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

16 **Fu L**, Fei J, Xu S, Xiang HX, Xiang Y, Tan ZX, Li MD, Liu FF, Li Y, Han MF, Li XY, Zhao H, Xu DX. Acute liver injury and its association with death risk of patients with COVID-19: a hospital-based prospective case-cohort study. *medRxiv* 2020 [DOI: 10.1101/2020.04.02.20050997]

17 **Chen T**, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H, Wang T, Guo W, Chen J, Ding C, Zhang X, Huang J, Han M, Li S, Luo X, Zhao J, Ning Q. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020; **368**: m1091 [PMID: 32217556 DOI: 10.1136/bmj.m1091]

18 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; and the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]

19 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]

20 **Li X**, Wang L, Yan S, Yang F, Xiang L, Zhu J, Shen B, Gong Z. Clinical characteristics of 25 death cases with COVID-19: A retrospective review of medical records in a single medical center, Wuhan, China. *Int J Infect Dis* 2020; **94**: 128-132 [PMID: 32251805 DOI: 10.1016/j.ijid.2020.03.053]

21 **Chau TN**, Lee KC, Yao H, Tsang TY, Chow TC, Yeung YC, Choi KW, Tso YK, Lau T, Lai ST, Lai CL. SARS-associated viral hepatitis caused by a novel coronavirus: report of three cases. *Hepatology* 2004; **39**: 302-310 [PMID: 14767982 DOI: 10.1002/hep.20111]

22 **Tian S**, Xiong Y, Liu H, Niu L, Guo J, Liao M, Xiao SY. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Mod Pathol* 2020; **33**: 1007-1014 [PMID: 32291399 DOI: 10.1038/s41379-020-0536-x]

23 **Sonzogni A**, Previtali G, Seghezzi M, Alessio MG, Gianatti A, Licini L, Zerbi P, Carsana L, Rossi R, Lauri E, Pellegrinelli A, Nebuloni M. Liver and COVID 19 infection: a very preliminary lesson learnt from histological post-mortem findings in 48 patients. Preprints 2020. [DOI: 10.20944/preprints202004.0438.v1]

24 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

25 **Souza LJ**, Alves JG, Nogueira RM, Gicovate Neto C, Bastos DA, Siqueira EW, Souto Filho JT, Cezário Tde A, Soares CE, Carneiro Rda C. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. *Braz J Infect Dis* 2004; **8**: 156-163 [PMID: 15361994 DOI: 10.1590/S1413-86702004000200006]

26 **Samanta J**, Sharma V. Dengue and its effects on liver. *World J Clin Cases* 2015; **3**: 125-131 [PMID: 25685758 DOI: 10.12998/wjcc.v3.i2.125]

27 **Escosteguy CC**, Pereira AGL, Marques MRVE, de Araujo Lima TR, Galliez RM, Medronho AR. Yellow fever: Profile of cases and factors associated with death in a hospital in the State of Rio de Janeiro, 2017-2018. *Rev Saude Publica* 2019; **53**: 1-12 [DOI: 10.11606/s1518-8787.2019053001434]

28 **Costa DS**, Moita LA, Alves EH, Sales AC, Rodrigues RR, Galeno JG, Gomes TN, Ferreira GP, Vasconcelos D. Dengue Virus and Yellow Fever Virus Damage the Liver: A Systematic Review About the Histopathological Profiles. *J Gastroenterol Hepatol Res* 2019; **8**: 2864-2870 [DOI: 10.17554/j.issn.2224-3992.2019.07.823]

29 **Chang HL**, Chen KT, Lai SK, Kuo HW, Su IJ, Lin RS, Sung FC. Hematological and biochemical factors predicting SARS fatality in Taiwan. *J Formos Med Assoc* 2006; **105**: 439-450 [PMID: 16801031 DOI: 10.1515/cclm-2020-0369]

30 **Saad M**, Omrani AS, Baig K, Bahloul A, Elzein F, Matin MA, Selim MA, Al Mutairi M, Al Nakhli D, Al Aidaroos AY, Al Sherbeeni N, Al-Khashan HI, Memish ZA, Albarrak AM. Clinical aspects and outcomes of 70 patients with Middle East respiratory syndrome coronavirus infection: a single-center experience in Saudi Arabia. *Int J Infect Dis* 2014; **29**: 301-306 [PMID: 25303830 DOI: 10.1016/j.ijid.2014.09.003]

31 **Cao B**, Li XW, Mao Y, Wang J, Lu HZ, Chen YS, Liang ZA, Liang L, Zhang SJ, Zhang B, Gu L, Lu LH, Wang DY, Wang C; National Influenza A Pandemic (H1N1) 2009 Clinical Investigation Group of China. Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. *N Engl J Med* 2009; **361**: 2507-2517 [PMID: 20007555 DOI: 10.1056/NEJMoa0906612]

32 **Sanders JM**, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* 2020 [PMID: 32282022 DOI: 10.1001/jama.2020.6019]

33 **Licata A**. Adverse drug reactions and organ damage: The liver. *Eur J Intern Med* 2016; **28**: 9-16 [PMID: 26827101 DOI: 10.1016/j.ejim.2015.12.017]

34 **Ellison CA**, Blackwell SB. Acute Hepatocellular Injury Associated With Azithromycin. *J Pharm Pract* 2020 [PMID: 31928122 DOI: 10.1177/0897190019894428]

35 **Martinez MA**, Vuppalanchi R, Fontana RJ, Stolz A, Kleiner DE, Hayashi PH, Gu J, Hoofnagle JH, Chalasani N. Clinical and histologic features of azithromycin-induced liver injury. *Clin Gastroenterol Hepatol* 2015; **13**: 369-376.e3 [PMID: 25111234 DOI: 10.1016/j.cgh.2014.07.054]

36 **Chalasani N**, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalkar J, Reddy KR, Watkins PB, Navarro V, Barnhart H, Gu J, Serrano J; United States Drug Induced Liver Injury Network. Features and Outcomes of 899 Patients With Drug-Induced Liver Injury: The DILIN Prospective Study. *Gastroenterology* 2015; **148**: 1340-52.e7 [PMID: 25754159 DOI: 10.1053/j.gastro.2015.03.006]

37 **Falcão MB**, Pamplona de Góes Cavalcanti L, Filgueiras Filho NM, Antunes de Brito CA. Case Report: Hepatotoxicity Associated with the Use of Hydroxychloroquine in a Patient with COVID-19. *Am J Trop Med Hyg* 2020; **102**: 1214-1216 [PMID: 32314698 DOI: 10.4269/ajtmh.20-0276]

38 **Abdel Galil SM**. Hydroxychloroquine-induced toxic hepatitis in a patient with systemic lupus erythematosus: a case report. *Lupus* 2015; **24**: 638-640 [PMID: 25424894 DOI: 10.1177/0961203314561667]

39 **Giner Galvañ V**, Oltra MR, Rueda D, Esteban MJ, Redón J. Severe acute hepatitis related to hydroxychloroquine in a woman with mixed connective tissue disease. *Clin Rheumatol* 2007; **26**: 971-972 [PMID: 16575495 DOI: 10.1007/s10067-006-0218-1]

40 **Makin AJ**, Wendon J, Fitt S, Portmann BC, Williams R. Fulminant hepatic failure secondary to hydroxychloroquine. *Gut* 1994; **35**: 569-570 [PMID: 8175002 DOI: 10.1136/gut.35.4.569]

41 **Wei CH**, Penunuri A, Karpouzas G, Fleishman W, Datta A, French SW. Troxis necrosis, a novel mechanism for drug-induced hepatitis secondary to immunomodulatory therapy. *Exp Mol Pathol* 2015; **99**: 341-343 [PMID: 26297838 DOI: 10.1016/j.yexmp.2015.08.006]

42 **Jamshidzadeh A**, Heidari R, Abazari F, Ramezani M, Khodaei F, Ommati MM, Ayarzadeh M, Firuzi R, Saeedi A, Azarpira N, Najibi A. Antimalarial drugs-induced hepatic injury in rats and the protective role of carnosine. *Pharm Sci* 2016; **22**: 170-180 [DOI: 10.15171/PS.2016.27]

43 **Niknahad H**, Heidari R, Firuzi R, Abazari F, Ramezani M, Azarpira N, Hosseinzadeh M, Najibi A, Saeedi A. Concurrent Inflammation Augments Antimalarial Drugs-Induced Liver Injury in Rats. *Adv Pharm Bull* 2016; **6**: 617-625 [PMID: 28101469 DOI: 10.15171/apb.2016.076]

44 **Andreani J**, Le Bideau M, Duflot I, Jardot P, Rolland C, Boxberger M, Wurtz N, Rolain JM, Colson P, La Scola B, Raoult D. In vitro testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. *Microb Pathog* 2020; **145**: 104228 [PMID: 32344177 DOI: 10.1016/j.micpath.2020.104228]

45 **Gautret P**, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, Mailhe M, Doudier B, Aubry C, Amrane S, Seng P, Hocquart M, Eldin C, Finance J, Vieira VE, Tissot-Dupont HT, Honoré S, Stein A, Million M, Colson P, La Scola B, Veit V, Jacquier A, Deharo JC, Drancourt M, Fournier PE, Rolain JM, Brouqui P, Raoult D. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study. *Travel Med Infect Dis* 2020; **34**: 101663 [PMID: 32289548 DOI: 10.1016/j.tmaid.2020.101663]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 423-435

DOI: 10.4254/wjh.v12.i8.423

ISSN 1948-5182 (online)

REVIEW

Review: Pathogenesis of cholestatic liver diseases

Raquel T Yokoda, Eduardo A Rodriguez

ORCID number: Raquel T Yokoda 0000-0003-1780-4446; Eduardo A Rodriguez 0000-0001-9684-8273.

Author contributions: Yokoda RT and Rodriguez EA wrote the paper and approved the final version.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Received: May 6, 2020
Peer-review started: May 6, 2020
First decision: May 24, 2020
Revised: June 7, 2020
Accepted: August 1, 2020
Article in press: August 1, 2020
Published online: August 27, 2020

P-Reviewer: Zhang XQ

Raquel T Yokoda, Department of Anatomic and Clinical Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY 10467, United States

Eduardo A Rodriguez, Department of Gastroenterology, Hepatology and Nutrition, University of Utah, Salt Lake City, UT 84132, United States

Corresponding author: Eduardo A Rodriguez, FACP, MD, Assistant Professor, Department of Gastroenterology, Hepatology and Nutrition, University of Utah, 30 N 1900 E, Room 4R118, Salt Lake City, UT 84132, United States. eduardo.rodriguez@hsc.utah.edu

Abstract

Cholestatic liver diseases (CLD) begin to develop after an impairment of bile flow start to affect the biliary tree. Cholangiocytes actively participate in the liver response to injury and repair and the intensity of this reaction is a determinant factor for the development of CLD. Progressive cholangiopathies may ultimately lead to end-stage liver disease requiring at the end orthotopic liver transplantation. This narrative review will discuss cholangiocyte biology and pathogenesis mechanisms involved in four intrahepatic CLD: Primary biliary cholangitis, primary sclerosing cholangitis, cystic fibrosis involving the liver, and polycystic liver disease.

Key words: Cholestasis; Cholangitis; Epigenomics; Immunogenetics; Pathogenesis; Bile acid

Core tip: Several factors can condition bile flow derangements including environmental triggering factors, bile transport obstruction and conditions that alter bile concentration. Sustained pro inflammatory signaling associated with genetic and/or epigenetic dysregulation can condition a chronic dysfunctional state that can lead to a fibrogenic state with loss of homeostasis and sometimes malignant transformation.

Citation: Yokoda RT, Rodriguez EA. Review: Pathogenesis of cholestatic liver diseases. *World J Hepatol* 2020; 12(8): 423-435
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/423.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.423

S-Editor: Gong ZM
L-Editor: A
P-Editor: Li JH

INTRODUCTION

Cholestatic liver diseases (CLD) encompasses progressive cholangiopathies, which may evolve to end-stage liver disease. In the United States from 1988 to 2018, this group of illness corresponded to 14.2% of all liver transplants[1]. Thus far, their high morbidity and mortality are an economic burden that evolved from the lack of effective treatments. Moreover, 10% to 40% of these patients will have a recurrence of the primary disease after liver transplantation (LT)[2].

New prospective therapeutic targets are an unmet necessity, a number of which are under preclinical development. To evaluate these potential therapies, it is essential to understand the primary target of these pathologies, the cholangiocytes. This review will reinforce the current understanding of the core concepts of CLD pathogenesis in the light of the last translational advancements that may impact clinical management.

CLD: COMMON PATHOGENIC MECHANISMS

Several factors can condition bile flow derangements (Figure 1). Although environmental triggering factors are mostly unknown, antigenic stimuli, exotoxins, endotoxins, xenobiotics, and microorganisms can promote cholangiocyte reaction that will evolve into a cholestatic state[3]. Bile transport obstruction is another predisposing factor. Intrahepatic and extrahepatic obstruction can take place due to extrinsic benign compression (cystic diseases), malignant mass effect (cholangiocarcinomas), and also as a consequence of cholelithiasis formation or migration throughout the biliary tree. Moreover, conditions that slow biliary flow promote a cholestatic state with increased bile acid (BA) concentration. Sepsis, hyperestrogenic states (pregnancy), congestive heart failure, and dysfunction of BA transporter genes may alter the main characteristics of BA, conditioning a more cytotoxic BA component.

Early cholangiocyte response may allow resolution of injury, however, sustained pro-inflammatory signaling associated with disregulation of genetic and/or epigenetic regulatory mechanisms could condition late dysfunctional permanent state. Eventually fibrogenic state with biliary and periportal fibrosis, loss of tissue homeostasis and autocrine and paracrine remodeling would be achieved. Ultimately, proliferation may lead to cell-cycle alteration, senescence, apoptosis, ductopenia, mesenchymal infiltration and sometimes malignant transformation. To date, new therapeutic targets are being developed for each CLD considering the core of this pathogenic process. The main framework will be analyzed along with the foundation for potential clinical development.

Ductular reaction: First core concept

Intra and extra-hepatic bile ductules of different sizes are lined by cholangiocytes, which are epithelial cells that regulate and modify bile volume and composition[3]. These vary in size, metabolic rate as well as proliferative and plasticity capabilities. Biliary differentiation pathways are being more thoroughly understood and so it is now known that hepatocytes and cholangiocytes have a common stem cell precursor, and trans differentiation may occur in massive parenchymal loss from one to another, although the exact mechanisms are not well understood[4].

Ductular reaction (DR) is part of the injury response. It is triggered by cholestasis which activates the hepatic progenitor cells in CLD[5]. The sonic-hedgehog pathway promotes both cholangiocyte maturation and deposition of fibronectin in ductular-reactive cells[6]. DR may induce injury resolution, or, biliary fibrosis in the presence of perpetuating transcriptional inflammatory addiction. The cytokine panel for this transcriptional impairment depends on the disease phenotype and ultimately will condition different histological classifications beyond the scope of this review[7]. Figure 2 lists the dominant spectrum of CLD.

Bile acid toxicity and mitochondrial dysfunction

The second core fundamental framework of CLD pathogenesis is BA cytotoxicity and mitochondrial dysfunction. Besides its functional role of converting lipid bilayers into mixed micelles, BA are endogenous ligands that activate a network of receptors including nuclear receptor farnesoid X (FXR), vitamin D3 receptor (VDR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), membrane G protein-coupled bile acid receptor-1, and Takeda-G-protein receptor5 (TGR5). Indeed, FXR and TGR5 provide an anti-inflammatory liver response in mouse models[8]. In fact, FXR mutations have been considered a cause of progressive familial intrahepatic cholestasis. Intestinal

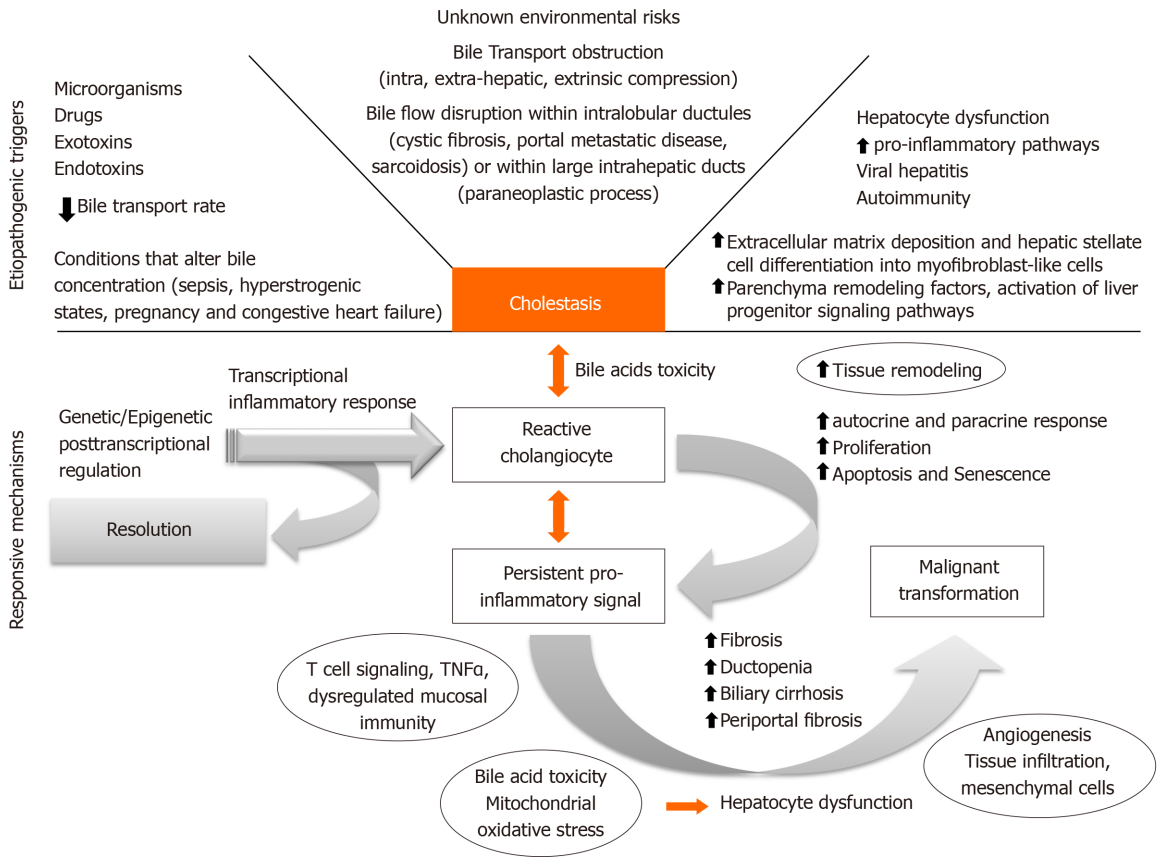

Figure 1 Core pathogenic mechanism of cholestatic liver diseases.

activation of FXR increases FGF15, a bile synthesis repressor through CYP7A1, a main regulatory enzyme, which reduces the pool size of BA and protects against escalating pro-inflammatory signaling in mouse models[9].

Likewise, BA hepatobiliary transport dysfunction may lead to several phenotypes of cholestatic diseases. Although transcellular BA transport details are mostly unknown, a number of apical and basolateral transporters have been identified. After synthesis of BA in the liver by CYP7A1 and hydroxylation by CYP8B1, bile acids and phospholipids are excreted and secreted across the canalicular membrane of hepatocytes into the biliary tree by BSEP (bile salt export pump/ABCB11) and ABCB4 (ATP binding cassette subfamily B member 4), respectively. BA are then re-uptaken in the terminal ileum by ASBT (apical sodium-dependent bile acid transporter/SCL10A2), and released into the portal system by a basolateral transporter (OSTα/β) and may later be re-uptaken by the liver *via* NTCP (Na+/taurocholate cotransporting polypeptide) or OATP (organic anion transporting polypeptides) transporters. Intrahepatic BA can further be processed by hydroxylation, glucuronidation or sulfation, and excreted back into sinusoidal and systemic circulation by OSTα/β and MRP3/4 bile acid transporters. Critical steps in the enterohepatic circulation are regulated by the BA receptor FXR, which limits BA uptake and synthesis by enhancing biliary and basolateral BA export. FGF19, a gut-derived FXR-dependent enterocrine hormone, suppresses hepatic bile acid synthesis and induces gallbladder filling when it is activated by high intestinal BA concentrations[10].

Recently, AMP-activated protein kinase (AMPK) signaling pathways have been implicated in the pathogenesis of drug-induced cholestasis[11]. An example of this pathway is metformin. An older study reported that after 2-3 wk of metformin usage, several patients developed portal inflammation and ductular proliferation[12].

Moreover, it is well-known that the hydrophilic profiles in BA spectrum protects against apoptosis (TCA and UDCA), while those in the hydrophobic range induce hepatic apoptosis and liver injury (TLCA and GCDCA). Additionally, accumulation of cytotoxic BA activates NF-κB-mediated inflammatory cytokines. This pathway is significant in intrahepatic cholestasis of pregnancy as it may arrest placental inflammation[13].

Several studies have described BA toxicities and established commonalities between

Figure 2 Cholestatic liver disease clinical spectrum.

this toxicity and mitochondrial dysfunction in extra-hepatic cholestasis[14]. *In vitro* studies demonstrated BA effect in normal liver cell line LO2. Glycochenodeoxycholic acid (GCDCA) stimulated cytotoxicity, disrupted the mitochondrial membrane potential, increasing production of reactive oxygen species (ROS), and leading to decreased mitochondrial mass and mitochondrial DNA content[14]. This feature can be fundamentally related to the development of anti-mitochondrial antibodies (AMA) in primary biliary cholangitis (PBC), consequence of infiltration by both CD4+ and CD8+ T cells reactive to conserved mitochondrial and nuclear antigens, particularly the E2 component of the pyruvate dehydrogenase complex — the principal target of circulating AMA[15]. Moreover, one study pointed deacetylation of the gene PGC-1α, peroxisome proliferator-activated receptor gamma, coactivator one alpha. PGC-1 α acts as an enzyme in mitochondria biogenesis[14]. In chronic intrahepatic cholestasis, the lipid peroxidation activates extracellular matrix cells, ROS, and aldehydes; which may exert direct fibrogenic effects on activated hepatic stellate cells[16].

Immunogenetic and epigenetic setpoints

The third fundamental aspect of the core framework is the influence of immunogenetics and epigenetics on immunoinflammatory response. Patients with CLD exhibit a variety of genetic alterations that account for the different elements of each CLD. However, some of those genes may be directly implicated in the progression rate of the cholestatic phenotype. Recently one study screened some of the progression-related candidate genes for primary biliary cholangitis[17]. They evaluated 315 DNA samples from patients for single nucleotide polymorphisms (SNPs) of 11 candidate genes involved in regulation of bile acid synthesis. Interestingly, genetic variants of CYP7A1, as well as its transcriptional activators (HNF4A and PPARGC1A), may activate bile acid synthesis in an escalating fashion leading to the progressing cholestasis in PBC[17]. It is significant that this gene could become a potential target for new therapeutics, or indirectly their transcriptional activators could serve as modulatory targets. This modulation is a type of epigenetic control of gene expression as a pathogenic mechanism.

Another study highlighted the central role of the IL-12-STAT4-Th1 pathway, a pro-inflammatory pathway in the progression of PBC, as well as the HLA associations and epigenetic effects[18,19]. Figure 3 shows a panel of immunogenetic genes, where those directly related to the T-cell function or the B-cells or the IL12-STAT4-Th1 are

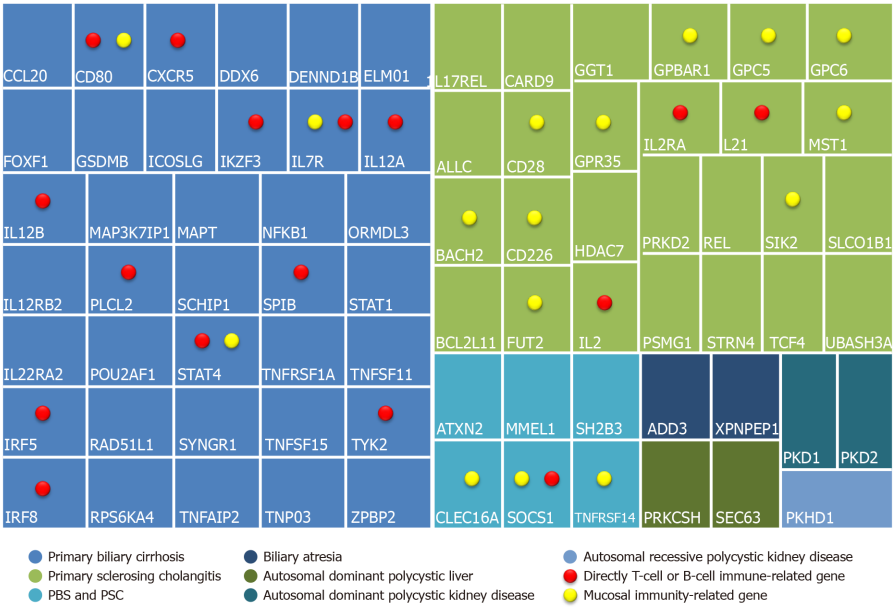

Figure 3 Immunogenetics related to the core of cholestatic liver diseases. PSC: Primary sclerosing cholangitis.

highlighted with a red dot. Additionally, genes associated with loss of immune-tolerance and epithelial permeability are marked with a yellow dot[20,21].

Dysfunctional matrix re-arrangements and fibrogenesis

To complete the core framework of CLD, dysfunctional matrix rearrangements and fibrogenesis are the fourth concept. Fibrogenesis is a dynamic process that appears intricate to immunoinflammatory mechanisms, secretion of tissue metalloproteinases, cytokine networks and derangements of mesenchymal cells infiltration with ultimate loss of tissue maintenance homeostasis[16]. The pattern of extra cellular matrix (ECM) accumulation in some CLD such as PBC is characterized by increased expression of mRNA encoding collagen type I, III, and IV, which in mesenchymal cells promotes the expansion of portal tracts, leading to deposition of excessive fibrillar ECM. In this way the fibrogenic processes involve damaged and non-damaged bile ducts as well as the periportal sinusoidal system, resulting in progressive cholestasis[16]. In contrast in patients with primary sclerosing cholangitis (PSC), the fibrogenic process has been compared to atherosclerosis onion-like concentric recruitment of pro-fibrogenic cells. Also animal models have reported vascular injury with ischemia of the bile duct epithelial cells during development of PSC lesion[22].

Hepatic stellate cells (HSC) are the primary source of myofibroblast during liver injury, however mesenchymal cells also give rise to myofibroblasts (portal myofibroblasts (PMF) as these cells are located in the portal tract)[23]. Studies in animal models of biliary cirrhosis (rat) reported that PMF use vascular endothelial growth factor A-containing microparticles signaling for newly formed vessels, driving scar progression, while acting as mural cells[24]. This type of fibrosis progression originating from the portal tract is crucial in cystic fibrosis-related liver fibrosis[25]. In PBC epigenetic influence has been observed in the discordance of monozygotic twins. The role of the CD40-CD40L interaction in T-cell and B-cell mechanisms has been reported in the decreased methylation of CD40L promoter regions amongst PBC patients compared with controls[18]. Similarly, X chromosome monosomy has been found on peripheral cells of PBC patients[26]. Recently the Milan PBC epigenetic Study Group reported demethylation of the CXCR3 promoter, which is negatively correlated with peripheral blood receptor expression in CD4+ T-cells[27]. The epigenetic role of demethylation is considered as CXCL9-11 is up-regulated in damaged bile ducts and it is a co-ligand for CXCR3, which is highly expressed in Th1 and Th17[28]. Another group evaluated the role of microRNA (miR), that can also promote downregulation of protein-coding gene expression. Down-Regulation of miR-122a and miR-26a was reported, as well as an increased expression of miR-328 and miR-299-5p. These microRNAs are known to affect cell proliferation, inflammation, oxidative stress metabolism, and apoptosis[29].

PRE-CLINICAL THERAPEUTIC DEVELOPMENTS

From a pathogenic standpoint, a number of therapeutic genetic and epigenetic targets can be considered. Some pathways already have one or more target drugs available. Table 1.

A number of preclinical studies may pave the way to new clinical advancements. A few of them are listed in Table 2, where we highlight the main pathogenic framework as described before.

CLINICAL TRIALS AND TRANSLATIONAL RESEARCH

The core fundamental concepts and pathogenic framework are platforms to build new models of clinical interventions for specific CLD. This section addresses the main cholestatic diseases individually.

Primary biliary cholangitis

PBC is characterized histologically by intralobular nonsuppurative bile duct destruction by lymphocytic cholangitis[30]. Patients with PBC often have a decreased quality of life as the disease progresses to hepatic fibrosis and end-stage liver disease. To date, one-third of the patients do not have a biochemical response to ursodeoxycholic acid (UDCA), which is primarily defined by bilirubin and alkaline phosphatase levels after one year of UDCA.

PBC inflammatory disarrays present with increased cholangiocyte chemokines released mainly CXCL10, CXCL9, CX3CL1, and CCL20, which involve the IL-12/IL23 pathways[31]. A number of novel therapeutics in immunomodulation such as fibrates and budesonide had promising results as an alternative to UDCA nonresponders, and recently obeticholic acid was approved by the FDA for UDCA non responders[32-34]. Advancements for PBC patients also include agonists for peroxisome proliferator-activated receptor alpha (PPARα), FXR, GR/PXR most often in combination with UDCA, fibrates, obeticholic acid (OCA) and budesonide, respectively[35]. Some of these translational therapeutics are mentioned in Table 3 and can also be used in PSC as discussed as follows.

Primary sclerosing cholangitis

There are currently no approved therapies for PSC. The disease causes a significant economic burden, and patients have high hospitalization and malignancy rates, often progressing to end-stage liver disease, requiring eventually liver transplantation. Table 3 summarizes the main translational research in the field. Novel approaches for PSC include transcriptional modifiers of bile formation, such as the agonists of FXR, PXR, GR and activation of PPARα. This activation can be promoted by fibrates as they decrease expression of inflammatory cytokines, also reducing hepatocyte BA synthesis. Another approach is the use of agonists of Takeda-G-protein 5 (TGR5), a BA membrane receptor expressed in various tissues as it can lower the levels of proinflammatory cytokines in bile ducts[36]. Other approaches include inhibitors of the ileal apical sodium BA transporter, derivatives of the FXR-induced fibroblast growth factor 19 (FXR-induced FGF19) from the ileum that suppress hepatic BA synthesis, and norursodesoxicholic acid (*nor*UDCA), a side chain shortened UDCA derivative.

Cystic fibrosis involving the liver – hepatobiliary spectrum

The frequency of biliary manifestations in cystic fibrosis (CF) is still unclear. Clinical phenotypes range from gallbladder dyskinesia, symptomatic cholelithiasis to sclerosing obstructive cholangitis. Early diagnosis can be challanging. Tools like the Aspartate Aminotransferase-to-Platelet Ratio Index (APRI) are reliable at predicting severe fibrosis, but not for differentiating fibrosis in early stages. Therefore, serum biomarkers are an unmet necessity thus far. Promising research areas include further investigating the role of intestinal bile salt malabsorption such as the plasma fibroblast growth factor 19 (FGF19) and the intermediate of CYP7A activity and the 4-cholesten-3-one (C4)[37]. Transient elastography may be useful as well, however appropriate validation in mild-to-moderate fibrosis is still pending[38]. Clinical trials for CF cholestasis, using the new generation of therapeutic targets beyond UDCA, would also provide benefits to patients. Some agents discussed previously had good results in preclinical research, such as NorUDCA, tested in mice[39].

Recent CF animal model investigations uncovered the underpinning relationship of the CF transmembrane conductance regulator and the control of biliary epithelial

Table 1 Potential pathways as targets for existing antibodies

Drug	Primary role of the pathway in specific cholestatic liver disease	Previous disease of drug-testing	Ref.
Anti-CD40 (dacetuzumab/lucatumumab)	T-cell-B-cell interactions in primary biliary cholangitis	Multiple sclerosis (pre-clinical)	[53]
Anti-CXCL10 (MDX-1100)	CXCR3-CXCL9/10/11 CXCR3 is upregulated on liver-infiltrating Th1 and Th17 in primary biliargy cholangitis	Rheumatoid arthritis	[54]
Anti-CXCL13 (Mab 5261)	T- and B-cell migration to germinal centers in primary biliary cholangitis	Preclinical development	[55]
Anti-CCR6	Recruitment of Th17 cells around inflamed biliary epithelial cells in primary biliary cholangitis	Preclinical development	[56]
Anti-GRP35	Activation of GPR35 reduces IL-4 release from natural killer T cells in primary sclerosing cholangitis	Antibody recently developed	[57]
Anti-PRKD2	SIK2 pathway in PSC, AMPK-related kinase PRKD2 polymorphism are seen in early inflammatory bowel disease in primary sclerosing cholangitis	Preclinical development	[58]

PSC: Primary sclerosing cholangitis.

inflammation and permeability mediated by TLR4-NF-κB[40]. Moreover, a number of studies have identified a dysfunctional PPAR-gamma (peroxisome proliferator-activated receptor gamma), that was partially recovered with PPAR-gamma ligands, as rosiglitazone, particularly attenuating biliary fibrosis in CF[41]. Another study, also in murine model, linked those PPAR-gamma as a limiting factor for NF-κB-dependent inflammation[42]. These findings can possibly be further studied as possible target for future therapies.

Polycystic liver disease

Polycystic liver diseases are autosomal dominant disorders that result from a mutation of PRKCSH or Sec63 genes; genes that are mainly expressed in cholangiocytes[43]. Cystogenesis in this scenario is due to benign cholangiocyte proliferation, with cell-cycle dysregulation and increased level of cAMP in cholangiocytes leading to cyst progression and abnormal fluid transport[44]. Over time, the cyst growth may compress the biliary tree impairing bile flow as well. Liver volume is a prognostic marker as complications may occur as the disease progresses, such as hepatic cyst infection, rupture, hemorrhage and hepatic venous outflow obstruction[45]. Therapeutic developments have focused in preclinical studies in lowering cAMP and stopping or reversing progression, usually evaluated by the organ size and hepatic cystic volume. Octreotide became an option for treatment *via* decrease in cAMP levels[46,47]. Recently an open-label clinical trial tested UDCA effect in cystic liver diseases and reported a reduction of liver cyst volume growth after 24 wk of treatment[48,49]. This effect was expected as UDCA decreases the concentration of cytotoxic BA and therefore diminishes proliferation stimuli[50]. Additionally, more than 50% of patients may have fibrosis[51].

CONCLUSION

Although CLD pathogenic features are becoming unveiled, and translational research is achieving success, some findings still challenge what we know about the basic molecular developments in CLD, such as the relationship of FXR agonists, synthesis of FGF19 and metabolism expression and cell survival[52], and ultimately possible carcinogenesis. To date, inhibitors of the FGF19/FGFR4 pathway are in development for the treatment of hepatocellular malignancies. This acknowledgment for the regular hepatology practice is essential, as for a number of cases, hepatologists and oncologist specialized in hepatobiliary tumors do not often work on the same cases at the same point in time. However, the same patient may experience interactions with these professionals on different occasions in the course of disease progression. For the current therapeutics of cholestatic disease, FXR agonists may represent a novel approach for PBC, and trigger experimentational use for PSC. In the long run, however, the aberrant expression of FGF19 in its oncogenic driver is not entirely presumed. The landscape of modulation of the fibroblast growth factor family, as well

Table 2 Preclinical research cholestatic liver diseases

Area of concern	Findings	Approach	Ref.
Mitochondrial damage by GCDCA	Mitofusin 2 protects hepatocyte mitochondrial function	*In vitro* (LO2 cell lines)	[59]
Immunomodulation in primary biliary cholangitis with CTLA-4-Ig (immunoglobulin) as an immunotherapeutic agent	Signaling by CTLA-4 can modulate costimulation and induce inhibitory signals	*In vivo* (murine models)	[60]
Immunomodulation in primary biliary cholangitis with anti-CD40L	Reduced liver inflammation significantly initial lowering of anti-mitochondrial antibodies was observed but non-sustained.	*In vivo* (murine models)	[61]
Action of nuclear bile acid receptor FXR in cholestasis	Hepatoprotection from cholestasis by inducing FGF-15	*In vivo* (murine model)	[9]
Immunomodulation Anti-CCR5/CCR2 in combination with all-trans-retinoic acid	Significant reduction in plasma liver enzymes, bilirubin, liver fibrosis, bile duct proliferation and hepatic infiltration of neutrophils and T cells and expression of cytokines	*In vivo* (murine model)	[62]
Curcumin acts through FXR signaling	Protection against alpha-naphthylisothiocyanate ANIT-induced cholestasis	*In vitro* and *in vivo* (murine model)	[63]
Modulation of bile duct proliferation, with Melatonin	GnRH stimulated fibrosis gene expression in Hepatic stellate cells; melatonin may improve outcomes of cholestasis by suppressing GnRH.	*In vivo* (murine model)	[64]
Apamin, an apitoxin (bee venom) derivate prevented tetrachloride-induced liver fibrosis	Apamin suppressed the deposition of collagen, the proliferation of BECs and expression of fibrogenic genes	*In vivo* (murine model)	[65]
Toxic bile acids induce mitochondrial fragmentation. Preventing fragmentation improved outcome	Decreasing mitochondrial fission substantially diminished ROS levels, liver injury, and fibrosis under cholestatic conditions	*In vivo* Knockout mouse models	[66]
Epigenetic approach Histone deacetylase 4 (HDAC4) restores prohibitin-1 (PHB1)	Genomic reprogramming, with regression of the fibrotic phenotype	*In vivo* Knockout mouse models	[67]
Anti-γ-glutamyl transpeptidase antibody for osteodystrophy in cholestatic liver disease	GGT inhibited mineral nodule formation and expression of alkaline phosphatase and bone sialoprotein in osteoblastic cells.	*In vivo* (murine model)	[68]
EGFR signaling protects from cholestatic liver injury and fibrosis.	STAT3 is a negative regulator of bile acids synthesis and protects from bile acid-induced apoptosis. Additionally, it regulates EGFR expression	*In vivo* Knockout mouse models	[69]
Necroptosis pathway in primary biliary cholangitis	Necroinflammatory pathways regulated by receptor-interacting protein 3 (RIP3), with deleterious progress in cholestatic diseases. RIP3 deficiency blocked bile-duct-ligation-induced (BDL) necroinflammation at 3 and 14 d post-BDL	*In vivo* Knockout mouse models	[70]
Tauroursodeoxycholic acid modulates apoptosis in mice	Significant reduction of liver fibrosis, accompanied by a slight decrease of liver damage	*In vivo* (murine model)	[71]

as its signal through the transmembrane tyrosine kinase receptors, needs an operable spotlight in cholestatic diseases.

Moreover, in pre-carcinogenic sclerosing conditions such as PSC, the agonistic effect of cell proliferation, differentiation, and tissue repair through a potential oncogenic signaling pathway demands further scrutiny. Besides, a possible role in therapeutic resistance for advanced metastatic hepatocellular carcinomas, once the pathway is wired up, is also concerning. Epigenetic modulation in the core of the CLD and the hepatostat growth activation through FGF19/FGFR4 may interface with the Hippo-Yap signaling and play an essential role in liver carcinogenesis.

It is expected that the current understanding of the multifactorial pathogenic process and the potential substantial role of epigenetics will drive further much needed basic research and introduce new concepts and prospective therapeutic targets to the world of CLD.

Table 3 Clinical trials and translational research

Area of concern and specific cholestatic liver disease	Findings	Phase, study description	Clinical trial number	Ref.
IL12/IL23 Inflammatory pathway and loss of self-tolerance (Primary biliary cholangitis)	After 28 wk of treatment modest decreases in alkaline phosphatase	Phase 2, open-label proof of concept using Ustekimunab for ursodeoxycholic acid non-responsive patients	NCT01389973	[72]
Ileal bile acid transporter (IBAT) (Primary biliary cholangiti, Alagille syndrome, progressive familial intrahepatic cholestasis)	Bile acid transporter inhibitor A4250 interrupts enterohepatic bile acid circulation at the terminal ileum	Phase 1 (40 individuals) completed Bile acids A4250 either as monotherapy or in combination with colonic release cholestyramine	NCT02963077	[73]
Modified bile acid and FXR agonist derived from chenodeoxycholic acid Obeticholic acid (OCA) (Primary biliary cholangitis)	Durable treatment response; the drug was approved by FDA in May 2017 for non-UDCA responders	Phase 4, double-blind, randomized, placebo-controlled, multicenter (428 patients) estimated completion by 2025 (COBALT study)	NCT02308111	[34]
IBAT inhibition by GSK2330672	After 14 d, GSK2330672 demonstrated to be safe, well tolerated and reduced pruritus severity	Phase 2 double-blind, randomized, placebo-controlled	NCT01899703	[74]
Bile acids	Significantly reduced ALT and the bile acid intermediate C4	Phase I: Combination of UDCA and ATRA	NCT01456468	[75]
Bile acids Obeticholic acid monotherapy (Primary biliary cholangitis)	With ursodiol or as monotherapy for 12 mo decreases from baseline in alkaline phosphatase and total bilirubin levels that differed significantly from the placebo. observed changes	Phase 3, double-blind, placebo-controlled trial and long-term safety extension of obeticholic acid (217 patients) (POISE study)	NCT01473524	[76]
Bezafibrate 400 mg alternative	PBC patients with inadequate response to ursodeoxycholic acid alone, treatment with bezafibrate in addition to ursodeoxycholic acid resulted in a rate of complete biochemical response that was significantly higher than the rate with placebo and ursodeoxycholic acid therapy	Phase 3 multi-center, randomized, placebo-controlled, parallel-group (100 patients) (BEZURSO study)	NCT01654731	[77]
Different doses of UDCA in primary sclerosing cholangitis	Significantly reduced ALP values dose-dependently	Phase 2 double-blind, randomized, multi-center, placebo-controlled (159 patients) (NUC3)	NCT01755507	[78]
Pentoxifylline as immunomodulator for primary biliary cholangitis	The study is small, and results were in clinicaltrials.gov, but due to study size no conclusion can be safely achieved	Phase 2, pilot study, open-label Pentoxifylline 400 mg TID for six months (20 participants)	NCT01249092	Results at clinicaltrials.gov
Umbilical cord-derived mesenchymal cells (UC-MSC)	A significant decrease in alkaline phosphatase	Phase1/2 study, randomized, parallel group (100 participants) 12 wk of treatment	NCT01662973	[79]
Mitomycin C in primary sclerosing cholangitis	Final results awaited	Phase 2, double-blind, randomized, parallel group (130 participants)	NCT01688024	-
Curcumin in primary sclerosing cholangitis	Final results awaited	Phase1/2 open-label pilot study Evaluating the safety and efficacy of curcumin (15 participants)	NCT02978339	-
Human monoclonal antibody (BTT1023) that targets the vascular adhesion protein (VAP-1) in primary sclerosing cholangitis	Recruiting	Phase 2, a single arm, two-stage, multicenter, open-label (41 participants)	NCT02239211	[80]
Cenicriviroc a CCR2/CCR5 inhibitor proof of concept in primary sclerosing cholangitis	Results awaited	Phase 2, proof of concept, open-label (24 participants) (PERSEUS study)	NCT02653625	-
Bile acids Maralixibat Apical bile acids transporter inhibition (ASBTi) in primary sclerosing cholangitis	Although results are online, complete information is still awaited	Phase 2, pilot, open-label	NCT02061540	Results available at clinicaltrial.gov

Immunomodulation Simtuzumab in primary sclerosing cholangitis Monoclonal antibody against lysyl oxidase-like 2 (LOXL2)	Results awaited	Phase 2b, dose-ranging, randomized, double-blind, placebo-controlled (235 participants)	NCT01672853	-
Bile acids Obethicolic acid in primary biliary cholangitis	Treatment with OCA 5-10 mg reduced serum ALP in patients with PSC. Mild to moderate dose-related pruritus was the most common adverse event	Phase 2, double-blind, placebo-controlled trial. Dose-Finding (AESOP)	NCT02177136	[80]

PSC: Primary sclerosing cholangitis.

REFERENCES

1 United Network for Organ Sharing - UNOS [Internet]. 2018. Available from: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/
2 **Khungar V**, Goldberg DS. Liver Transplantation for Cholestatic Liver Diseases in Adults. *Clin Liver Dis* 2016; **20**: 191-203 [PMID: 26593299 DOI: 10.1016/j.cld.2015.08.011]
3 **Lazaridis KN**, LaRusso NF. The Cholangiopathies. *Mayo Clin Proc* 2015; **90**: 791-800 [PMID: 25957621 DOI: 10.1016/j.mayocp.2015.03.017]
4 **De Assuncao TM**, Sun Y, Jalan-Sakrikar N, Drinane MC, Huang BQ, Li Y, Davila JI, Wang R, O'Hara SP, Lomberk GA, Urrutia RA, Ikeda Y, Huebert RC. Development and characterization of human-induced pluripotent stem cell-derived cholangiocytes. *Lab Invest* 2015; **95**: 684-696 [PMID: 25867762 DOI: 10.1038/labinvest.2015.51]
5 **Sclair SN**, Fiel MI, Wu HS, Doucette J, Aloman C, Schiano TD. Increased hepatic progenitor cell response and ductular reaction in patients with severe recurrent HCV post-liver transplantation. *Clin Transplant* 2016; **30**: 722-730 [PMID: 27027987 DOI: 10.1111/ctr.12740]
6 **Jalan-Sakrikar N**, De Assuncao TM, Lu J, Almada LL, Lomberk G, Fernandez-Zapico ME, Urrutia R, Huebert RC. Hedgehog Signaling Overcomes an EZH2-Dependent Epigenetic Barrier to Promote Cholangiocyte Expansion. *PLoS One* 2016; **11**: e0168266 [PMID: 27936185 DOI: 10.1371/journal.pone.0168266]
7 **Li Y**, Ayata G, Baker SP, Banner BF. Cholangitis: a histologic classification based on patterns of injury in liver biopsies. *Pathol Res Pract* 2005; **201**: 565-572 [PMID: 16259109 DOI: 10.1016/j.prp.2005.06.004]
8 **Chiang JYL**, Ferrell JM. Bile Acid Metabolism in Liver Pathobiology. *Gene Expr* 2018; **18**: 71-87 [PMID: 29325602 DOI: 10.3727/105221618X15156018385515]
9 **Modica S**, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, Di Tullio G, Palasciano G, Moustafa T, Halilbasic E, Trauner M, Moschetta A. Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. *Gastroenterology* 2012; **142**: 355-65.e1-4 [PMID: 22057115 DOI: 10.1053/j.gastro.2011.10.028]
10 **Fickert P**, Wagner M. Biliary bile acids in hepatobiliary injury - What is the link? *J Hepatol* 2017; **67**: 619-631 [PMID: 28712691 DOI: 10.1016/j.jhep.2017.04.026]
11 **Li X**, Liu R, Zhang L, Jiang Z. The emerging role of AMP-activated protein kinase in cholestatic liver diseases. *Pharmacol Res* 2017; **125**: 105-113 [PMID: 28889972 DOI: 10.1016/j.phrs.2017.09.002]
12 **Nammour FE**, Fayad NF, Peikin SR. Metformin-induced cholestatic hepatitis. *Endocr Pract* 2003; **9**: 307-309 [PMID: 14561576 DOI: 10.4158/EP.9.4.307]
13 **Zhang Y**, Pan Y, Lin C, Zheng Y, Sun H, Zhang H, Wang J, Yuan M, Duan T, Du Q, Chen J. Bile acids evoke placental inflammation by activating Gpbar1/NF-κB pathway in intrahepatic cholestasis of pregnancy. *J Mol Cell Biol* 2016; **8**: 530-541 [PMID: 27402811 DOI: 10.1093/jmcb/mjw025]
14 **Tan M**, Tang C, Zhang Y, Cheng Y, Cai L, Chen X, Gao Y, Deng Y, Pan M. SIRT1/PGC-1α signaling protects hepatocytes against mitochondrial oxidative stress induced by bile acids. *Free Radic Res* 2015; **49**: 935-945 [PMID: 25789761 DOI: 10.3109/10715762.2015.1016020]
15 **Hirschfield GM**, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. *Annu Rev Pathol* 2013; **8**: 303-330 [PMID: 23347352 DOI: 10.1146/annurev-pathol-020712-164014]
16 **Pinzani M**, Luong TV. Pathogenesis of biliary fibrosis. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1279-1283 [PMID: 28754450 DOI: 10.1016/j.bbadis.2017.07.026]
17 **Inamine T**, Higa S, Noguchi F, Kondo S, Omagari K, Yatsuhashi H, Tsukamoto K, Nakamura M. Association of genes involved in bile acid synthesis with the progression of primary biliary cirrhosis in Japanese patients. *J Gastroenterol* 2013; **48**: 1160-1170 [PMID: 23354620 DOI: 10.1007/s00535-012-0730-9]
18 **Webb GJ**, Siminovitch KA, Hirschfield GM. The immunogenetics of primary biliary cirrhosis: A comprehensive review. *J Autoimmun* 2015; **64**: 42-52 [PMID: 26250073 DOI: 10.1016/j.jaut.2015.07.004]
19 **Sato K**, Hall C, Glaser S, Francis H, Meng F, Alpini G. Pathogenesis of Kupffer Cells in Cholestatic Liver Injury. *Am J Pathol* 2016; **186**: 2238-2247 [PMID: 27452297 DOI: 10.1016/j.ajpath.2016.06.003]
20 **Trivedi PJ**, Hirschfield GM. The Immunogenetics of Autoimmune Cholestasis. *Clin Liver Dis* 2016; **20**: 15-31 [PMID: 26593288 DOI: 10.1016/j.cld.2015.08.002]
21 **Hirschfield GM**, Chapman RW, Karlsen TH, Lammert F, Lazaridis KN, Mason AL. The genetics of complex cholestatic disorders. *Gastroenterology* 2013; **144**: 1357-1374 [PMID: 23583734 DOI: 10.1053/j.gastro.2013.03.053]
22 **Fickert P**, Pollheimer MJ, Beuers U, Lackner C, Hirschfield G, Housset C, Keitel V, Schramm C, Marschall HU, Karlsen TH, Melum E, Kaser A, Eksteen B, Strazzabosco M, Manns M, Trauner M; International PSC Study Group (IPSCSG). Characterization of animal models for primary sclerosing cholangitis (PSC). *J Hepatol* 2014; **60**: 1290-1303 [PMID: 24560657 DOI: 10.1016/j.jhep.2014.02.006]

23 **Kinnman N**, Francoz C, Barbu V, Wendum D, Rey C, Hultcrantz R, Poupon R, Housset C. The myofibroblastic conversion of peribiliary fibrogenic cells distinct from hepatic stellate cells is stimulated by platelet-derived growth factor during liver fibrogenesis. *Lab Invest* 2003; **83**: 163-173 [PMID: 12594232 DOI: 10.1097/01.lab.0000054178.01162.e4]

24 **Lemoinne S**, Cadoret A, Rautou PE, El Mourabit H, Ratziu V, Corpechot C, Rey C, Bosselut N, Barbu V, Wendum D, Feldmann G, Boulanger C, Henegar C, Housset C, Thabut D. Portal myofibroblasts promote vascular remodeling underlying cirrhosis formation through the release of microparticles. *Hepatology* 2015; **61**: 1041-1055 [PMID: 25043701 DOI: 10.1002/hep.27318]

25 **Debray D**, Narkewicz MR, Bodewes FAJA, Colombo C, Housset C, de Jonge HR, Jonker JW, Kelly DA, Ling SC, Poynard T, Sogni P, Trauner M, Witters P, Baumann U, Wilschanski M, Verkade HJ. Cystic Fibrosis-related Liver Disease: Research Challenges and Future Perspectives. *J Pediatr Gastroenterol Nutr* 2017; **65**: 443-448 [PMID: 28753176 DOI: 10.1097/MPG.0000000000001676]

26 **Invernizzi P**, Miozzo M, Battezzati PM, Bianchi I, Grati FR, Simoni G, Selmi C, Watnik M, Gershwin ME, Podda M. Frequency of monosomy X in women with primary biliary cirrhosis. *Lancet* 2004; **363**: 533-535 [PMID: 14975617 DOI: 10.1016/S0140-6736(04)15541-4]

27 **Lleo A**, Zhang W, Zhao M, Tan Y, Bernuzzi F, Zhu B, Liu Q, Tan Q, Malinverno F, Valenti L, Jiang T, Tan L, Liao W, Coppel R, Invernizzi P, Lu Q, Adams DH, Gershwin ME; PBC Epigenetic Study Group. DNA methylation profiling of the X chromosome reveals an aberrant demethylation on CXCR3 promoter in primary biliary cirrhosis. *Clin Epigenetics* 2015; **7**: 61 [PMID: 26150899 DOI: 10.1186/s13148-015-0098-9]

28 **Chuang YH**, Lian ZX, Cheng CM, Lan RY, Yang GX, Moritoki Y, Chiang BL, Ansari AA, Tsuneyama K, Coppel RL, Gershwin ME. Increased levels of chemokine receptor CXCR3 and chemokines IP-10 and MIG in patients with primary biliary cirrhosis and their first degree relatives. *J Autoimmun* 2005; **25**: 126-132 [PMID: 16243485 DOI: 10.1016/j.jaut.2005.08.009]

29 **Padgett KA**, Lan RY, Leung PC, Lleo A, Dawson K, Pfeiff J, Mao TK, Coppel RL, Ansari AA, Gershwin ME. Primary biliary cirrhosis is associated with altered hepatic microRNA expression. *J Autoimmun* 2009; **32**: 246-253 [PMID: 19345069 DOI: 10.1016/j.jaut.2009.02.022]

30 **Tabibian JH**, Lindor KD. Primary biliary cirrhosis: safety and benefits of established and emerging therapies. *Expert Opin Drug Saf* 2015; **14**: 1435-1444 [PMID: 26212223 DOI: 10.1517/14740338.2015.1073260]

31 **Yang CY**, Ma X, Tsuneyama K, Huang S, Takahashi T, Chalasani NP, Bowlus CL, Yang GX, Leung PS, Ansari AA, Wu L, Coppel RL, Gershwin ME. IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy. *Hepatology* 2014; **59**: 1944-1953 [PMID: 24375552 DOI: 10.1002/hep.26979]

32 **Mousa HS**, Lleo A, Invernizzi P, Bowlus CL, Gershwin ME. Advances in pharmacotherapy for primary biliary cirrhosis. *Expert Opin Pharmacother* 2015; **16**: 633-643 [PMID: 25543678 DOI: 10.1517/14656566.2015.998650]

33 **Ali AH**, Lindor KD. Obeticholic acid for the treatment of primary biliary cholangitis. *Expert Opin Pharmacother* 2016; **17**: 1809-1815 [PMID: 27468093 DOI: 10.1080/14656566.2016.1218471]

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

35 **Chazouillères O**. Novel Aspects in the Management of Cholestatic Liver Diseases. *Dig Dis* 2016; **34**: 340-346 [PMID: 27170387 DOI: 10.1159/000444544]

36 **Duboc H**, Taché Y, Hofmann AF. The bile acid TGR5 membrane receptor: from basic research to clinical application. *Dig Liver Dis* 2014; **46**: 302-312 [PMID: 24411485 DOI: 10.1016/j.dld.2013.10.021]

37 **Bodewes FA**, Verkade HJ, Taminiau JA, Borowitz D, Wilschanski M; Working group Câ€‹ystic Fibrosis and Pancreatic Disease of the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN). Cystic fibrosis and the role of gastrointestinal outcome measures in the new era of therapeutic CFTR modulation. *J Cyst Fibros* 2015; **14**: 169-177 [PMID: 25677689 DOI: 10.1016/j.jcf.2015.01.006]

38 **Sadler MD**, Crotty P, Fatovich L, Wilson S, Rabin HR, Myers RP. Noninvasive methods, including transient elastography, for the detection of liver disease in adults with cystic fibrosis. *Can J Gastroenterol Hepatol* 2015; **29**: 139-144 [PMID: 25855877 DOI: 10.1155/2015/138530]

39 **Trauner M**, Halilbasic E, Claudel T, Steinacher D, Fuchs C, Moustafa T, Pollheimer M, Krones E, Kienbacher C, Traussnigg S, Kazemi-Shirazi L, Munda P, Hofer H, Fickert P, Paumgartner G. Potential of nor-Ursodeoxycholic Acid in Cholestatic and Metabolic Disorders. *Dig Dis* 2015; **33**: 433-439 [PMID: 26045280 DOI: 10.1159/000371904]

40 **Fiorotto R**, Villani A, Kourtidis A, Scirpo R, Amenduni M, Geibel PJ, Cadamuro M, Spirli C, Anastasiadis PZ, Strazzabosco M. The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity. *Hepatology* 2016; **64**: 2118-2134 [PMID: 27629435 DOI: 10.1002/hep.28817]

41 **Harmon GS**, Dumlao DS, Ng DT, Barrett KE, Dennis EA, Dong H, Glass CK. Pharmacological correction of a defect in PPAR-gamma signaling ameliorates disease severity in Cftr-deficient mice. *Nat Med* 2010; **16**: 313-318 [PMID: 20154695 DOI: 10.1038/nm.2101]

42 **Scirpo R**, Fiorotto R, Villani A, Amenduni M, Spirli C, Strazzabosco M. Stimulation of nuclear receptor peroxisome proliferator-activated receptor-γ limits NF-κB-dependent inflammation in mouse cystic fibrosis biliary epithelium. *Hepatology* 2015; **62**: 1551-1562 [PMID: 26199136 DOI: 10.1002/hep.28000]

43 **Masyuk T**, Masyuk A, LaRusso N. Cholangiociliopathies: genetics, molecular mechanisms and potential therapies. *Curr Opin Gastroenterol* 2009; **25**: 265-271 [PMID: 19349863 DOI: 10.1097/MOG.0b013e328328f4ff]

44 **Banales JM**, Masyuk TV, Gradilone SA, Masyuk AI, Medina JF, LaRusso NF. The cAMP effectors Epac and protein kinase a (PKA) are involved in the hepatic cystogenesis of an animal model of autosomal recessive polycystic kidney disease (ARPKD). *Hepatology* 2009; **49**: 160-174 [PMID: 19065671 DOI: 10.1002/hep.22636]

45 **van Aerts RMM**, van de Laarschot LFM, Banales JM, Drenth JPH. Clinical management of polycystic liver disease. *J Hepatol* 2018; **68**: 827-837 [PMID: 29175241 DOI: 10.1016/j.jhep.2017.11.024]

46 **Hogan MC**, Masyuk TV, Page LJ, Kubly VJ, Bergstralh EJ, Li X, Kim B, King BF, Glockner J, Holmes DR 3rd, Rossetti S, Harris PC, LaRusso NF, Torres VE. Randomized clinical trial of long-acting somatostatin for autosomal dominant polycystic kidney and liver disease. *J Am Soc Nephrol* 2010; **21**: 1052-1061 [PMID: 20431041 DOI: 10.1681/ASN.2009121291]

47 **Hogan MC**, Masyuk TV, Page L, Holmes DR 3rd, Li X, Bergstralh EJ, Irazabal MV, Kim B, King BF, Glockner JF, Larusso NF, Torres VE. Somatostatin analog therapy for severe polycystic liver disease: results after 2 years. *Nephrol Dial Transplant* 2012; **27**: 3532-3539 [PMID: 22773240 DOI: 10.1093/ndt/gfs152]

48 **Lammert F**, Méndez-Sánchez N. The effect of ursodeoxycholic acid in cystic cholangiopathies. *Ann Hepatol* 2016; **15**: 949-950 [PMID: 27740534 DOI: 10.5604/16652681.1222121]

49 **D'Agnolo HM**, Kievit W, Takkenberg RB, Riaño I, Bujanda L, Neijenhuis MK, Brunenberg EJ, Beuers U, Banales JM, Drenth JP. Ursodeoxycholic acid in advanced polycystic liver disease: A phase 2 multicenter randomized controlled trial. *J Hepatol* 2016; **65**: 601-607 [PMID: 27212247 DOI: 10.1016/j.jhep.2016.05.009]

50 **Perugorria MJ**, Labiano I, Esparza-Baquer A, Marzioni M, Marin JJ, Bujanda L, Banales JM. Bile Acids in Polycystic Liver Diseases: Triggers of Disease Progression and Potential Solution for Treatment. *Dig Dis* 2017; **35**: 275-281 [PMID: 28249268 DOI: 10.1159/000450989]

51 **Barbier L**, Ronot M, Aussilhou B, Cauchy F, Francoz C, Vilgrain V, Soubrane O, Paradis V, Belghiti J. Polycystic liver disease: Hepatic venous outflow obstruction lesions of the noncystic parenchyma have major consequences. *Hepatology* 2018; **68**: 652-662 [PMID: 29023812 DOI: 10.1002/hep.29582]

52 **Massafra V**, Milona A, Vos HR, Burgering BM, van Mil SW. Quantitative liver proteomics identifies FGF19 targets that couple metabolism and proliferation. *PLoS One* 2017; **12**: e0171185 [PMID: 28178326 DOI: 10.1371/journal.pone.0171185]

53 **'t Hart BA**, Hintzen RQ, Laman JD. Preclinical assessment of therapeutic antibodies against human CD40 and human interleukin-12/23p40 in a nonhuman primate model of multiple sclerosis. *Neurodegener Dis* 2008; **5**: 38-52 [PMID: 18075274 DOI: 10.1159/000109937]

54 **Yellin M**, Paliienko I, Balanescu A, Ter-Vartanian S, Tseluyko V, Xu LA, Tao X, Cardarelli PM, Leblanc H, Nichol G, Ancuta C, Chirieac R, Luo A. A phase II, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of MDX-1100, a fully human anti-CXCL10 monoclonal antibody, in combination with methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum* 2012; **64**: 1730-1739 [PMID: 22147649 DOI: 10.1002/art.34330]

55 **Klimatcheva E**, Pandina T, Reilly C, Torno S, Bussler H, Scrivens M, Jonason A, Mallow C, Doherty M, Paris M, Smith ES, Zauderer M. CXCL13 antibody for the treatment of autoimmune disorders. *BMC Immunol* 2015; **16**: 6 [PMID: 25879435 DOI: 10.1186/s12865-015-0068-1]

56 **Liston A**, Kohler RE, Townley S, Haylock-Jacobs S, Comerford I, Caon AC, Webster J, Harrison JM, Swann J, Clark-Lewis I, Korner H, McColl SR. Inhibition of CCR6 function reduces the severity of experimental autoimmune encephalomyelitis via effects on the priming phase of the immune response. *J Immunol* 2009; **182**: 3121-3130 [PMID: 19234209 DOI: 10.4049/jimmunol.0713169]

57 **Jenkins L**, Harries N, Lappin JE, MacKenzie AE, Neetoo-Isseljee Z, Southern C, McIver EG, Nicklin SA, Taylor DL, Milligan G. Antagonists of GPR35 display high species ortholog selectivity and varying modes of action. *J Pharmacol Exp Ther* 2012; **343**: 683-695 [PMID: 22967846 DOI: 10.1124/jpet.112.198945]

58 **Harikumar KB**, Kunnumakkara AB, Ochi N, Tong Z, Deorukhkar A, Sung B, Kelland L, Jamieson S, Sutherland R, Raynham T, Charles M, Bagherzadeh A, Foxton C, Boakes A, Farooq M, Maru D, Diagaradjane P, Matsuo Y, Sinnett-Smith J, Gelovani J, Krishnan S, Aggarwal BB, Rozengurt E, Ireson CR, Guha S. A novel small-molecule inhibitor of protein kinase D blocks pancreatic cancer growth in vitro and in vivo. *Mol Cancer Ther* 2010; **9**: 1136-1146 [PMID: 20442301 DOI: 10.1158/1535-7163.MCT-09-1145]

59 **Chen Y**, Lv L, Jiang Z, Yang H, Li S, Jiang Y. Mitofusin 2 protects hepatocyte mitochondrial function from damage induced by GCDCA. *PLoS One* 2013; **8**: e65455 [PMID: 23755235 DOI: 10.1371/journal.pone.0065455]

60 **Dhirapong A**, Yang GX, Nadler S, Zhang W, Tsuneyama K, Leung P, Knechtle S, Ansari AA, Coppel RL, Liu FT, He XS, Gershwin ME. Therapeutic effect of cytotoxic T lymphocyte antigen 4/immunoglobulin on a murine model of primary biliary cirrhosis. *Hepatology* 2013; **57**: 708-715 [PMID: 22996325 DOI: 10.1002/hep.26067]

61 **Tanaka H**, Yang GX, Iwakoshi N, Knechtle SJ, Kawata K, Tsuneyama K, Leung P, Coppel RL, Ansari AA, Joh T, Bowlus C, Gershwin ME. Anti-CD40 ligand monoclonal antibody delays the progression of murine autoimmune cholangitis. *Clin Exp Immunol* 2013; **174**: 364-371 [PMID: 23981074 DOI: 10.1111/cei.12193]

62 **Yu D**, Cai SY, Mennone A, Vig P, Boyer JL. Cenicriviroc, a cytokine receptor antagonist, potentiates all-trans retinoic acid in reducing liver injury in cholestatic rodents. *Liver Int* 2018; **38**: 1128-1138 [PMID: 29356312 DOI: 10.1111/liv.13698]

63 **Yang F**, Tang X, Ding L, Zhou Y, Yang Q, Gong J, Wang G, Wang Z, Yang L. Curcumin protects ANIT-induced cholestasis through signaling pathway of FXR-regulated bile acid and inflammation. *Sci Rep* 2016; **6**: 33052 [PMID: 27624003 DOI: 10.1038/srep33052]

64 **McMillin M**, DeMorrow S, Glaser S, Venter J, Kyritsi K, Zhou T, Grant S, Giang T, Greene JF Jr, Wu N, Jefferson B, Meng F, Alpini G. Melatonin inhibits hypothalamic gonadotropin-releasing hormone release and reduces biliary hyperplasia and fibrosis in cholestatic rats. *Am J Physiol Gastrointest Liver Physiol* 2017; **313**: G410-G418 [PMID: 28751425 DOI: 10.1152/ajpgi.00421.2016]

65 **Kim JY**, An HJ, Kim WH, Park YY, Park KD, Park KK. Apamin suppresses biliary fibrosis and activation of hepatic stellate cells. *Int J Mol Med* 2017; **39**: 1188-1194 [PMID: 28405682 DOI: 10.3892/ijmm.2017.2922]

66 **Yu T**, Wang L, Lee H, O'Brien DK, Bronk SF, Gores GJ, Yoon Y. Decreasing mitochondrial fission prevents cholestatic liver injury. *J Biol Chem* 2014; **289**: 34074-34088 [PMID: 25342755 DOI: 10.1074/jbc.M114.588616]

67 **Barbier-Torres L**, Beraza N, Fernández-Tussy P, Lopitz-Otsoa F, Fernández-Ramos D, Zubiete-Franco I, Varela-Rey M, Delgado TC, Gutiérrez V, Anguita J, Pares A, Banales JM, Villa E, Caballería J, Alvarez L,

Lu SC, Mato JM, Martínez-Chantar ML. Histone deacetylase 4 promotes cholestatic liver injury in the absence of prohibitin-1. *Hepatology* 2015; **62**: 1237-1248 [PMID: 26109312 DOI: 10.1002/hep.27959]

68 **Kawazoe Y**, Miyauchi M, Nagasaki A, Furusho H, Yanagisawa S, Chanbora C, Inubushi T, Hyogo H, Nakamoto T, Suzuki K, Moriwaki S, Tazuma S, Niida S, Takata T. Osteodystrophy in Cholestatic Liver Diseases Is Attenuated by Anti-γ-Glutamyl Transpeptidase Antibody. *PLoS One* 2015; **10**: e0139620 [PMID: 26418133 DOI: 10.1371/journal.pone.0139620]

69 **Svinka J**, Pflügler S, Mair M, Marschall HU, Hengstler JG, Stiedl P, Poli V, Casanova E, Timelthaler G, Sibilia M, Eferl R. Epidermal growth factor signaling protects from cholestatic liver injury and fibrosis. *J Mol Med (Berl)* 2017; **95**: 109-117 [PMID: 27568040 DOI: 10.1007/s00109-016-1462-8]

70 **Afonso MB**, Rodrigues PM, Simão AL, Ofengeim D, Carvalho T, Amaral JD, Gaspar MM, Cortez-Pinto H, Castro RE, Yuan J, Rodrigues CM. Activation of necroptosis in human and experimental cholestasis. *Cell Death Dis* 2016; **7**: e2390 [PMID: 27685634 DOI: 10.1038/cddis.2016.280]

71 **Paridaens A**, Raevens S, Devisscher L, Bogaerts E, Verhelst X, Hoorens A, Van Vlierberghe H, van Grunsven LA, Geerts A, Colle I. Modulation of the Unfolded Protein Response by Tauroursodeoxycholic Acid Counteracts Apoptotic Cell Death and Fibrosis in a Mouse Model for Secondary Biliary Liver Fibrosis. *Int J Mol Sci* 2017; **18** [PMID: 28117681 DOI: 10.3390/ijms18010214]

72 **Hirschfield GM**, Gershwin ME, Strauss R, Mayo MJ, Levy C, Zou B, Johanns J, Nnane IP, Dasgupta B, Li K, Selmi C, Marschall HU, Jones D, Lindor K; PURIFI Study Group. Ustekinumab for patients with primary biliary cholangitis who have an inadequate response to ursodeoxycholic acid: A proof-of-concept study. *Hepatology* 2016; **64**: 189-199 [PMID: 26597786 DOI: 10.1002/hep.28359]

73 **Graffner H**, Gillberg PG, Rikner L, Marschall HU. The ileal bile acid transporter inhibitor A4250 decreases serum bile acids by interrupting the enterohepatic circulation. *Aliment Pharmacol Ther* 2016; **43**: 303-310 [PMID: 26527417 DOI: 10.1111/apt.13457]

74 **Hegade VS**, Kendrick SF, Dobbins RL, Miller SR, Thompson D, Richards D, Storey J, Dukes GE, Corrigan M, Oude Elferink RP, Beuers U, Hirschfield GM, Jones DE. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. *Lancet* 2017; **389**: 1114-1123 [PMID: 28187915 DOI: 10.1016/S0140-6736(17)30319-7]

75 **Assis DN**, Abdelghany O, Cai SY, Gossard AA, Eaton JE, Keach JC, Deng Y, Setchell KD, Ciarleglio M, Lindor KD, Boyer JL. Combination Therapy of All-Trans Retinoic Acid With Ursodeoxycholic Acid in Patients With Primary Sclerosing Cholangitis: A Human Pilot Study. *J Clin Gastroenterol* 2017; **51**: e11-e16 [PMID: 27428727 DOI: 10.1097/MCG.0000000000000591]

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

77 **Patel A**, Seetharam A. Primary Biliary Cholangitis: Disease Pathogenesis and Implications for Established and Novel Therapeutics. *J Clin Exp Hepatol* 2016; **6**: 311-318 [PMID: 28003721 DOI: 10.1016/j.jceh.2016.10.001]

78 **Fickert P**, Hirschfield GM, Denk G, Marschall HU, Altorjay I, Färkkilä M, Schramm C, Spengler U, Chapman R, Bergquist A, Schrumpf E, Nevens F, Trivedi P, Reiter FP, Tornai I, Halilbasic E, Greinwald R, Pröls M, Manns MP, Trauner M; European PSC norUDCA Study Group. norUrsodeoxycholic acid improves cholestasis in primary sclerosing cholangitis. *J Hepatol* 2017; **67**: 549-558 [PMID: 28529147 DOI: 10.1016/j.jhep.2017.05.009]

79 **Wang L**, Li J, Liu H, Li Y, Fu J, Sun Y, Xu R, Lin H, Wang S, Lv S, Chen L, Zou Z, Li B, Shi M, Zhang Z, Wang FS. Pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 85-92 [PMID: 23855301 DOI: 10.1111/jgh.12029]

80 **Kowdley KV**, Vuppalanchi R, Levy C, Floreani A, Andreone P, LaRusso NF, Shrestha R, Trotter J, Goldberg D, Rushbrook S, Hirschfield GM, Schiano T, Jin Y, Pencek R, MacConell L, Shapiro D, Bowlus CL; AESOP Study Investigators. A randomized, placebo-controlled, phase II study of obeticholic acid for primary sclerosing cholangitis. *J Hepatol* 2020; **73**: 94-101 [PMID: 32165251 DOI: 10.1016/j.jhep.2020.02.033]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 436-450

DOI: 10.4254/wjh.v12.i8.436

ISSN 1948-5182 (online)

REVIEW

Lipidomics in non-alcoholic fatty liver disease

Sofia Kartsoli, Christina E Kostara, Vasilis Tsimihodimos, Eleni T Bairaktari, Dimitrios K Christodoulou

ORCID number: Sofia Kartsoli 0000-0002-7053-9162; Christina E Kostara 0000-0001-7045-1323; Vasilis Tsimihodimos 0000-0003-1708-3415; Eleni T Bairaktari 0000-0003-3231-8649; Dimitrios K Christodoulou 0000-0001-9694-1160.

Author contributions: Kartsoli S and Kostara C performed the literature review and drafted the initial manuscript; Tsimihodimos V, Bairaktari E, and Christodoulou D contributed to manuscript analysis, editing, and critical revision; all authors approved the submitted version of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Manuscript source: Invited

Sofia Kartsoli, Dimitrios K Christodoulou, Department of Gastroenterology, School of Health Sciences, Faculty of Medicine, University of Ioannina, Ioannina 45110, Greece

Christina E Kostara, Eleni T Bairaktari, Laboratory of Clinical Chemistry, School of Health Sciences, Faculty of Medicine, University of Ioannina, Ioannina 45110, Greece

Vasilis Tsimihodimos, Department of Internal Medicine, School of Health Sciences, Faculty of Medicine, University of Ioannina, Ioannina 45110, Greece

Corresponding author: Dimitrios K Christodoulou, MD, PhD, Professor of Gastroenterology, Department of Gastroenterology, School of Health Sciences, University Hospital of Ioannina, Faculty of Medicine, University of Ioannina, PO Box 1186, Ioannina 45110, Greece. dchristo@uoi.gr

Abstract

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disorder in Western countries, comprises steatosis to nonalcoholic steatohepatitis (NASH), with the latter having the potential to progress to cirrhosis. The transition from isolated steatosis to NASH is still poorly understood, but lipidomics approach revealed that the hepatic lipidome is extensively altered in the setting of steatosis and steatohepatitis and these alterations correlate with disease progression. Recent data suggest that both quantity and quality of the accumulated lipids are involved in pathogenesis of NAFLD. Changes in glycerophospholipid, sphingolipid, and fatty acid composition have been described in both liver biopsies and plasma of patients with NAFLD, implicating that specific lipid species are involved in oxidative stress, inflammation, and cell death. In this article, we summarize the findings of main human lipidomics studies in NAFLD and delineate the currently available information on the pathogenetic role of each lipid class in lipotoxicity and disease progression.

Key words: Lipidomics; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Lipotoxicity; Fatty acids; Ceramides

Core tip: Lipidomics is a new rapidly growing field that allows the overall and detailed investigation of the whole lipid composition in a given biology matrix. Lipid profiling of liver biopsies of patients with non-alcoholic fatty liver disease (NAFLD) has previously

manuscript

Received: February 28, 2020
Peer-review started: February 28, 2020
First decision: May 20, 2020
Revised: June 3, 2020
Accepted: June 20, 2020
Article in press: June 20, 2020
Published online: August 27, 2020

P-Reviewer: Musumeci G, Tiribelli C
S-Editor: Gong ZM
L-Editor: Wang TQ
P-Editor: Wang LL

revealed several changes in glycerophospholipids and sphingolipids concentrations and alterations in fatty acid pattern compared to healthy control. However, findings from lipidomics studies in plasma samples are inconsistent. We review the main findings of lipidomics studies and the important pathophysiological role of specific lipid species in lipotoxicity and development of NAFLD.

Citation: Kartsoli S, Kostara CE, Tsimihodimos V, Bairaktari ET, Christodoulou DK. Lipidomics in non-alcoholic fatty liver disease. *World J Hepatol* 2020; 12(8): 436-450
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/436.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.436

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver diseases in the Western countries, affecting approximately 25% of the general population[1]. NAFLD encompasses a wide spectrum of liver histological features, ranging from mild hepatic steatosis (non-alcoholic fatty liver, NAFL) to nonalcoholic steatohepatitis (NASH)[2]. The hallmark of NAFLD is the hepatic intracellular accumulation of lipids and the subsequent formation of lipid droplets in hepatocytes[3]. NASH, the more progressive form of the disease, is characterized by the presence of hepatic steatosis accompanied by lobular inflammation, hepatocellular damage, and fibrosis and associated with an increased risk of developing cirrhosis and hepatocellular carcinoma[4]. In fact, NASH-related cirrhosis is believed to become the leading cause of liver transplantation in the future[5].

NAFLD is commonly associated with insulin resistance and type 2 diabetes mellitus and is considered an independent risk factor for cardiovascular disease[6]. Obesity, physical inactivity, consumption of nutritionally imbalanced food, and unhealthy dietary and other lifestyle habits are also associated with NAFLD, and lifestyle modifications involving physical activity and diet have been shown to improve hepatic steatosis and liver fibrosis[6-8]. Although there has been remarkable progress in the elucidation of NAFLD pathogenesis, the pathophysiological pathways underlying lipotoxicity and transition of simple steatosis to NASH are still incompletely understood[9]. Recent lipidomic studies revealed marked changes in the fatty acid pattern and phospholipid composition in liver samples of NAFLD patients, suggesting that perturbations in lipid metabolism are a key factor in the pathogenesis and progression of NAFLD[10,11]. Furthermore, liver biopsy remains the only reliable but invasive method to diagnose NAFLD and differentiates NASH from simple steatosis. Thus, the non-invasive diagnosis of NASH is still an unmet need. Alterations occurring in plasma lipid molecules identified by lipidomic techniques which cannot be determined in every day clinical practice, may have utility as non-invasive biomarkers of disease progression[12].

The present review article focuses on the main findings of the alterations occurring in lipidome in NAFLD patients and the interpretation of pathophysiological role of several identified lipid classes in the development and progression of NAFLD.

PATHOGENESIS OF NAFLD AND ROLE OF LIPIDS

The pathogenesis of NAFLD is considered to be a multifactorial process and the underlying mechanisms involved in the progression of the disease are complex. Intrahepatic fat accumulation, the hallmark of the disease, is the result of increased uptake of fatty acids, increased *de novo* lipogenesis, and impairment in export and oxidation of fatty acids[3]. Obesity through expansion and dysfunction of adipose tissue and insulin resistance through subsequent reduction of adipose tissue lipolysis lead to increased efflux of free fatty acids[13]. Moreover, the hyperinsulinemia associated with insulin resistance promotes *de novo* fatty acid synthesis in the liver by activating the sterol regulatory element binding protein-1c (SREBP-1c), a transcriptional regulator of lipogenic genes[14]. These free fatty acids as well as those from dietary sources either undergo β-oxidation or are esterified with glycerol to form triglycerides. Then, triglycerides are stored in hepatocytes and form lipid droplets or are packaged and exported as very-low-density lipoprotein (VLDL)[3]. Thus, a dietary overload and

insulin resistance promote the hepatic fat accumulation, as observed in NAFLD[15].

Intracellular deposition of lipids in NAFLD and the subsequent increased demand for metabolism of excess fatty acids lead to production of reactive oxygen species (ROS), elevation of oxidative or endoplasmic reticulum (ER) stress, and activation of Jun N-terminal kinase, all of which result in mitochondrial dysfunction and cell death[16]. Cell injury, in the setting of steatosis, is also largely attributed to activation of inflammatory pathways. Adipose tissue dysfunction leads to secretion of pro-inflammatory cytokines and alters the production and secretion of adipokines, such as leptin and adiponectin that are involved in the modulation of inflammation and insulin resistance[15]. Hepatic inflammation in fatty liver is considered to be triggered by a variety of compounds, such as damage-associated molecular patterns (DAMPs) released from hepatocytes, gut-derived bacterial endotoxin, free fatty acids, and free cholesterol[17]. Cytokine-induced liver inflammation, the subsequent activation of Kupffer and hepatic stellate cells, and lipotoxicity induced by free fatty acids and other lipotoxic bioactive lipids are involved in chronic liver injury and are thought to be responsible for progression from NAFL to NASH and development of fibrosis[18].

Over the past decade, our knowledge regarding lipotoxicity has been greatly expanded and recent progress in lipidomics analyses has given new insights into lipid profiling and pathophysiological mechanisms involved in chronic inflammation and cell injury. Investigation of liver and serum lipidome in patients with NAFLD has disclosed that perturbations in lipid metabolism are a key factor for the development of NAFLD and that several complex lipid species, including sphingolipids and glycerophospholipids, are involved in lipotoxicity and the pathogenesis of NASH.

LIPIDOMICS STUDIES IN NAFLD

Lipidomics is defined as the detailed characterization of lipid molecular species and of their structure and biological role in a given matrix including cell, tissue, and biological fluid[19]. This relatively new research field is a subset of metabolomics and represents a powerful approach to obtain a comprehensive overview of whole lipid metabolism in a biological system or even in specific disease state[20]. Lipidomics includes the identification and characterization as well as the quantification of thousands of lipid molecular species in a biological matrix[21]. This rapidly growing advanced field incorporates analytical techniques that are utilized for lipid separation and detection, such as high-performance liquid chromatography (HPLC), electrospray ionization mass spectroscopy (ESI MS), and nuclear magnetic spectroscopy (NMR)[19,22].

The first lipidomics studies in NAFLD patients, as seen in Table 1, were conducted in liver biopsies and focused mainly on the analysis of fatty acid composition. Araya *et al*[10] was the first to report an increased n-6:n-3 ratio in liver lipids of NAFLD patients accompanied by a decrease of the long chain polyunsaturated fatty acid (PUFA) of n-3 and n-6 series in liver TAG, such as arachidonic, eicosapentaenoic, and docosahexanoic acid. A depletion of long chain n-3 and n-6 PUFA in NASH patients has also been reported by a later study, regardless of the dietary FA intake, suggesting that the biosynthetic pathways of these lipids are impaired[23]. Indeed, later studies on enzymatic activities confirmed the decreased activity of Δ5 desaturase, a key enzyme in essential n-3 and n-6 PUFA synthesis[24]. However, the first most comprehensive lipidomic study in liver biopsies, which included quantification of major lipid classes, was carried by Puri *et al*[11]. In this study, lipidomic analyses identified marked changes not only in the fatty acid composition but also in the total phospholipid content[11]. Alterations of phospholipid content in liver biopsies of NASH patients have also been reported by other studies, implicating that phospholipid synthesis is impaired in NASH and is associated with disease progression[24].

The research later focused on the study of the alterations occurring in plasma and serum samples of patients with NAFLD. In view of the fact that the liver is the key organ of metabolism and that plasma lipids under fasting conditions reflect mainly the lipids exported from the liver, changes in the circulating lipidome could be correlated with those in the liver during NAFLD progression. Interestingly, the changes observed in plasma fatty acid and phospholipid composition were discrepant from those reported in liver samples[25,26]. Moreover, as seen in Table 2, the findings of lipidomic studies conducted on plasma samples are inconsistent. According to Puri *et al*[26], no significant differences were observed in the plasma phospholipid subclasses of patients with NAFLD compared to healthy controls. However, recent studies report statistically significant changes in plasma phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidyloinositol (PI), phosphatidylcholine (PC),

Table 1 Summary of main liver lipidomics studies in non-alcoholic fatty liver disease

Ref.	Tissue	Main findings in NAFLD patients compared to healthy controls	Main findings in NASH patients compared to NAFL patients
Puri *et al*[11], 2007	Liver	**Increased**: DAG, TAG, total SFA, total PUFA; stepwise increase in the mean TAG/DAG ratio, FC/PC ratio and hepatic FC from normal livers to NAFL to NASH. **Decreased:** Total PC in both NAFL and NASH; AA in FFA, TAG, and PC in NASH; EPA and DHA in TAG in NASH.	The n-6:n-3 FFA ratio increased in NASH
Araya *et al*[10], 2004	Liver, adipose tissue (fatty acid composition)	**Increased:** n-6:n-3 ratio, n-6 LCPUFA in liver phospholipids, total MUFA. **Decreased:** Long-chain PUFA of the n-6 and n-3 series in liver TAG, AA/LA ratio, EPA + DHA)/ALA in liver TAG, n-3 LCPUFA in phospholipids, total PUFA, n-3 PUFA, n-6 PUFA, AA, EPA, DHA.	The n-6:n-3 ratio increased in NASH
Allard *et al*[23], 2008	Liver, red blood cells (fatty acid composition)	**Increased:** MUFAs, palmitoleic acid (16:1 n9), and oleic acid (18:1 n9) in NASH compared to control group. **Decreased:** Total n-3 PUFA, long-chain n-3 (EPA + DHA) and long-chain n - 6 (AA) PUFA in NASH compared to control; RBC-FA composition similar among the three groups.	**Decreased:** Total n- 6-PUFA in NASH compared to NAFL
Chiappini *et al*[24], 2017	Liver	**Increased:** C14:0, C16:0, C16:1n-7, C18:1n-7, C18:1n-9, and C18:2n-6 in NASH. **Decreased:** Total SM, PI, PS, PE, PC in NASH.	Lipid signature of NASH (32 lipids). **Decreased:** AA, EPA, and DHA; total Cer.

NAFLD: Non-alcoholic fatty liver disease; NAFL: Nonalcoholic fatty liver; NASH: Nonalcoholic steatohepatitis; DAG: Diacylglycerol; TAG: Triacylglycerol; SFA: Saturated Fatty acids; PUFA: Polyunsaturated fatty acids; FC: Free cholesterol; PC: Phosphatidylcholine; FFA : Free fatty acids; LCPUFA: Long chain polyunsaturated fatty acid; MUFA: Monounsaturated fatty acid; RBC-FA: Red blood cell-fatty acids; SM: Sphingomyelin; PI: Phosphatidylinositol; PS: Phosphatidylserine; PE: Phosphatidylethanolamine; EPA: Eicosapentaenoic acid (C20:5n-3); DHA: Docosahexanoic acid (C22:6n-3); AA: Arachidonic acid (C20:4n-6); LA: Linoleic acid (C18:2n-6); ALA: α-linolenic acid (C18:3n-3); Cer: Ceramides.

and sphingomyelin contents among healthy subjects and NAFL and NASH patients[25,27].

Due to discrepancy between the findings in plasma lipidomic analyses and the need to discover novel non-invasive biomarkers to distinguish NASH from NAFL, several studies for lipidomics analysis were performed in both plasma and liver biopsy samples[28,29]. A total of 48 common analytes with an overlap in both tissues were identified in a comprehensive lipidomic study conducted both in liver and plasma samples of patients with NAFLD. These analytes were mainly sphingolipid species, such as dihydroceramides, 1-deoxydihydroceramides, and longer chain ceramides, suggesting that perturbation of sphingolipid metabolism is involved in the pathogenesis of NAFLD[28].

The alterations occurring in each lipid class as well as the possible mechanisms underlying these changes in NAFLD will be discussed below.

GLYCEROPHOSPHOLIPIDS

Glycerophospholipids are major components of cellular membranes and a source of physiologically active compounds. They serve as signaling molecules and as anchors for proteins in cell membranes.

Phosphatidylcholine (PC) is one of the most abundant phospholipids in mammals and a major component of cellular membrane lipids. PC levels were reported to be decreased in the liver samples of patients with NAFLD[11,24]. However, there are conflicting data concerning the changes occurring in serum PC[25-27].

From a metabolic point of view, in most mammalian cells, PC is produced *de novo* from dietary choline *via* the cytidine 5′-diphosphate CDP-choline pathway[30]. In hepatocytes, up to 30% of PC comes from the conversion of phosphatidylethanolamine (PE) to PC, a reaction which is catalyzed by the enzyme phosphatidylethanolamine N-methyltransferase (PEMT)[31]. The synthesis of PE occurs *via* a CDP-ethanolamine pathway and *via* decarboxylation of phosphatidylserine (PS). Up to now, a few number of lipidomic studies mentioned alterations in PE in NAFLD patients. Liver PE content was found to be decreased among subjects with NASH, but in another study

Table 2 Summary of main lipidomics studies in plasma and serum in non-alcoholic fatty liver disease

Ref.	Tissue	Main findings in NAFLD patients compared to healthy control	Main findings in NASH patients compared to NAFL patients
Puri *et al*[26], 2009	Plasma	**Increased:** DAG, TAG, MUFA, dihomo-gamma-linolenic acid, palmitoleic acid, oleic acid, palmitoleic acid to palmitic acid ratio in NAFLD; stepwise increase in lipoxygenase (LOX) metabolites 5-HETE, 8-HETE, and 15-HETE from healthy controls to NAFL to NASH; 11-HETE in NASH compared with controls. **Decreased:** LA; total plasmalogen levels in NASH compared with controls.	
Zheng *et al*[81], 2012	Plasma phospholipids fatty acid composition	**Increased:** Dihomo-gamma-linolenic acid (C20: 3n-6), total SFA in phospholipids. **Decreased:** Eicosanoic acid (C20: 0), cis-11-octadecenoic acid (C18: 1n-7), DHA in PL.	
Loomba *et al*[89], 2015	plasma eicosanoid lipidomic profile	**Increased:** 15-HETE, 5,6-diHETrE. **Decreased:** 12,13-diHOME.	**Increased:** 11,12-diHETrE, dhk PGD2, and 20-COOH AA. **Decreased:**
Walle *et al*[80], 2016	Serum (fatty acid composition)	**Increased:** Palmitoleic acid in CE in individuals with NAFLD. **Decreased:** LA and total n-6 fatty acids in TAG in individuals with NASH.	**Increased:** SFA in TAG were higher in subjects with NASH, myristic acid in CE and TAG, Stearic acid in TAG. **Decreased:**
Tiwari-Heckler *et al*[27], 2018	Serum	**Increased:** PC and SM in NAFL and NASH. **Decreased:** Lysophospatidylethanolamine in NAFL and NASH individuals.	**Increased:** PE in patients with NASH.
Ma *et al*[27], 2016	Plasma	**Increased:** PS and PI in NAFL and NASH, DHA and AA in PS in NAFL and NASH.	

NAFLD: Non-alcoholic fatty liver disease; NAFL: nonalcoholic fatty liver; NASH: Nonalcoholic steatohepatitis; DAG: Diacylglycerol; TAG: Triacylglycerol; SFA: Saturated Fatty acids; MUFA: Monounsaturated fatty acids; PC: Phosphatidylcholine; HETE: Hydroxyeicosatetraenoic acid; 5,6-diHETrE : 5,6 dihydroxy- eicosatrienoic acid; 12,13-diHOME: 12,13-dihydroxy-9- octadecenoic acid; CE: Cholesteryl ester; PE: Phosphatidylethanolamine; LA: Linoleic acid (C18:2n-6); DHA: 11,12-diHETrE: 11,12-dihydroxy- eicosatrienoic acid; dhk PGD2: 13,14-dihydro-15-keto prostaglandin D2; 20-COOH AA: 20-carboxy arachidonic acid; SM: Sphingomyelin; PE: Phosphatidylethanolamine; PS: Phosphatidylserine; PI: Phosphatidylinositol.

serum PE levels were increased in these patients[24,27].

The ratio of PC/PE in the liver reflects the activity of PEMT[32]. In a shotgun MS-based targeted lipidomic analysis, researchers observed a statistically significant decrease of the hepatic PC/PE ratio in NAFLD patients[32]. Similarly, a low PC/PE ratio was also reported in red blood cell membrane of NAFLD patients and is considered as a biomarker of NAFLD. Additionally, a loss-of-function polymorphism in the *PEMT* gene seems to be associated with susceptibility in NAFLD[33]. However, when this parameter was calculated in plasma of NAFLD patients, no significant differences were observed among the healthy controls and NAFL and NASH patients, suggesting that compensatory mechanisms are activated in an attempt to maintain the plasma PC/PE ratio[25].

The low hepatic PC levels and the altered hepatic PC/PE ratio seem to have major implications in the development of NAFLD, but the pathophysiology of the lipid-induced processes is not fully understood. PC is the only phospholipid molecule that is known to regulate the assembly and secretion of lipoproteins[34]. Low hepatic levels

of PC, due to its synthesis impairment, have been found to impair the VLDL secretion and reduce significantly the levels of circulating VLDL lipoproteins. A dysfunction of VLDL secretion results in hepatic accumulation of TGs, as observed in many animal model studies[35,36]. Moreover, low PC levels have been previously described to activate sterol regulatory element-binding protein 1 (SREBP1)[37]. The activation of SREBP1, as mentioned above, leads to upregulation of lipogenic gene expression, thus resulting in increased *de novo* lipogenesis and formation of lipid droplets in hepatocytes.

From a structural point of view, disturbances in the proportion of PC and PE possibly affect the structure of the phospholipid bilayer of cell membrane. PC has a cylindrical shape and is distributed mainly in the outer monolayer of plasma membrane. On the contrary, PE is described as conical, and is located mostly in the inner monolayer[38]. A low PC/PE ratio possibly leads to rearrangement of PE in the outer monolayer, resulting in a loss of membrane integrity and increased permeability to pro-inflammatory molecules such as cytokines. Thus, the release of cellular contents, such as calcium, accompanied by an increase in influx of cytokines, initiates the inflammation in NAFLD[39].

As far as the rest of the glycerophospholipids is concerned, only a small number of lipidomics studies have previously reported statistically significant changes of their abundance in NAFLD[11,24,25]. Likewise, the findings from lipidomics studies conducted on liver samples were inconsistent with those from plasma samples of NAFLD patients.

Chiappini *et al*[24] found that the levels of PS and PI were decreased in liver biopsy samples of patients with NASH compared with control individuals, whereas in a recent lipidomic study, no statistically significant differences were found in hepatic PS and PI among the control group, patients with NAFL, and those with NASH[24,29]. On the contrary, plasma PS and PI were found to be increased in NAFL and NASH compared with the control, while another study reported only an increase of serum PI in NASH patients compared to patients with simple steatosis[25,40]. Tiwari-Heckler *et al*[27], on the other hand, reported no significant changes in the amount of circulating PI among controls, NAFL patients, and NASH patients, but it is worth noting that in this study liver biopsy was not performed in all included subjects. These glycerophospholipids are also components of cellular membrane and are associated with cellular signaling and cellular apoptosis[41,42]. Given the important role of these lipids, differences observed in their hepatic or plasma levels may be involved in the development and progression of NAFLD.

Lysophosphatidylocholine (LPC) is a biologically active lipid and is considered an important mediator of hepatic lipotoxicity[43]. In liver biopsies from patients with NASH, LPC was found to be increased and this elevation seems to follow the disease severity[11,44]. However, several plasma and serum lipidomic studies failed to detect any statistically significant changes in the LPC content in patients with NAFL or NASH[25-27]. Interestingly, a recent study in biopsy proven patients with NAFLD found that plasma LPC species were decreased in patients with NASH[45]. Furthermore, another study reported that LPC diminished in patients with NAFLD[46]. This finding combined with an increase of TGs with low carbon number and double-bond content and a decrease of ether phospholipids has been proposed as a useful biomarker capable of estimating the percentage of liver fat in patients with NAFLD.

LPC is generated from PC by the action of secretory or lipoprotein-bound phospholipase A2 (PLA2). Also, LPC in plasma originates by the activity of lecithin-cholesterol acyltransferase (LCAT) as well as the activity of endothelial lipase. Hepatic secretion is also considered as a source of plasma LPC[47]. The increased hepatic LPC content could be attributable to an increase in hepatic biosynthesis or to an increase of total LPCs transported back to the liver by albumin or alpha 1-acid glycoprotein (AGP)[48]. As concerns the LPC levels in plasma, an impairment either on LCAT activity or PLA2 activity, as well as an increased turnover of LPC to PC or lysophosphatidic acid and sphingosine-1-phosphate are probable causes of diminished LPC levels in plasma. In fact, lipoprotein associated phospholipase A2 levels were found to be decreased in patients with NAFLD, whereas LCAT activity was higher in subjects with NAFLD, as inferred from a Fatty Liver Index > 60[49,50]. Moreover, a study in mice reported lower levels of palmitoyl-, stearoyl-, and oleoyl-LPCs in NASH compared to animals with NAFL, suggesting that the activity of lyso-PC acyltransferase, that catalyzes the recycle of LPCs to PC, is elevated in NASH[51].

LPC as a bioactive molecule, seems to be involved in the pathogenesis of NAFLD and the transition from simple steatosis to NASH. LPC affects the whole liver lipid metabolism and has been found to downregulate genes involved in fatty acid oxidation and upregulate genes involved in cholesterol biosynthesis[52]. Furthermore, LPC has been demonstrated *in vitro* to trigger apoptosis of hepatocytes, probably

through disruption of mitochondrial integrity, whereas inhibitors of phospholipase A2 were shown to decrease palmitate-induced lipotoxicity and cell apoptosis[52,53]. Lastly, lipotoxicity induced by LPC could be mediated by release of proinflammatory and pro-fibrogenic molecules from hepatocytes or the enhanced turnover of LPC to profibrogenic lysophosphatidic acid[54].

Plasmalogens are a class of glycerophospholipids carrying a vinyl ether bond in sn-1 and an ester bond in sn-2 position of their glycerol backbone. The biosynthesis of plasmalogens is a complex multistep process that takes place in peroxisomes and the endoplasmic reticulum[55]. Circulating plasma plasmalogens levels have been previously found to be decreased in patients with NASH and were negatively associated with obesity[26,56]. Furthermore, a depletion of total ether phospholipids has also been found in patients with NAFLD[46]. Lipidomic studies in liver biopsies of patients with NAFLD, however, failed to detect any changes in plasmalogen levels, probably due to their significantly lower liver concentrations compared to the rest of glycerophospholipids[57]. The liver contains low amounts of plasmalogens, although the enzymes involved in their synthesis are active in this tissue. This reduction might be attributable to their synthesis in the liver, and subsequent transport by lipoproteins to other tissues[57]. More interestingly, lipidomic analyses in NAFLD patients carrying the GG-genotype of *PNPLA3*, who are at a higher risk for more advanced disease and fibrosis, revealed lower levels of total plasma plasmalogens compared to subjects with CC- and CG-allele[27].

Plasmalogens represent a key structural component of the cell membrane and may be involved in ion transport and cholesterol efflux. They have been described as signaling molecules and may also serve as precursors for eicosanoid biosynthesis[58]. Several studies have shown that plasmalogens, by virtue of their vinyl ether, function as endogenous antioxidants[59]. The deficiency in plasmalogens, which has been reported in plasma of NASH patients, could be attributed to oxidative stress-induced peroxisome damage and subsequent impairment of plasmalogen biosynthesis[55]. In fact, a recent study reported that endogenous hepatic plasmalogens, through a PPARa-dependent mechanism, prevent the development of hepatic steatosis and NASH in mice[60].

SPHINGOLIPIDS

Sphingolipids are a special group of phospholipids which contain a sphingosine backbone. Even though sphingolipids are very low in abundance compared with glycerophospholipids, they are considered important structural components of cell membrane[61,62]. They are involved in the arrangement of membrane lipid domains and cell signaling of major biological processes, such as cell survival and immune responses[62]. Lipidomic studies revealed changes in levels of sphingomyelin (SM), ceramides, and diydroceramides in plasma and liver biopsies of patients with NAFL and NASH, implicating that alterations in sphingolipid metabolism are associated with the development and severity of NAFLD[24,28,45].

SM is the most abundant sphingolipid and its plasma levels have been previously reported to correlate with body mass index (BMI)[56,61]. In NAFLD, the results from lipid profiling of liver and plasma are inconsistent. SM was found to be decreased in liver biopsies of patients with biopsy proven NASH[24], but Puri *et al*[11] reported a non-statistically significant increase of this sphingolipid in patients with NASH. In other lipidomic studies, in which the control group was also morbidly obese, no significant differences were observed in the total sphingomyelin levels among the control, NAFL, and NASH groups[25,29,40]. Tiwari-Heckler *et al*[27], however, reported an increase of total serum SM in NAFL and NASH patients compared to healthy controls. Moreover, individual sphingomyelin species, specifically SM (36:3), (d18:2/16:0), (d18:2/14:0), (d18:1/18:0), (d18:1/16:0), (d18:1/12:0), and (d18:0/16:0), were found to be increased in serum of patients with NAFLD compared to healthy subjects[63], whereas Zhou *et al*[45] reported that circulating sphingomyelin cluster with representatives SM (d18:1/24:1), SM (d18:1/16:0), SM (d18:1/22:0), SM (d18:1/24:0), SM (d18:1/18:0), SM (d18:1/20:0), SM (d18:1/23:0), SM (d18:0/16:0), and SM (d18:0/20:4) was decreased in NASH patients compared to non-NASH subjects. Although there is no consensus on whether SM increases or decreases along with disease severity, studies in transgenic mice lacking the sphingomyelin synthase gene, revealed a strong association between liver SM levels and insulin resistance[64]. Further studies are needed to assess the relationship between SM metabolism and progression of NAFLD.

Numerous studies suggest that ceramide is a major contributing factor to insulin

resistance[65]. Ceramides and ceramide-derived sphingolipids are structural constituents of cell membranes, which also possess cell-signaling properties. Even though ceramide synthesis occurs in many organs, the liver is a key site for ceramide synthesis and in fact data from several studies suggest that sphingolipids, such as SM and ceramides, are found in higher quantity in the liver compared to other tissues[65,66]. Moreover, ceramide levels have been reported to be increased in the plasma of patients with prediabetes and ceramides were also increased in plasma and liver biopsies of patients with NAFLD[28,40,67].

Ceramide synthesis can occur through three different pathways: (1) A *de novo* pathway that includes four sequential reactions with serine palmitoyl-CoA transferase (SPT) representing the rate-limiting enzyme of this pathway; (2) Through hydrolysis of SM catalyzed by sphingomyelinase (SMase); and (3) A salvage pathway[68]. *De novo* synthesis has been described to be stimulated by a diet rich in saturated fat[69]. Furthermore, increased hepatic free fatty acid influx, inflammation induced by TNFα and IL1, and oxidative stress can all increase the activity of SPT and activate *de novo* synthesis of ceramides[68,70]. All these three conditions are involved in the etiopathogenesis of NAFLD and represent important regulators of *de novo* ceramide synthesis[3]. Aside from the activation of *de novo* synthesis, inflammation increases ceramides by up-regulating the activity of sphingomyelinase[71]. Adiponectin, an adipokine involved in NAFLD pathophysiology, affects also the ceramide production. Adiponectin *via* receptors appears to upregulate the expression of ceramidase, the enzyme that converts ceramides to sphingosine-1-phosphate (S1P). Patients with NAFLD exhibit lower adiponectin levels than healthy subjects and this seems to contribute to the already increased concentration of ceramides[72].

Ceramides, through their function as signaling molecules, have several physiological effects that contribute to the pathogenesis of steatosis and steatohepatitis. In particular, ceramides have been previously reported to decrease insulin sensitivity in skeletal muscle and hepatocytes[65]. In fact, a previous animal study reported that administration of inhibitors of ceramide biosynthesis resulted in a significant improvement of insulin resistance[70]. While increase of inflammatory cytokines leads to increased ceramide production, it is likely that ceramides through feedback mechanisms lead to increased production of cytokines and induce further processes of inflammation[65]. In addition, ceramides are involved in increased oxidative stress, mitochondrial dysfunction, and cell apoptosis[65,73]. Finally, there is evidence that ceramides may regulate the synthesis of HDL lipoproteins and thereby affect the reverse cholesterol transport. In a study in Western diet rat models, administration of myriosine - an inhibitor of ceramide biosynthesis – not only improved insulin resistance and steatosis, but also increased ApoAI production rate and consequently the production rate of HDL lipoprotein[74].

NEUTRAL LIPIDS

As far as neutral lipid classes are concerned, a limited number of studies have been conducted to investigate whether quantitative changes in their content are observed in patients with NAFLD. Triacylglycerols (TG), as expected, were found to be increased in liver biopsies of patients with NAFLD, whereas no statistically significant differences were observed in free fatty acid (FFA) hepatic content[11,29]. Diacylglycerols (DG) were also increased in the liver and interestingly, the ratio of TG/DG was increased in a stepwise manner from NAFL to NASH, suggesting that diacylglycerol acyl transferase (DGAT) is possibly involved in the pathogenesis of NAFLD[11]. In fact, inhibitors of DGAT-2 decreased hepatic steatosis, ballooning, and fibrosis in mice[75]. Moreover, recently this study was extended in phase 1 clinical trial in humans and steatosis and clinical markers of liver function were improved[76].

Several studies have demonstrated that cholesterol homeostasis is disturbed in NAFLD[77,78]. Hepatic free cholesterol accumulation has been correlated with disease progression from simple steatosis to NASH without an increase in cholesterol esters[11], whereas the findings about esterified cholesterol are contradictory[11,29]. Free cholesterol is considered a cytotoxic lipid that is involved in hepatotoxicity by disrupting membrane integrity and inducing oxidative stress, mitochondrial dysfunction, and apoptosis[79]. Thus, the observed increase of free cholesterol might contribute to liver injury and disease progression.

FATTY ACIDS

Numerous studies have demonstrated that the fatty acid composition of lipids is altered in patients with simple steatosis and NASH. Total saturated fatty acids were found to be increased in liver biopsies of patients with NAFLD[11]. Especially, an increase in individual saturated fatty acids such as myristic acid and palmitic acid was found in liver samples of patients with NASH[24]. Walle *et al*[80] conducted a comprehensive study in serum fatty acid composition and reported an increase in total saturated fatty acids in triacyglycerols in NASH patients compared to patients with simple steatosis. Furthermore, serum levels of myristic acid in cholesterol esters and triacyglycerols and those of stearic acid in triacylgylerols were found to be increased in patients with NASH[80]. Total saturated fatty acids were reported also to be increased in plasma phospholipids in patients with NAFLD[81]. The increased *de novo* lipogenesis occurring in NAFLD as well a diet enriched in those types of fatty acids might be the main cause for the increase of saturated fatty acids in the liver and serum of patients with NAFLD[82]. In addition, saturated fatty acids exhibit pro-apoptotic properties and also, are involved in the pathogenesis of steatosis. The increase of saturated fatty acids in hepatocytes results in endoplasmatic reticulum stress, increased caspase activation, and hepatocellular apoptosis[83].

Total monounsaturated fatty acids were also found to be increased in the liver and plasma of NAFLD patients[10,23,26,29]. In some cases, this increase was driven by palmitoleic acid and oleic acid[23,26]. These individual fatty acids are generated by the enzyme stearoyl-Coa desaturase (SCD1) from saturated fatty acids. The increase of monounsaturated fatty acids could be attributable to increased *de novo* lipogenesis activity and increased activity of SCD1[84]. In fact, Chiappini *et al*[24] demonstrated that the gene expression of *SCD1* was significantly increased in NASH patients in accordance with the increase of oleic and palmitoleic acid. Monounsaturated fatty acids are considered to contribute to the development of steatosis, but are more efficient in incorporating into hepatocyte triglycerides, thus they are less lipotoxic than saturated fatty acids. A potential protective role of monounsaturated fatty acids against lipotoxicity has also been suggested through the promotion of triglycerides accumulation in hepatocytes[85].

The most common finding in lipidomic studies is the decrease of long chain PUFA. Specifically, a decrease in eicosapentaenoic acid, docosahexanoic acid, and arachidonic acid was reported in several lipidomic studies performed in the liver and plasma of patients with NAFLD[10,11,23,25]. The depletion of these n-3 and n-6 PUFA may be attributed to either a dietary deficiency or impaired biosynthesis. The generation of these PUFA is a multistep process in which several elongase and desaturases enzyme are involved. In NASH patients, the activities of fatty acid desaturase 1 (FADS1) and fatty acid elongase 6 (ELOVL6) were decreased[24]. Furthermore, the decreased activity of FADS1 is considered a key pathogenetic factor in the progression of simple steatosis to NASH. Another interesting finding is the increased n-6/n-3 ratio observed in liver biopsies of patients with NASH[10,11]. PUFA, especially n-3, are involved several biological processes and exhibit a protective role against lipotoxicity and insulin resistance[86]. Restoration of hepatic n-3 content improved steatosis and insulin resistance and decreased lipid peroxidation and necroinflammation in a mouse model of steatohepatitis[86]. Moreover, PUFA interact with transcription factors and modulate the expression of genes involved in lipid metabolism and fibrogenesis[87,88].

PUFA serve also as precursors for the synthesis of proinflammatory eicosanoids and specialized pro-resolving mediators (SPMs). The biosynthesis of these lipid species involves several enzymes such as cyclooxygenases and lipoxygenases. Puri *et al*[26] reported a stepwise increase of lipoxygenase metabolites of arachidonic acid in plasma from control to NAFL and NASH, whereas no significant differences were observed in the plasma cyclooxygenase products of arachidonic acid among the study groups. Specifically, the lipoxygenase metabolites 5-HETE, 8-HETE, 11-HETE, and 15-HETE were found to be increased in plasma of patients with NASH[26]. Later, Loomba *et al*[89] investigated the plasma lipidomic profile of eicosanoid in patients with NAFLD and reported a significant increase of arachidonic acid-derived metabolites 11,12-diHETrE, dhk PGD2, and 20-COOH AA in plasma of patients with NASH compared to subjects with NAFL.

LIMITATIONS OF PLASMA LIPIDOMICS STUDIES IN NAFLD

The findings of lipidomics studies conducted in plasma or serum of patients with

NAFLD, as mentioned before, are inconsistent. Lack of consistency is observed also between findings from plasma and liver studies. Interestingly, the discrepancies between liver and plasma findings regard mainly glycerophospholipid composition rather than fatty acid composition. In general, liver lipidomics studies revealed a decrease in glycerophospholipid species, such as PC, PE, PS, and PI, in NAFL patients and in some cases this alteration was profound only in the setting of NASH. On the contrary, most plasma lipidomic studies failed to detect depletion of these lipids and in some cases plasma glycerophospholipids were found to be increased in patients with NAFLD compared to the control group. Plasma glycerophospholipids are carried and distributed in lipoprotein classes. Plasma PC and PE are mainly distributed in HDL lipoprotein and 50% of hepatic PC is derived from circulation probably though hepatic uptake of HDL-PC[90,91]. Hence, low hepatic glycerophospholipid content, in an attempt to maintain adequate levels of these lipids, could lead to activation of unknown compensatory processes resulting in increased delivery of HDL-associated phospholipids and subsequent increase in plasma levels.

Moreover, findings regarding SM content in the liver and plasma are also inconsistent. Approximately 50% of plasma SM is found in LDL and 40% in HDL, and it is worth noticing that plasma SM levels correlate with BMI[56,90]. Differences in lipidomics study design including the selection of obese study population as a control group could explain the discordant findings. Furthermore, alteration in SM content in lipoprotein particles due to dietary factors, obesity, and unknown compensatory mechanism could be responsible for the differences observed in liver and plasma studies regarding sphingolipid species.

Further lipidomic studies focused on phospholipid content of lipoproteins in NAFLD patients should address this issue and delineate the changes observed in the setting of NAFLD.

NONINVASIVE DIAGNOSIS OF NASH THROUGH LIPIDOMICS

At present, the diagnosis of NAFLD and the distinction of NASH from simple steatosis require liver biopsy and histological assessment. Nevertheless, liver biopsy is an invasive, costly, and time-consuming procedure. Hence, there is a growing interest in developing noninvasive methods for differential diagnosis of NASH and evaluation of treatment outcomes. Lipidomic studies carried out in liver biopsies of patients with NAFL and NASH patients reported alterations of hepatic lipid profile and several studies investigated if these changes were also observed in plasma or serum. Plasma lipidomic studies reported changes in the concentration of several lipids between patients with NASH and NAFL, but as highlighted above the results are inconsistent. As seen in Table 2, saturated fatty acids in TGs, such as myristic acid and stearic acid, were found to be increased in patients with NASH compared to subjects with NAFL[80]. Moreover, plasma eicosanoid lipidomics analyses revealed a significant increase of arachidonic acid-derived metabolites (11,12-diHETrE, dhk PGD2, and 20-COOH AA) in patients with NASH compared to subjects with NAFL and researchers suggested that these eicosanoids may have a utility as biomarkers for the noninvasive diagnosis of NASH[89]. Lipoxygenase metabolites 5-HETE, 8-HETE, 11-HETE, and 15-HETE were also found to be increased in plasma of patients with NASH and these metabolites seem promising predictive biomarkers of NASH[26].

Gorden *et al*[28] investigated the alterations of liver and plasma lipidomic profiles in patients with NAFLD categorized in three subgroups of disease progression. The study population included healthy subjects, patients with simple steatosis, patients with NASH, and subjects with cirrhosis. Lipidomic analyses in combination with aqueous metabolites analyses led to identification of 48 common analytes, which presented variation across disease stage and an overlap in both tissues. These analytes were sphingolipid species, such as dihydroceramides, 1-deoxydihydroceramides, and longer chain ceramides, implicating that sphingolipid metabolism is impaired and additionally involved in disease progression and transition of simple steatosis to NASH. Furthermore, Gorden *et al*[28] identified a panel of 20 plasma lipids that can be used to distinguish NASH from simple steatosis. This panel included dihydrosphingolipids, ether phospatidylicholines, and other individual species. However, the number of patients that participated in this study is relatively small and validation of these findings in larger cohort of patients is needed[28]. Later, Zhou and his team developed an MS-based model and diagnostic score for NASH with an area under the receiver operating characteristic of 0.86. The NASH ClinLipMet score included AST, fasting glucose, glutamate, isoleucine, glycine, lysophospatidylcholine

16:0, and phospoethanolamine 40:6 along with *PNPLA3* genotype. This score needs also external validation[45].

CONCLUSION

Recent advances in lipidomics technology have made it possible to profile lipidome of liver tissues and plasma in NAFLD and compare the findings among the different stages of disease. Lipidomic profiling accompanied by experimental studies using pharmacological reagents to alter synthesis or metabolism of certain lipids, has given additional insights into mechanisms governing lipotoxicity and disease progression. In this review, the most interesting findings of lipodomics analyses are summarized and the interpretation of these findings in the pathogenesis of NAFLD is discussed. The inconsistencies observed between the findings of plasma and liver lipidomics studies in NAFLD have also been underlined and future studies will need to address this issue. Moreover, even if a small number of studies identified specific lipids or a panel of lipids as biomarkers of disease progression, these findings need further external validation from a large cohort of patients.

REFERENCES

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

2 **Contos MJ**, Sanyal AJ. The clinicopathologic spectrum and management of nonalcoholic fatty liver disease. *Adv Anat Pathol* 2002; **9**: 37-51 [PMID: 11756758 DOI: 10.1097/00125480-200201000-00005]

3 **Dowman JK**, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. *QJM* 2010; **103**: 71-83 [PMID: 19914930 DOI: 10.1093/qjmed/hcp158]

4 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362 [PMID: 10573511 DOI: 10.1002/hep.510300604]

5 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

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

7 **Trovato FM**, Castrogiovanni P, Malatino L, Musumeci G. Nonalcoholic fatty liver disease (NAFLD) prevention: role of Mediterranean diet and physical activity. *Hepatobiliary Surg Nutr* 2019; **8**: 167-169 [PMID: 31098370 DOI: 10.21037/hbsn.2018.12.05]

8 **Trovato FM**, Martines GF, Brischetto D, Catalano D, Musumeci G, Trovato GM. Fatty liver disease and lifestyle in youngsters: diet, food intake frequency, exercise, sleep shortage and fashion. *Liver Int* 2016; **36**: 427-433 [PMID: 26346413 DOI: 10.1111/liv.12957]

9 **Malhi H**, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 360-369 [PMID: 18956292 DOI: 10.1055/s-0028-1091980]

10 **Araya J**, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli P, Poniachik J. Increase in long-chain polyunsaturated fatty acid n - 6/n - 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci (Lond)* 2004; **106**: 635-643 [PMID: 14720121 DOI: 10.1042/CS20030326]

11 **Puri P**, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 1081-1090 [PMID: 17654743 DOI: 10.1002/hep.21763]

12 **Sa R**, Zhang W, Ge J, Wei X, Zhou Y, Landzberg DR, Wang Z, Han X, Chen L, Yin H. Discovering a critical transition state from nonalcoholic hepatosteatosis to nonalcoholic steatohepatitis by lipidomics and dynamical network biomarkers. *J Mol Cell Biol* 2016; **8**: 195-206 [PMID: 26993042 DOI: 10.1093/jmcb/mjw016]

13 **Bugianesi E**, Moscatiello S, Ciaravella MF, Marchesini G. Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm Des* 2010; **16**: 1941-1951 [PMID: 20370677 DOI: 10.2174/138161210791208875]

14 **Foretz M**, Guichard C, Ferré P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci USA* 1999; **96**: 12737-12742 [PMID: 10535992 DOI: 10.1073/pnas.96.22.12737]

15 **Jung UJ**, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci* 2014; **15**: 6184-6223 [PMID: 24733068 DOI: 10.3390/ijms15046184]

16 **Cusi K**. Role of insulin resistance and lipotoxicity in non-alcoholic steatohepatitis. *Clin Liver Dis* 2009; **13**: 545-563 [PMID: 19818304 DOI: 10.1016/j.cld.2009.07.009]

17 **Schuster S**, Cabrera D, Arrese M, Feldstein AE. Triggering and resolution of inflammation in NASH. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 349-364 [PMID: 29740166 DOI: 10.1038/s41575-018-0009-6]

18 **Trovato FM**, Catalano D, Musumeci G, Trovato GM. 4Ps medicine of the fatty liver: the research model of predictive, preventive, personalized and participatory medicine-recommendations for facing obesity, fatty liver and fibrosis epidemics. *EPMA J* 2014; **5**: 21 [PMID: 25937854 DOI: 10.1186/1878-5085-5-21]

19 **Han X**, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J Lipid Res* 2003; **44**: 1071-1079 [PMID: 12671038 DOI: 10.1194/jlr.R300004-JLR200]

20 **Oresic M**, Hänninen VA, Vidal-Puig A. Lipidomics: a new window to biomedical frontiers. *Trends Biotechnol* 2008; **26**: 647-652 [PMID: 18951641 DOI: 10.1016/j.tibtech.2008.09.001]

21 **Naudí A**, Cabré R, Jové M, Ayala V, Gonzalo H, Portero-Otín M, Ferrer I, Pamplona R. Lipidomics of human brain aging and Alzheimer's disease pathology. *Int Rev Neurobiol* 2015; **122**: 133-189 [PMID: 26358893 DOI: 10.1016/bs.irn.2015.05.008]

22 **Nicholson JK**, Buckingham MJ, Sadler PJ. High resolution 1H n.m.r. studies of vertebrate blood and plasma. *Biochem J* 1983; **211**: 605-615 [PMID: 6411064 DOI: 10.1042/bj2110605]

23 **Allard JP**, Aghdassi E, Mohammed S, Raman M, Avand G, Arendt BM, Jalali P, Kandasamy T, Prayitno N, Sherman M, Guindi M, Ma DW, Heathcote JE. Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol* 2008; **48**: 300-307 [PMID: 18086506 DOI: 10.1016/j.jhep.2007.09.009]

24 **Chiappini F**, Coilly A, Kadar H, Gual P, Tran A, Desterke C, Samuel D, Duclos-Vallée JC, Touboul D, Bertrand-Michel J, Brunelle A, Guettier C, Le Naour F. Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. *Sci Rep* 2017; **7**: 46658 [PMID: 28436449 DOI: 10.1038/srep46658]

25 **Ma DW**, Arendt BM, Hillyer LM, Fung SK, McGilvray I, Guindi M, Allard JP. Plasma phospholipids and fatty acid composition differ between liver biopsy-proven nonalcoholic fatty liver disease and healthy subjects. *Nutr Diabetes* 2016; **6**: e220 [PMID: 27428872 DOI: 10.1038/nutd.2016.27]

26 **Puri P**, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, Contos MJ, Sterling RK, Fuchs M, Zhou H, Watkins SM, Sanyal AJ. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology* 2009; **50**: 1827-1838 [PMID: 19937697 DOI: 10.1002/hep.23229]

27 **Tiwari-Heckler S**, Gan-Schreier H, Stremmel W, Chamulitrat W, Pathil A. Circulating Phospholipid Patterns in NAFLD Patients Associated with a Combination of Metabolic Risk Factors. *Nutrients* 2018; **10** [PMID: 29883377 DOI: 10.3390/nu10050649]

28 **Gorden DL**, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, Min J, Spann NJ, McDonald JG, Kelly SL, Duan J, Sullards MC, Leiker TJ, Barkley RM, Quehenberger O, Armando AM, Milne SB, Mathews TP, Armstrong MD, Li C, Melvin WV, Clements RH, Washington MK, Mendonsa AM, Witztum JL, Guan Z, Glass CK, Murphy RC, Dennis EA, Merrill AH Jr, Russell DW, Subramaniam S, Brown HA. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res* 2015; **56**: 722-736 [PMID: 25598080 DOI: 10.1194/jlr.P056002]

29 **Peng KY**, Watt MJ, Rensen S, Greve JW, Huynh K, Jayawardana KS, Meikle PJ, Meex RCR. Mitochondrial dysfunction-related lipid changes occur in nonalcoholic fatty liver disease progression. *J Lipid Res* 2018; **59**: 1977-1986 [PMID: 30042157 DOI: 10.1194/jlr.M085613]

30 **Vance DE**, Vance JE. Physiological consequences of disruption of mammalian phospholipid biosynthetic genes. *J Lipid Res* 2009; **50** Suppl: S132-S137 [PMID: 18955728 DOI: 10.1194/jlr.R800048-JLR200]

31 **Sundler R**, Akesson B. Regulation of phospholipid biosynthesis in isolated rat hepatocytes. Effect of different substrates. *J Biol Chem* 1975; **250**: 3359-3367 [PMID: 1123345]

32 **Arendt BM**, Ma DW, Simons B, Noureldin SA, Therapondos G, Guindi M, Sherman M, Allard JP. Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine. *Appl Physiol Nutr Metab* 2013; **38**: 334-340 [PMID: 23537027 DOI: 10.1139/apnm-2012-0261]

33 **Song J**, da Costa KA, Fischer LM, Kohlmeier M, Kwock L, Wang S, Zeisel SH. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J* 2005; **19**: 1266-1271 [PMID: 16051693 DOI: 10.1096/fj.04-3580com]

34 **Jacobs RL**, Devlin C, Tabas I, Vance DE. Targeted deletion of hepatic CTP:phosphocholine cytidylyltransferase alpha in mice decreases plasma high density and very low density lipoproteins. *J Biol Chem* 2004; **279**: 47402-47410 [PMID: 15331603 DOI: 10.1074/jbc.M404027200]

35 **Waite KA**, Cabilio NR, Vance DE. Choline deficiency-induced liver damage is reversible in Pemt(-/-) mice. *J Nutr* 2002; **132**: 68-71 [PMID: 11773510 DOI: 10.1093/jn/132.1.68]

36 **Jacobs RL**, Zhao Y, Koonen DP, Sletten T, Su B, Lingrell S, Cao G, Peake DA, Kuo MS, Proctor SD, Kennedy BP, Dyck JR, Vance DE. Impaired de novo choline synthesis explains why phosphatidylethanolamine N-methyltransferase-deficient mice are protected from diet-induced obesity. *J Biol Chem* 2010; **285**: 22403-22413 [PMID: 20452975 DOI: 10.1074/jbc.M110.108514]

37 **Walker AK**, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, Shioda T, Hansen M, Yang F, Niebergall LJ, Vance DE, Tzoneva M, Hart AC, Näär AM. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell* 2011; **147**: 840-852 [PMID: 22035958 DOI: 10.1016/j.cell.2011.09.045]

38 **Bigay J**, Antonny B. Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular territories in determining specificity. *Dev Cell* 2012; **23**: 886-895 [PMID: 23153485 DOI: 10.1016/j.devcel.2012.10.009]

39 **Li Z**, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab* 2006; **3**: 321-331 [PMID: 16679290 DOI: 10.1016/j.cmet.2006.03.007]

40 **Anjani K**, Lhomme M, Sokolovska N, Poitou C, Aron-Wisnewsky J, Bouillot JL, Lesnik P, Bedossa P, Kontush A, Clement K, Dugail I, Tordjman J. Circulating phospholipid profiling identifies portal contribution to NASH signature in obesity. *J Hepatol* 2015; **62**: 905-912 [PMID: 25450212 DOI: 10.1016/j.jhep.2014.11.002]

41 **Balla T**. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol Rev* 2013; **93**: 1019-1137 [PMID: 23899561 DOI: 10.1152/physrev.00028.2012]

42 **Fadok VA**, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000; **405**: 85-90 [PMID: 10811223 DOI: 10.1038/35011084]

43 **Han MS**, Park SY, Shinzawa K, Kim S, Chung KW, Lee JH, Kwon CH, Lee KW, Lee JH, Park CK, Chung WJ, Hwang JS, Yan JJ, Song DK, Tsujimoto Y, Lee MS. Lysophosphatidylcholine as a death effector in the lipoapoptosis of hepatocytes. *J Lipid Res* 2008; **49**: 84-97 [PMID: 17951222 DOI: 10.1194/jlr.M700184-JLR200]

44 **García-Cañaveras JC**, Donato MT, Castell JV, Lahoz A. A comprehensive untargeted metabonomic analysis of human steatotic liver tissue by RP and HILIC chromatography coupled to mass spectrometry reveals important metabolic alterations. *J Proteome Res* 2011; **10**: 4825-4834 [PMID: 21830829 DOI: 10.1021/pr200629p]

45 **Zhou Y**, Orešič M, Leivonen M, Gopalacharyulu P, Hyysalo J, Arola J, Verrijken A, Francque S, Van Gaal L, Hyötyläinen T, Yki-Järvinen H. Noninvasive Detection of Nonalcoholic Steatohepatitis Using Clinical Markers and Circulating Levels of Lipids and Metabolites. *Clin Gastroenterol Hepatol* 2016; **14**: 1463-1472.e6 [PMID: 27317851 DOI: 10.1016/j.cgh.2016.05.046]

46 **Orešič M**, Hyötyläinen T, Kotronen A, Gopalacharyulu P, Nygren H, Arola J, Castillo S, Mattila I, Hakkarainen A, Borra RJ, Honka MJ, Verrijken A, Francque S, Iozzo P, Leivonen M, Jaser N, Juuti A, Sørensen TI, Nuutila P, Van Gaal L, Yki-Järvinen H. Prediction of non-alcoholic fatty-liver disease and liver fat content by serum molecular lipids. *Diabetologia* 2013; **56**: 2266-2274 [PMID: 23824212 DOI: 10.1007/s00125-013-2981-2]

47 **Sekas G**, Patton GM, Lincoln EC, Robins SJ. Origin of plasma lysophosphatidylcholine: evidence for direct hepatic secretion in the rat. *J Lab Clin Med* 1985; **105**: 190-194 [PMID: 3973457]

48 **Ojala PJ**, Hermansson M, Tolvanen M, Polvinen K, Hirvonen T, Impola U, Jauhiainen M, Somerharju P, Parkkinen J. Identification of alpha-1 acid glycoprotein as a lysophospholipid binding protein: a complementary role to albumin in the scavenging of lysophosphatidylcholine. *Biochemistry* 2006; **45**: 14021-14031 [PMID: 17115697 DOI: 10.1021/bi061657l]

49 **Liu Z**, Li H, Zheng Y, Gao Z, Cong L, Yang L, Zhou Y. Association of Lipoprotein-Associated Phospholipase A2 with the Prevalence of Nonalcoholic Fatty Liver Disease: A Result from the APAC Study. *Sci Rep* 2018; **8**: 10127 [PMID: 29973631 DOI: 10.1038/s41598-018-28494-8]

50 **Nass KJ**, van den Berg EH, Gruppen EG, Dullaart RPF. Plasma lecithin:cholesterol acyltransferase and phospholipid transfer protein activity independently associate with nonalcoholic fatty liver disease. *Eur J Clin Invest* 2018; **48**: e12988 [PMID: 29947103 DOI: 10.1111/eci.12988]

51 **Tanaka N**, Matsubara T, Krausz KW, Patterson AD, Gonzalez FJ. Disruption of phospholipid and bile acid homeostasis in mice with nonalcoholic steatohepatitis. *Hepatology* 2012; **56**: 118-129 [PMID: 22290395 DOI: 10.1002/hep.25630]

52 **Hollie NI**, Cash JG, Matlib MA, Wortman M, Basford JE, Abplanalp W, Hui DY. Micromolar changes in lysophosphatidylcholine concentration cause minor effects on mitochondrial permeability but major alterations in function. *Biochim Biophys Acta* 2014; **1841**: 888-895 [PMID: 24315825 DOI: 10.1016/j.bbalip.2013.11.013]

53 **Kakisaka K**, Cazanave SC, Fingas CD, Guicciardi ME, Bronk SF, Werneburg NW, Mott JL, Gores GJ. Mechanisms of lysophosphatidylcholine-induced hepatocyte lipoapoptosis. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G77-G84 [PMID: 21995961 DOI: 10.1152/ajpgi.00301.2011]

54 **Hirsova P**, Ibrahim SH, Krishnan A, Verma VK, Bronk SF, Werneburg NW, Charlton MR, Shah VH, Malhi H, Gores GJ. Lipid-Induced Signaling Causes Release of Inflammatory Extracellular Vesicles From Hepatocytes. *Gastroenterology* 2016; **150**: 956-967 [PMID: 26764184 DOI: 10.1053/j.gastro.2015.12.037]

55 **Wallner S**, Schmitz G. Plasmalogens the neglected regulatory and scavenging lipid species. *Chem Phys Lipids* 2011; **164**: 573-589 [PMID: 21723266 DOI: 10.1016/j.chemphyslip.2011.06.008]

56 **Weir JM**, Wong G, Barlow CK, Greeve MA, Kowalczyk A, Almasy L, Comuzzie AG, Mahaney MC, Jowett JB, Shaw J, Curran JE, Blangero J, Meikle PJ. Plasma lipid profiling in a large population-based cohort. *J Lipid Res* 2013; **54**: 2898-2908 [PMID: 23868910 DOI: 10.1194/jlr.P035808]

57 **Vance JE**. Lipoproteins secreted by cultured rat hepatocytes contain the antioxidant 1-alk-1-enyl-2-acylglycerophosphoethanolamine. *Biochim Biophys Acta* 1990; **1045**: 128-134 [PMID: 2116174 DOI: 10.1016/0005-2760(90)90141-j]

58 **Braverman NE**, Moser AB. Functions of plasmalogen lipids in health and disease. *Biochim Biophys Acta* 2012; **1822**: 1442-1452 [PMID: 22627108 DOI: 10.1016/j.bbadis.2012.05.008]

59 **Zoeller RA**, Lake AC, Nagan N, Gaposchkin DP, Legner MA, Lieberthal W. Plasmalogens as endogenous antioxidants: somatic cell mutants reveal the importance of the vinyl ether. *Biochem J* 1999; **338**: 769-776 [PMID: 10051451 DOI: 10.1042/bj3380769]

60 **Jang JE**, Park HS, Yoo HJ, Baek IJ, Yoon JE, Ko MS, Kim AR, Kim HS, Park HS, Lee SE, Kim SW, Kim SJ, Leem J, Kang YM, Jung MK, Pack CG, Kim CJ, Sung CO, Lee IK, Park JY, Fernández-Checa JC, Koh EH, Lee KU. Protective role of endogenous plasmalogens against hepatic steatosis and steatohepatitis in mice. *Hepatology* 2017; **66**: 416-431 [PMID: 28073164 DOI: 10.1002/hep.29039]

61 **Quehenberger O**, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 2010; **51**: 3299-3305 [PMID: 20671299 DOI: 10.1194/jlr.M009449]

62 **Rodriguez-Cuenca S**, Pellegrinelli V, Campbell M, Oresic M, Vidal-Puig A. Sphingolipids and glycerophospholipids - The "ying and yang" of lipotoxicity in metabolic diseases. *Prog Lipid Res* 2017; **66**: 14-29 [PMID: 28104532 DOI: 10.1016/j.plipres.2017.01.002]

63 **Barr J**, Vázquez-Chantada M, Alonso C, Pérez-Cormenzana M, Mayo R, Galán A, Caballería J, Martín-Duce A, Tran A, Wagner C, Luka Z, Lu SC, Castro A, Le Marchand-Brustel Y, Martínez-Chantar ML, Veyrie N, Clément K, Tordjman J, Gual P, Mato JM. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative biomarkers associated with the progression of nonalcoholic fatty liver disease. *J Proteome Res* 2010; **9**: 4501-4512 [PMID: 20684516 DOI: 10.1021/pr1002593]

64 **Li Z**, Zhang H, Liu J, Liang CP, Li Y, Li Y, Teitelman G, Beyer T, Bui HH, Peake DA, Zhang Y, Sanders PE, Kuo MS, Park TS, Cao G, Jiang XC. Reducing plasma membrane sphingomyelin increases insulin

sensitivity. *Mol Cell Biol* 2011; **31**: 4205-4218 [PMID: 21844222 DOI: 10.1128/MCB.05893-11]
65 **Holland WL**, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. *Endocr Rev* 2008; **29**: 381-402 [PMID: 18451260 DOI: 10.1210/er.2007-0025]
66 **Kotronen A**, Seppänen-Laakso T, Westerbacka J, Kiviluoto T, Arola J, Ruskeepää AL, Yki-Järvinen H, Oresic M. Comparison of lipid and fatty acid composition of the liver, subcutaneous and intra-abdominal adipose tissue, and serum. *Obesity (Silver Spring)* 2010; **18**: 937-944 [PMID: 19798063 DOI: 10.1038/oby.2009.326]
67 **Meikle PJ**, Wong G, Barlow CK, Weir JM, Greeve MA, MacIntosh GL, Almasy L, Comuzzie AG, Mahaney MC, Kowalczyk A, Haviv I, Grantham N, Magliano DJ, Jowett JB, Zimmet P, Curran JE, Blangero J, Shaw J. Plasma lipid profiling shows similar associations with prediabetes and type 2 diabetes. *PLoS One* 2013; **8**: e74341 [PMID: 24086336 DOI: 10.1371/journal.pone.0074341]
68 **Gault CR**, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol* 2010; **688**: 1-23 [PMID: 20919643 DOI: 10.1007/978-1-4419-6741-1_1]
69 **Frangioudakis G**, Garrard J, Raddatz K, Nadler JL, Mitchell TW, Schmitz-Peiffer C. Saturated- and n-6 polyunsaturated-fat diets each induce ceramide accumulation in mouse skeletal muscle: reversal and improvement of glucose tolerance by lipid metabolism inhibitors. *Endocrinology* 2010; **151**: 4187-4196 [PMID: 20660065 DOI: 10.1210/en.2010-0250]
70 **Holland WL**, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasis SK, Fontenot GK, Birnbaum MJ, Summers SA. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab* 2007; **5**: 167-179 [PMID: 17339025 DOI: 10.1016/j.cmet.2007.01.002]
71 **Nikolova-Karakashian M**, Karakashian A, Rutkute K. Role of neutral sphingomyelinases in aging and inflammation. *Subcell Biochem* 2008; **49**: 469-486 [PMID: 18751923 DOI: 10.1007/978-1-4020-8831-5_18]
72 **Holland WL**, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, Davis KE, Bikman BT, Halberg N, Rutkowski JM, Wade MR, Tenorio VM, Kuo MS, Brozinick JT, Zhang BB, Birnbaum MJ, Summers SA, Scherer PE. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* 2011; **17**: 55-63 [PMID: 21186369 DOI: 10.1038/nm.2277]
73 **Marí M**, Colell A, Morales A, Caballero F, Moles A, Fernández A, Terrones O, Basañez G, Antonsson B, García-Ruiz C, Fernández-Checa JC. Mechanism of mitochondrial glutathione-dependent hepatocellular susceptibility to TNF despite NF-kappaB activation. *Gastroenterology* 2008; **134**: 1507-1520 [PMID: 18343380 DOI: 10.1053/j.gastro.2008.01.073]
74 **Kasumov T**, Li L, Li M, Gulshan K, Kirwan JP, Liu X, Previs S, Willard B, Smith JD, McCullough A. Ceramide as a mediator of non-alcoholic Fatty liver disease and associated atherosclerosis. *PLoS One* 2015; **10**: e0126910 [PMID: 25993337 DOI: 10.1371/journal.pone.0126910]
75 **Choi CS**, Savage DB, Kulkarni A, Yu XX, Liu ZX, Morino K, Kim S, Distefano A, Samuel VT, Neschen S, Zhang D, Wang A, Zhang XM, Kahn M, Cline GW, Pandey SK, Geisler JG, Bhanot S, Monia BP, Shulman GI. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. *J Biol Chem* 2007; **282**: 22678-22688 [PMID: 17526931 DOI: 10.1074/jbc.M704213200]
76 **Amin NB**, Carvajal-Gonzalez S, Purkal J, Zhu T, Crowley C, Perez S, Chidsey K, Kim AM, Goodwin B. Targeting diacylglycerol acyltransferase 2 for the treatment of nonalcoholic steatohepatitis. *Sci Transl Med* 2019; **11** [PMID: 31776293 DOI: 10.1126/scitranslmed.aav9701]
77 **Min HK**, Kapoor A, Fuchs M, Mirshahi F, Zhou H, Maher J, Kellum J, Warnick R, Contos MJ, Sanyal AJ. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. *Cell Metab* 2012; **15**: 665-674 [PMID: 22560219 DOI: 10.1016/j.cmet.2012.04.004]
78 **Caballero F**, Fernández A, De Lacy AM, Fernández-Checa JC, Caballería J, García-Ruiz C. Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. *J Hepatol* 2009; **50**: 789-796 [PMID: 19231010 DOI: 10.1016/j.jhep.2008.12.016]
79 **Gan LT**, Van Rooyen DM, Koina ME, McCuskey RS, Teoh NC, Farrell GC. Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *J Hepatol* 2014; **61**: 1376-1384 [PMID: 25064435 DOI: 10.1016/j.jhep.2014.07.024]
80 **Walle P**, Takkunen M, Männistö V, Vaittinen M, Lankinen M, Kärjä V, Käkelä P, Ågren J, Tiainen M, Schwab U, Kuusisto J, Laakso M, Pihlajamäki J. Fatty acid metabolism is altered in non-alcoholic steatohepatitis independent of obesity. *Metabolism* 2016; **65**: 655-666 [PMID: 27085774 DOI: 10.1016/j.metabol.2016.01.011]
81 **Zheng JS**, Xu A, Huang T, Yu X, Li D. Low docosahexaenoic acid content in plasma phospholipids is associated with increased non-alcoholic fatty liver disease in China. *Lipids* 2012; **47**: 549-556 [PMID: 22527845 DOI: 10.1007/s11745-012-3671-4]
82 **Musso G**, Gambino R, De Michieli F, Cassader M, Rizzetto M, Durazzo M, Fagà E, Silli B, Pagano G. Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 2003; **37**: 909-916 [PMID: 12668986 DOI: 10.1053/jhep.2003.50132]
83 **Li ZZ**, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-CoA desaturase. *J Biol Chem* 2009; **284**: 5637-5644 [PMID: 19119140 DOI: 10.1074/jbc.M807616200]
84 **Chong MF**, Hodson L, Bickerton AS, Roberts R, Neville M, Karpe F, Frayn KN, Fielding BA. Parallel activation of de novo lipogenesis and stearoyl-CoA desaturase activity after 3 d of high-carbohydrate feeding. *Am J Clin Nutr* 2008; **87**: 817-823 [PMID: 18400702 DOI: 10.1093/ajcn/87.4.817]
85 **Listenberger LL**, Han X, Lewis SE, Cases S, Farese RV Jr, Ory DS, Schaffer JE. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci USA* 2003; **100**: 3077-3082 [PMID: 12629214 DOI: 10.1073/pnas.0630588100]
86 **López-Vicario C**, González-Périz A, Rius B, Morán-Salvador E, García-Alonso V, Lozano JJ, Bataller R, Cofán M, Kang JX, Arroyo V, Clària J, Titos E. Molecular interplay between Δ5/Δ6 desaturases and long-

chain fatty acids in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2014; **63**: 344-355 [PMID: 23492103 DOI: 10.1136/gutjnl-2012-303179]

87 **Sui YH**, Luo WJ, Xu QY, Hua J. Dietary saturated fatty acid and polyunsaturated fatty acid oppositely affect hepatic NOD-like receptor protein 3 inflammasome through regulating nuclear factor-kappa B activation. *World J Gastroenterol* 2016; **22**: 2533-2544 [PMID: 26937141 DOI: 10.3748/wjg.v22.i8.2533]

88 **Lytle KA**, Depner CM, Wong CP, Jump DB. Docosahexaenoic acid attenuates Western diet-induced hepatic fibrosis in Ldlr-/- mice by targeting the TGFβ-Smad3 pathway. *J Lipid Res* 2015; **56**: 1936-1946 [PMID: 26315048 DOI: 10.1194/jlr.M061275]

89 **Loomba R**, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *J Lipid Res* 2015; **56**: 185-192 [PMID: 25404585 DOI: 10.1194/jlr.P055640]

90 **Wiesner P**, Leidl K, Boettcher A, Schmitz G, Liebisch G. Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. *J Lipid Res* 2009; **50**: 574-585 [PMID: 18832345 DOI: 10.1194/jlr.D800028-JLR200]

91 **van der Veen JN**, Lingrell S, Vance DE. The membrane lipid phosphatidylcholine is an unexpected source of triacylglycerol in the liver. *J Biol Chem* 2012; **287**: 23418-23426 [PMID: 22610093 DOI: 10.1074/jbc.M112.381723]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 451-474

DOI: 10.4254/wjh.v12.i8.451

ISSN 1948-5182 (online)

REVIEW

Update on diagnosis and management of sepsis in cirrhosis: Current advances

Cyriac Abby Philips, Rizwan Ahamed, Sasidharan Rajesh, Tom George, Meera Mohanan, Philip Augustine

ORCID number: Cyriac Abby Philips 0000-0002-9587-336X; Rizwan Ahamed 0000-0003-4747-6359; Sasidharan Rajesh 0000-0002-3293-1817; Tom George 0000-0002-3515-6457; Meera Mohanan 0000-0002-8752-4530; Philip Augustine 0000-0003-0787-0984.

Author contributions: Philips CA designed and wrote the original draft and was involved in the revision; Ahamed R, Rajesh S, and George T were involved in the writing; Philips CA, Ahamed R, Rajesh S, George T, Mohanan M, and Augustine P were involved in the editing of the manuscript; Rajesh S, Mohanan M, and Augustine P were involved in the review of the manuscript; George T was involved in the reformatting of the manuscript; all authors have read and approve the final manuscript.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Cyriac Abby Philips, The Liver Unit and Monarch Liver Lab, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682028, Kerala, India

Rizwan Ahamed, Philip Augustine, Gastroenterology and Advanced G.I Endoscopy, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682028, Kerala, India

Sasidharan Rajesh, Tom George, Division of Hepatobiliary Interventional Radiology, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682028, Kerala, India

Meera Mohanan, Anaesthesia and Critical Care, Cochin Gastroenterology Group, Ernakulam Medical Center, Kochi 682028, Kerala, India

Corresponding author: Cyriac Abby Philips, MBBS, MD, Associate Chief Physician, The Liver Unit and Monarch Liver Lab, Cochin Gastroenterology Group, Ernakulam Medical Center, 10th Floor, E.M.C Hospital, NH-Bypass, Palarivattom, Kochi 682028, Kerala, India. abbyphilips@gmail.com

Abstract

Sepsis and septic shock are catastrophic disease entities that portend high mortality in patients with cirrhosis. In cirrhosis, hemodynamic perturbations, immune dysregulation, and persistent systemic inflammation with altered gut microbiota in the background of portal hypertension enhance the risk of infections and resistance to antimicrobials. Patients with cirrhosis develop recurrent life-threatening infections that progress to multiple organ failure. The definition, pathophysiology, and treatment options for sepsis have been ever evolving. In this exhaustive review, we discuss novel advances in the understanding of sepsis, describe current and future biomarkers and scoring systems for sepsis, and delineate newer modalities and adjuvant therapies for the treatment of sepsis from existing literature to extrapolate the same concerning the management of sepsis in cirrhosis. We also provide insights into the role of gut microbiota in initiation and progression of sepsis and finally, propose a treatment algorithm for management of sepsis in patients with cirrhosis.

Key words: Portal hypertension; Sequential organ failure assessment; Acute on chronic liver failure; Predisposition insult response organ-dysfunction model; Intensive care unit; Shock

Manuscript source: Invited manuscript

Received: March 22, 2020
Peer-review started: March 22, 2020
First decision: April 12, 2020
Revised: May 18, 2020
Accepted: June 27, 2020
Article in press: June 27, 2020
Published online: August 27, 2020

P-Reviewer: Pavides M, Takata H
S-Editor: Yan JP
L-Editor: Filipodia
P-Editor: Wang LL

Core tip: Advances in understanding sepsis have led to an uncomplicated and robust definition with prognostic importance. What has emerged is a redefinition of the clinical protocols for early and aggressive management of sepsis at hour 1 of patient presentation and identification of a novel combination of biomarkers. In addition, antimicrobial resistance has been addressed and adjuvant therapies have been identified through deep data mining, metagenomics, and machine learning-based tools for improving clinical outcomes. These advances have the potential to be extrapolated and studied in patients with cirrhosis and sepsis to improve notable catastrophic clinical outcomes seen in this unique and challenging patient population.

Citation: Philips CA, Ahamed R, Rajesh S, George T, Mohanan M, Augustine P. Update on diagnosis and management of sepsis in cirrhosis: Current advances. *World J Hepatol* 2020; 12(8): 451-474
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/451.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.451

INTRODUCTION

Critical illness in the presence or absence of overwhelming infection leading to multiple organ failure in patients with cirrhosis is a rapid, complex, and catastrophic process that, in the majority, does not respond to conventional treatment practices laid down for the general population. In advanced cirrhosis, hyperdynamic circulation with high cardiac output, subclinical cardiomyopathy, central hypovolemia, third space fluid accumulation, and low systemic vascular resistance prevail. In decompensated patients with cirrhosis, a small proportion develops the syndrome acute on chronic liver failure (ACLF) with sepsis, which is characterized by extrahepatic organ failures requiring intensive care management with rapid progression to multiple organ failure. Such events increase in-hospital mortality and result in treatment futility even with the best supportive care.

Sepsis is defined as a "life-threatening organ dysfunction caused by a dysregulated host response to infection," that can cause critical illness in patients with cirrhosis with different clinical consequences. In a compensated cirrhosis, the development of sepsis can cause acute decompensation that can progress to ACLF. Sepsis can develop as an intercurrent event in decompensated cirrhosis, leading to worsening of existing or new-onset decompensation, both of which can lead to ACLF. Sepsis can also develop during acute decompensation or ACLF, all of which can lead to organ failures. Sepsis is established in the presence of suspected or documented infection and an acute increase of ≥ two sequential organ failure assessment (SOFA) (a proxy for organ dysfunction) points[1-3].

In cirrhosis, an associated immune dysfunction exists with worsening severity, depending on the stages and severity of decompensation. This cirrhosis-associated immune dysfunction (CAID) is dynamic and affects both the innate and acquired immune functions, because of changes and deficiencies in both the local milieu of the liver microenvironment and systemic immunity. CAID depends on the increased and persistent systemic inflammation, liver disease severity, and portal hypertension and is central to both acute and chronic decompensation. Besides, increased gut permeability, reduction in gut motility, and altered gut microbiota promote increased bacterial translocation and subsequent endotoxemia, leading to worsening systemic inflammation in cirrhosis. Sepsis by itself is a state of profound immune dysregulation in which, during the early phase, a pro-inflammatory state, counterbalanced by an anti - inflammatory response, affects immune functions (compensatory anti - inflammatory response syndrome). In patients with dysregulated immune functions, such as those with cirrhosis, this initial phase goes unchecked, progressing to sepsis - induced immunosuppression and a stage of immune-paralysis with subsequent organ failure development. In the following sections of this review, we aim to discuss current and future aspects in the diagnosis and treatment of sepsis and extrapolate recent advances in the management of sepsis concerning critically ill patients with cirrhosis with sepsis[4,5].

DEFINING SEPSIS, SEPTIC SHOCK, AND RELATED COMPONENTS IN CIRRHOSIS

Early on, the pathogenesis of sepsis and its systemic consequences were considered a hyper-inflammatory response to microbial invasion (infection) accompanied by an evolving cytokine storm. Because of this, sepsis was defined as "a systemic inflammatory response syndrome (SIRS) to infection" (Sepsis-1 definition). A decade later, expert consensus concluded that such a general definition did not allow for staging of sepsis-related events, and hence prediction of the host response to infection remained vague for clinical and research purposes. The SIRS criteria, even though useful for easy identification of sepsis, remained non-specific and too sensitive. This paved the way for the Sepsis-2 definition that included the PIRO [P: Predisposition; I: The type and extent of insult (infection in sepsis); R: The type and extent of host response; and O: The type and extent of organ dysfunction] model[6,7]. With PIRO, morbidity (primary organ dysfunction) because of the infection itself and morbidity developing during host response (secondary organ dysfunction) were identifiable. For example, in a patient with cirrhosis with the development of bacterial pneumonia, type 1 respiratory failure occurring early during illness can be considered primary organ dysfunction (because of the infection). In the same patient, the development of ascites and acute kidney injury during the later course of the disease can be secondary organ dysfunction (hepatorenal syndrome) because of predisposition (cirrhosis), insult (pneumonia), and host response (decompensation of cirrhosis). In such a situation, stratifying patients at risk of death depending on early and late events along with components of the PIRO model can help in improving prognostication and define specific timeframes for therapeutic interventions.

Acceptance and attempt at including the PIRO model in patients with cirrhosis are lacking in the literature. Jalan and colleagues studied the prognostic value of the PIRO model on outcomes in patients with ACLF. The authors found that in patients with organ failures, previous hospitalization (predisposition), persistence and severity of inflammation (response), and severity of organ failure (organ dysfunction) were associated with higher mortality[8,9]. Maiwall *et al*[10] in a prospective study of ACLF patients showed that serum creatinine, bilirubin, potassium, and blood urea at baseline (predisposition); nephrotoxic medications (insult); SIRS (response), and circulatory failure (organ dysfunction) identified those at risk of developing acute kidney injury during the disease course and death. The PIRO model could help identify patients with cirrhosis at risk of sepsis, those at risk of developing specific organ failure, and those at risk of recurrent sepsis. This could help define specific therapeutic "windows" to improve further deterioration and reduce organ failures in patients with cirrhosis.

An expert consensus meeting re-defined sepsis (Sepsis – 3), with the omission of the terms SIRS and severe sepsis. According to the new consensus, in the absence of organ dysfunction, the event is termed an "infection". Septic shock was defined as hypotension unresponsive to fluid boluses and with lactate > 2 mmol/L. For defining and grading organ failures, the SOFA score was identified as the best tool. Acute organ dysfunction is identified when the SOFA score increased by two points from baseline (considered 0 before admission), and parallel identification of an infective focus defined sepsis. A new screening tool for early recognition of sepsis called the "quick"-SOFA (qSOFA) was provided, intended for primary use in the non-intensive unit care. According to qSOFA, patients meeting two of three criteria (altered mental status, respiratory rate > 22 per min, systolic blood pressure < 100 mmHg) were suspected of having new-onset or worsening sepsis. The SOFA score was developed from the general intensive care unit population rather than from patients with cirrhosis, and hence, some of the core components (such as Glasgow coma scale and platelet count) could be influenced by the severity of the underlying liver disease[11,12].

To improve on the prediction of SOFA score in patients with cirrhosis developing acute decompensation, the European Association for Study of Liver-Chronic Liver Failure Consortium (EASL - CLIF Consortium) changed the SOFA score into CLIF - SOFA score and defined ACLF according to the new score. In the CLIF - SOFA score, six organ systems with specific changes applied regarding patients with end - stage liver disease were designated. Platelet count was replaced by the international normalized ratio of prothrombin time and the Glasgow coma scale with hepatic encephalopathy as the central nervous system criterion. It also modified the use of terlipressin as part of the cardiovascular component and renal replacement therapy within the renal parameter[13-15]. The CLIF - SOFA score also added peripheral capillary oxygen saturation/fraction of inspired oxygen in the air as an alternative to respiration

parameter for patients without arterial line placed (Table 1).

Small studies have shown that the Acute Physiology And Chronic Health Evaluation II (APACHE II) and subsequent modifications were superior to other scoring and definition systems of sepsis in cirrhosis. However, no study has shown its superiority to CLIF-SOFA. Hence, further studies pending, application of either of these scoring systems, as per operator ease is acceptable in critically ill patients with cirrhosis. To summarize, in a patient with cirrhosis, sepsis is identified when there is the fulfilment of at least two SOFA score points at presentation, in the presence of an infection, the latter proved either radiologically or microbiologically with or without blood biomarker correlation (Figure 1).

THE LACTATE QUANDARY IN CIRRHOSIS

Serum lactate levels play a major role in defining patients with septic shock. It was shown that lactate levels were elevated in patients with cirrhosis, compared to healthy controls, related to portal pressures, and increased with severity of the liver disease. This meant that lactate kinetics in the cirrhosis population differed greatly from other patient populations[16]. A study tested lactate levels in patients with acute circulatory failure with hepatic dysfunction. No differences in pertinent variables, such as lowest systolic blood pressure, serum creatinine, and simplified APACHE scores, were noted. However, the lactate levels were higher in the group with liver disease (8.24 mmol/L *vs* 4.29 mmol/L, $P < 0.001$), with a positive correlation between lactate and aspartate aminotransferase levels. The authors concluded that there was no apparent correlation between liver dysfunction and the severity of shock as a confounder[17]. Kruse *et al*[18] tested the significance of blood lactate in critically ill patients with liver disease. They found that arterial lactate > 2.2 mmol/L was associated with clinical evidence of shock and significant in-hospital mortality.

In a systematic review of blood lactate as a predictor for in-hospital mortality in acutely ill patients, venous or arterial lactate > 2.5 mmol/L at admission was associated with the progression of clinical deterioration[18-20]. Sun *et al*[21] showed that the serum lactate levels were predictive of extrahepatic organ failure (acute kidney injury) in critically ill patients with cirrhosis. The mortality rate increased with a rise in serum lactate. In a multinational study, Drolz *et al*[22] showed that lactate levels reflected the severity of disease and organ failure and was independently associated with a high risk of death in the brief term in critically ill cirrhosis patients.

The addition of lactate into the CLIF-C score for ACLF patients improved its prognostic power. A serum lactate ≥ 5 mmol/L had high predictive power for short term mortality, and lactate clearance predicted 28-d mortality. Admission and 12-h lactate clearance in those with admission lactate ≥ 5 mmol/L predicted 1-y mortality. In summary, including lactate above 2 mmol/L can be extrapolated to define patients of cirrhosis with septic shock. The admission and serial lactate measurements and lactate clearance are useful in identifying those with poor prognosis even though the complex lactate dynamics remain undefined in patients with advanced cirrhosis. Thus, septic shock in cirrhosis can be identified in the presence of sepsis, the onset of hypotension requiring vasopressor support [mean arterial pressure (MAP) < 65 mmHg], and lactate > 2 mmol/L despite adequate fluid resuscitation.

TOLERANCE TO SEPSIS – A BROKEN DEAL IN CIRRHOSIS

There are three essential strategies for dealing with disease because of pathogens - avoidance, resistance, and tolerance. Of these, the first two are notable among animals (who are mobile), while the third strategy is clear in plants (since they are stationary). Tolerance results in the ability to maintain health in the presence of a pathogen(s). An example of tolerance to infection or pathogen is the case of Ms. Mary Mallon ("typhoid Mary"), causing severe Salmonellosis in persons consuming dishes she prepared and tolerance to malaria among persons with sickle-cell anaemia. In sepsis, at the core, there occurs complete dysregulation of local and systemic inflammatory and associated metabolic processes that lead to organ failure. Yet another major event in sepsis is the destruction of red blood cells through direct or indirect pathogen-based hemolysin effect. Hence, in sepsis, heme production is overwhelming, and the removal of free heme results in the formation of divalent iron (Fe^{2+}). Excess production of Fe^{2+} leads to the overproduction of reactive free radicals through the Fenton reaction, resulting in the release of trivalent iron (Fe^{3+}), which is a hydroxyl radical that

Table 1 Modification of sequential organ failure assessment score for patients with cirrhosis, the chronic liver failure-sequential organ failure assessment scoring system[13,15]

Score	1	2	3	4
Respiration PaO_2/FiO_2 or SpO_2/FiO_2, mmHg	> 300 to ≤ 400 or > 357 to ≤ 512	> 200 to ≤ 300 or > 214 to ≤ 357	> 100 to ≤ 200 or 89 to ≤ 214	< 100 or ≤ 89
Liver bilirubin, mg/dL	1.2-1.9	2.0-5.9	6.0-11.9	> 12
Cardiovascular hypotension	Mean arterial pressure < 70 mmHg	Dopamine ≤ 5 or any dobutamine or terlipressin	Dopamine > 5 or noradrenaline ≤ 0.1	Dopamine > 15 or noradrenaline > 0.1
Cerebral HE grades	I	II	III	IV
Renal creatinine (mg/dL) or urine output	1.2-1.9	2.0-3.4	3.5-4.9 or use of renal replacement therapy	≥ 5.0
Coagulation - INR	≥ 1.1 to < 1.25	≥ 1.25 to < 1.5	≥ 1.5 to < 2.5	≥ 2.5 or platelet count ≤ 20000/μL

FiO_2: Fraction of inspired oxygen; HE: Hepatic encephalopathy; INR: International normalized ratio; PaO_2: Arterial oxygen pressure.

promotes various secondary metabolic reactions. To prevent toxic secondary reactions, oxidized iron is removed by ferritin. Ferritin thus confers tolerance towards infections[23].

In the seminal work by Weis *et al*[24] on sepsis tolerance, mice with pre-deleted ferritin subunit (FTH) and those expressing FTH, underwent cecal ligation and puncture (an animal model of sepsis). The authors found that the survival of the mice depended on FTH expression on hepatocytes and macrophages. Those with FTH deficiency had inferior survival with the development of sepsis. In both FTH deficient and sufficient groups, the microbial burden and cytokine production were similar but without overt sepsis in the latter, showing tolerance to sepsis development in the presence of ferritin expression. In FTH deficient mice, the bodyweight loss was extensive, with lower body temperatures, and correlated with hypoglycaemia. Thus, the link between FTH expression and maintenance of blood glucose levels was notable in this study. When heme was infused into FTH deficient mice with sepsis, death was inevitable; with the infusion of glucose, health status, and survival improved. At the gene expression level, the activity of glucose-6-phosphatase catalytic subunit-1 (G6PC-1) was reduced, leading to curtailment of gluconeogenesis. The authors found that in the absence of ferritin expression, free heme downregulated G6PC-1 expression and reduced hepatic gluconeogenesis and glycogenolysis, leading to an increase in mortality. Use of iron-chelators, antioxidants, and iron-free ferritin restored G6PC-1 activity and induced gluconeogenesis, leading to an improvement in survival[23,24]. In an animal model of listeriosis, Medzhitov *et al*[25] showed that correction of hypoglycaemia using glucose infusions worsened survival because of the promotion of neurotoxicity by exacerbation of reactive oxidative species. In virus-infected mice, glucose infusions improved sepsis and survival and reduced neuronal endoplasmic reticulum stress responses. These two studies showcase an important aspect of sepsis – adaptive tolerance to sepsis in the host that was dependent on the pathogen type[25,26].

In patients with decompensated cirrhosis, Changani *et al*[27] showed impairment in gluconeogenesis in advanced liver disease but not in stable patients with cirrhosis. In the early stages of cirrhosis, hepatic gluconeogenesis and fatty acid oxidation are increased in the presence of reduced hepatic glycogen content, resulting in lactate and alanine production through muscle breakdown and protein degradation. With the progression of cirrhosis, liver failure sets in, leading to a reduction in gluconeogenesis, depletion of glycogen stores, amelioration in glycogenesis, and loss of muscle mass (sarcopenia) leading to a diminution in pro-glucogenic substrates[28]. Heme-oxygenase has anti-inflammatory and anti-apoptotic properties, and induction of heme-oxygenase-1 in animal models of acute or chronic liver injury showed a reduction in hepatic inflammation and fibrosis progression and partial resolution of existing fibrosis. In animal models of cirrhosis and humans with decompensated cirrhosis, the expression of heme-oxygenase increased with increasing severity of liver disease and portal hypertension[29,30]. Patients with cirrhosis have excessive erythrocyte destruction because of splenomegaly, reduced red blood cell survival, reduced red cell mass, suppression of bone marrow function, blood loss because of acute and chronic gastrointestinal bleeding events associated with portal hypertension and blunted response to erythropoietin[31,32].

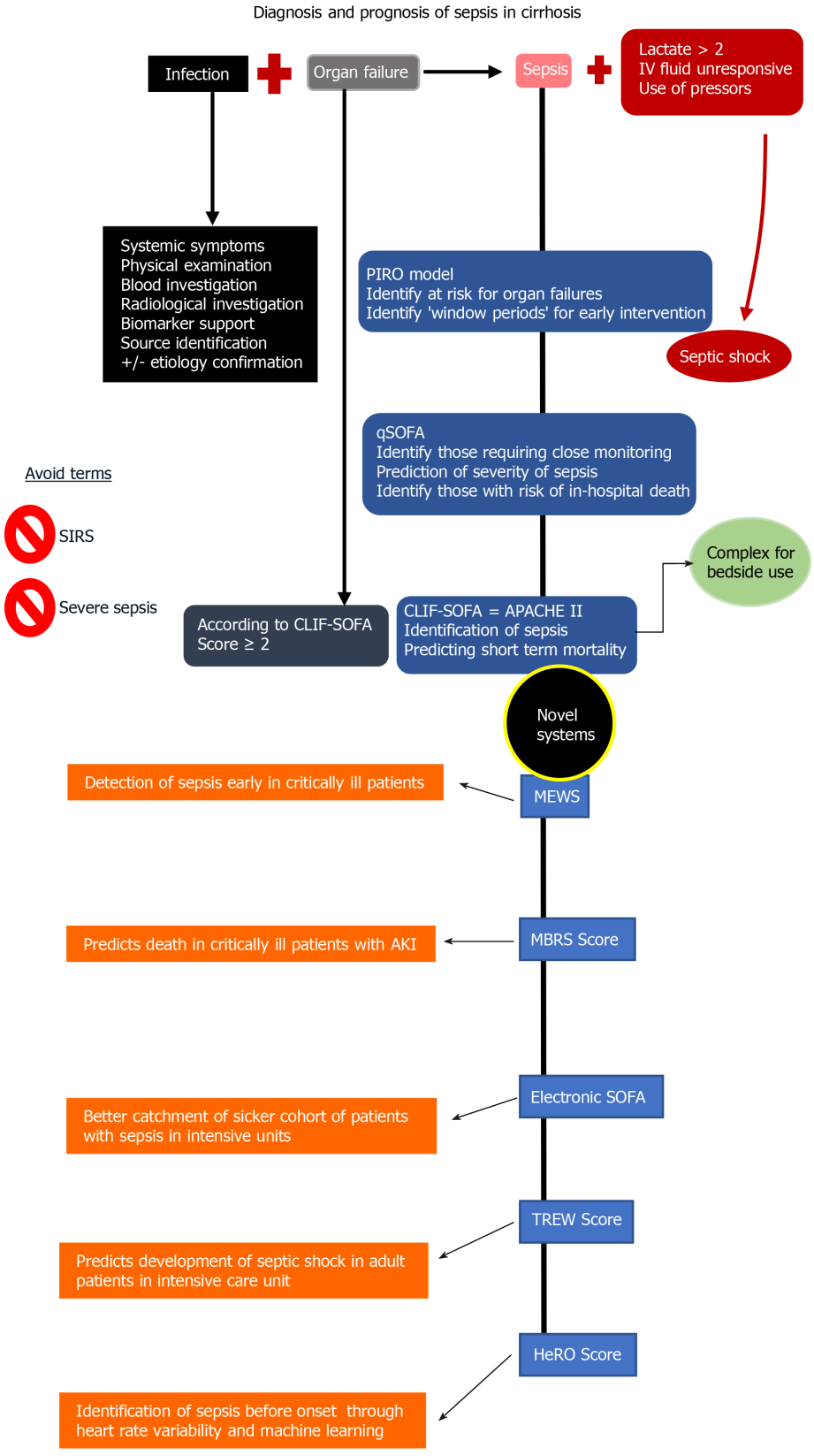

Figure 1 Definitions, diagnosis, and summary of prognostic scoring systems of sepsis[2,4,6,58,63-65]. Sepsis is defined as presence of suspected or confirmed infection in the presence of an organ failure as defined by the sequential organ failure assessment tool. After diagnosis of sepsis, a prognostic tool is utilized to identify patients at risk of worsening or death. APACHE: Acute physiology and chronic health evaluation; CLIF: Chronic liver failure; HeRO: Heart rate index; MBRS: Mean arterial pressure, bilirubin, respiratory failure, sepsis; MEWS: Modified early warning score; PIRO: Predisposition, insult, response, organ failure; qSOFA: Quick sequential organ failure assessment; SIRS: Systemic inflammatory response syndrome; SOFA: Sequential organ failure assessment; TREW: Targeted real-time early warning score.

Ferritin, a marker of stored iron, can be elevated in decompensated cirrhosis

patients with inflammation and in those patients whose cirrhosis aetiology is secondary to alcohol use or chronic hepatitis C infection. It has been shown that even in the presence of high ferritin levels in patients with decompensated cirrhosis, the transferrin levels were low, and transferrin saturation elevated. Lower transferrin levels that represent malnutrition, the severity of cirrhosis, and inflammation are associated with poor transplant-free survival in patients with decompensated cirrhosis[33,34]. To summarize, in cirrhosis, there occurs increased red cell destruction, reduction in hepatic gluconeogenesis, lower levels of transferrin, and high dysfunctional levels of ferritin, which leads to a state of perturbed tolerance to infections and a higher risk of sepsis, loss of muscle mass, lower body temperature, and dysfunctional control over inflammation. Measures to improve tolerance to sepsis in patients with advanced cirrhosis could become an important component in the armamentarium of therapeutic options against cirrhosis with sepsis and improving survival outcomes (Figure 2).

FASTING METABOLISM AND DEFENSE AGAINST INFECTIONS – IS THERE A ROLE IN EARLY CIRRHOSIS?

A proverb goes, "feed a cold, starve a fever". In the presence of infection, animals develop specific behavioural changes that include anorexia, sleep pattern variations, and withdrawal from social activities – asymptomatic complex referred to as "sickness behaviours". These patterns were considered flawed consequences of the host response to infection. Newer evidence suggests that sickness behaviours are strategic evolution in the host to ward off the harmful effects of infection and improve survival. Of these, the most important is anorexia. Anorexia modulates host metabolic requirements of stress responses pertinent for tolerance to bacterial inflammatory states.

In the seminal work by Wang *et al*[26], it was demonstrated in a small animal model that fasting metabolism was protective in bacterial but not virus-induced inflammation. The ketosis that develops during fasting limited the reactive oxygen species induced neuronal damage during bacterial infection-related inflammation while non-fasting or glucose infusion prevented neuronal damage in viral inflammation, showcasing the importance of host responses to aetiology of infection during fasting[26,35,36]. Force-feeding mice with lipopolysaccharide induced endotoxemia increased mortality. Intermittent fasting was shown to increase acute immune and behavioural sickness responses leading to worse outcomes in mouse models of viral infections and inflammation.

Metabolic processes in the liver microenvironment are firmly regulated by neuronal and hormonal systems such as the sympathetic and parasympathetic systems and insulin-glucagon related systems[37,38]. Patients with cirrhosis are a unique population regarding nutritional and metabolic disorders. In advanced cirrhosis, the liver cannot synthesize and store required amounts of glycogen, which creates a "glucose deficient" state in times of stress. In this scenario, the utilization of non-carbohydrate sources for gluconeogenesis, such as glycerols from fatty tissue and amino acids from muscles, becomes remarkable. Dietary improvements rather than restriction are well known to improve outcomes in this stage, even though dietary or nutritional interventions in special situations such as in an obese patient with cirrhosis remain controversial. An overnight fast in a patient with cirrhosis patient is akin to 3-d fasting in an average person[39]. Owen *et al*[40] showed that after an overnight fast, hepatic glucose production in patients with cirrhosis was diminished because of low-rate glycogenolysis, but hepatic gluconeogenesis and ketogenesis were increased. After 3 d of starvation, patients with cirrhosis were found to have hepatic gluconeogenic and ketogenic profiles comparable to those of healthy patients undergoing deprivation of a similar duration. García-Compeán *et al*[41] showed that subclinical abnormal glucose tolerance was a predictor of death in patients with liver cirrhosis. In the study by Wang *et al*[26], the authors found that fasting metabolism protected against sepsis and that protection was suppressed by infused glucose[35], and Weis *et al*[24] discovered that mice have to maintain minimal glucose levels through gluconeogenesis for tolerating bacterial sepsis. Thus, there occurs an upper and lower limit of glucose homeostasis and blood glucose level that must be maintained through the reduced intake (anorexia) and by endogenous hepatic glucose production (gluconeogenesis) to improve outcomes in sepsis. This is a matter of further research in patients with cirrhosis.

In animal models of cirrhosis and sepsis, we need to identify outcomes related to

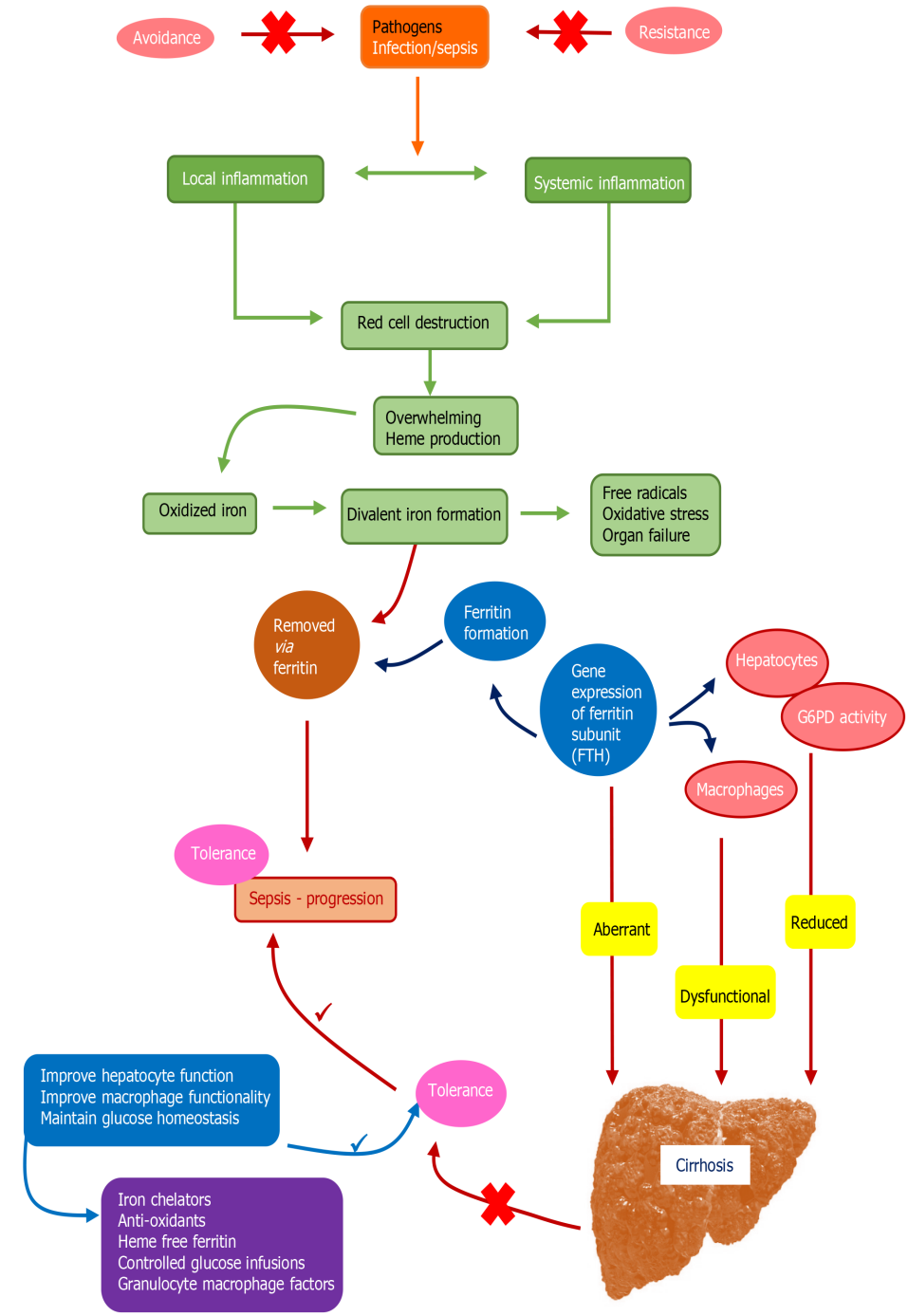

Figure 2 Insights into pathophysiology of tolerance toward sepsis and loss of tolerance leading to higher risk of sepsis in cirrhosis patients[4,23-25]. Apart from tolerance, loss of resistance and exposure to pathogens (shown as red crosses in the upper part of the figure) can initiate infections that can lead to development of sepsis. In patients with infections who develop sepsis, local and systemic inflammation lead to dysregulated red cell homeostasis and development of toxic oxidants especially iron ligands that are removed by ferritin. Ferritin formation and oxidant sweep are regulated systematically through hepatocyte and macrophage functions in the healthy liver through expression of glucose-6-phosphatase (G6PD) and ferritin H gene subunit (FTH). In cirrhosis, liver dysfunction results in aberrant FTH activity, defective macrophage and hepatocyte functions and reduction in G6PD activity, resulting in increased oxidant stress and loss of tolerance to infection, leading to progression of sepsis through reduction in functional ferritin (shown as red crosses at the bottom). The blue and purple boxes demonstrate steps and measures for correction of dysregulated responses in a patient with cirrhosis, respectively, so as to improve tolerance to infection and prevention of sepsis. FTH: Ferritin H gene subunit; G6PD: Glucose-6-phosphatase.

fasting and non-fasting states. Studies on intermittent or prolonged fasting on immune functions in patients with cirrhosis and associated clinical outcomes remain an unmet need. In summary, in patients with compensated cirrhosis, the role of different modes/methods of fasting for prevention or treatment of bacterial infection could be an exciting area of research – one that needs bench work in small cirrhotic animal

models for further consideration in humans (Figure 3).

ADVANCES IN GENERAL PATHOPHYSIOLOGY OF SEPSIS AND RELEVANCE IN CIRRHOSIS

With the intrusion of a microbial entity, the initial host response is the activation of innate immunity that is comprised of macrophages, monocytes, neutrophils, and natural killer cells. This cellular activation is a result of the binding of pathogen-associated molecular patterns, which include endotoxins and fungal elements such as beta-glucans and other microbial degradation components. Apart from these direct pathogen-related activator molecules, damage-associated molecular patterns, which include intracellular material, components of dead or damaged host cells, and microbial DNA, potentiate the host response to infection. All of these activator molecules bind to specific receptors on cells (such as monocytes and macrophages) associated with mounting a counteractive immune response through toll-like receptors, C-type leptin receptors retinoic-acid inducible gene-1-like receptors and nucleotide-binding oligomerization domain-like receptors[42]. In sepsis, close interactions between the inflammatory and haemostatic pathways also affect host responses at the cellular, tissue, and organ levels. With perpetuation of host inflammatory response to an overwhelming or under controlled infection, toxicity at local and systemic levels due to inflammatory components as well as microthrombi formation in organ systems in the initial phase leads to hypoperfusion and decreased delivery of and utilization of oxygen by cellular components, leading to organ dysfunction seen in sepsis[43].

In cirrhosis, the systemic inflammation is mediated through the activation of all innate and adaptive immune cells, with the tipping of the balance towards pro-inflammatory cytokines. In compensated cirrhosis, the progression of fibrosis and hepatocyte loss release damage-associated molecular patterns that activate the immune system causing sterile systemic inflammation. In decompensated cirrhosis, worsening portal hypertension leads to bacterial translocation and release of pathogen-associated molecular patterns into the systemic circulation from the intestinal lumen into the circulation. The continuous influx of immune and inflammation activating molecules leads to a state of persistent inflammation in the host. As cirrhosis progresses and patients start developing complications of portal hypertension, and ultimately liver failure, exhaustion of the immune system occurs, along with loss of tolerance to infections, leading to the inability to mount functional innate and adaptive immune responses. This defines CAID state in which increased levels of anti-inflammatory cytokines and leukocyte inhibitory antigens predominate with loss of immune cell function. In its most extreme form, ACLF, a state of immune paralysis that is also notable in advanced stages of sepsis, is appreciable. In advanced cirrhosis with CAID, an infectious insult can rapidly lead to a state of immune exhaustion that is much more burdensome compared to a non-cirrhotic patient population. A study showed that lymphocytopenia on the 4th day after a diagnosis of sepsis was predictive of both 28-d and 1-y mortality in sepsis[42,44].

Systemic inflammation plays a central role in defining landmark events in patients with cirrhosis. Even in the absence of infection or sepsis, patients with cirrhosis are at baseline, in a state of persistent systemic inflammation. In the presence of non-infectious causes for worsening or acute severe systemic inflammatory states (for example, alcoholic hepatitis, drug-induced liver injury, or reactivation of chronic hepatitis B virus infection), acute decompensation can develop in patients with compensated cirrhosis. In cirrhosis patients with bacterial infections who developed acute decompensation and ACLF, the inflammatory markers interleukin (IL)-6, tumour necrosis factor-alpha, and IL-1 receptor antagonist were found to increase much higher than those with other stress/insults[45]. In patients with compensated and decompensated cirrhosis, in the absence of infections, persistent systemic inflammation leads to a prothrombotic or hypercoagulable state. This baseline hypercoagulability worsens organ dysfunction in patients with cirrhosis who develop infections, and in advanced stages of cirrhosis, once organ failures take full form, disseminated intravascular coagulation develops that leads to a haemorrhagic phenotype in critically ill patients with cirrhosis with septic shock[46,47]. In summary, targeting sepsis in cirrhosis is not merely targeting the pathogen but, in early stages, improving tolerance to infection and correcting of hypercoagulability; in middle stages, keeping in control the unhealthy proinflammatory storm; and late stages, improving immune regulation and abolishing immune paralysis. Timing of treatments

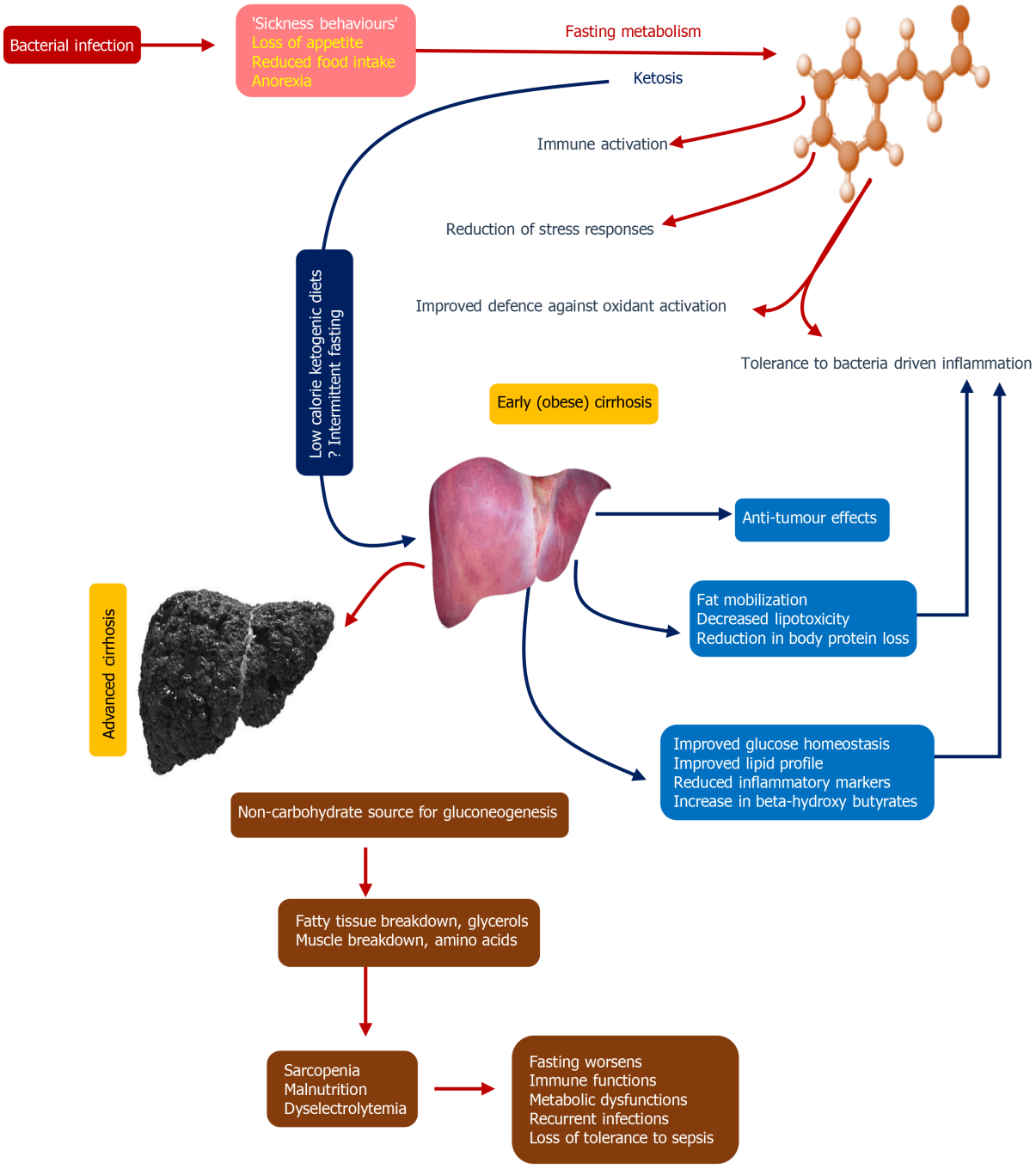

Figure 3 Fasting metabolism and its impact on immune homeostasis and enhanced tolerance to infections[26-28]. The figure demonstrates the potential mechanisms associated with fasting metabolism on immune functions that ultimately prove beneficial for prevention of and combating infections. This could be hypothesized to have benefits in patients with early cirrhosis, especially in those who are obese, pending bench to bedside translational studies. Nonetheless, in advanced cirrhosis, on the contrary, nutritional management to improve immune functions, prevention of infections, and boosting tolerance to sepsis is of importance.

and targeted therapy for these important events in sepsis and cirrhosis remain an unmet need and require multicentre collaboration and specific focused groups working on each aspect to define each therapeutic component that would ultimately become a primary protocol that can be generalized world over.

NOVEL BIOMARKERS FOR SEPSIS DIAGNOSIS AND PROGNOSIS: USE IN CIRRHOSIS

A multitude of biomarkers has been identified that help in the diagnosis and prognosis of sepsis. Of these, the C-reactive protein (CRP), procalcitonin (PCT), and IL-6 have been most extensively studied and of clinical use currently. However, these markers have high levels of heterogeneity concerning the population studied and lack homogeneity in displaying diagnostic value under special circumstances. The Surviving Sepsis Campaign guidelines advocate that measuring PCT can help reduce the duration and promote early discontinuation, escalation, or de-escalation of antimicrobial therapy in patients with diagnosed sepsis. Even though single PCT measurements do not have strong prognostic value, serial measurements can help identify patients at risk of death due to the progression of sepsis and the emergence of septic shock[48,49].

Mid-regional pro adrenomedullin (MR-proADM), a fragment of adrenomedullin precursor (amino acids 45 to 92) with vasodilator and natriuretic properties, was found to be superior to current biomarkers and scoring systems in predicting 28-d mortality in patients with sepsis, septic shock, critically ill with new-onset fever, and respiratory tract infection. In patients with cirrhosis, MR-proADM was found to relate to portal pressures and systemic hemodynamics. It was recently shown that MR-proADM was reliable in identifying cirrhosis patients with complicated bacterial infections as well as those with a very high risk of short-term death independent of bacterial infections or SIRS criteria[50].

Remmler *et al*[51], in a retrospective observational study in end-stage liver disease patients, found that the model for end-stage liver disease (MELD) scores, IL-6 level, and CRP level were associated with mortality risk. The 1-y mortality was zero among patients with IL-6 levels < 5.3 pg/mL but 68% among those with IL-6 > 37.0 pg/mL. The predictive performance for 90-d mortality was excellent (area under the curve, 0.94) for IL-6 and similar to those of MELD and MELD-sodium scores and superior to those of CRP and white blood cell levels. The authors also found that the IL-6 level was an independent predictor of mortality after adjustment for the other markers[51].

Recently, the changes associated with the expression of the neutrophilic CD64 surface marker were found to predict severe inflammation and sepsis efficiently. Neutrophilic dysfunction, a hallmark of sepsis, is that in which neutrophils lose their ability to respond to chemokines leading to an alteration in the microbicidal activity. Such neutrophils have a specific motility signature that can be captured using microfluidic-based assays. From these motility signatures, the Sepsis Score was determined by [$N \times (O + P + R + AD)/10^3$], where N is the neutrophil count; O the number of oscillations exhibited within the migration channels; P the time spent pausing during spontaneous motility; R the reverse migration of cells out of the device, and AD the average distance migrated by the cells. Among patients without cirrhosis, the scoring system generated an area under the curve of 0.98 for non-sepsis and sepsis patients with 96.8% sensitivity and 97.6% specificity[52].

Apart from the clinical definitions that guide diagnosing sepsis at the outset, several novel investigational tools have improved diagnosis and prognostication of sepsis. A novel biomarker, the intensive care infection score (ICIS) composed of five blood-cell-derived parameters [mean fluorescence intensity of mature (segmented) neutrophils, the difference in haemoglobin concentration between newly formed and mature red blood cells, the total segmented neutrophil count, the antibody-secreting lymphocytes, and the accurate immature granulocytes count] characterizing the early innate immune response can be routinely obtained from blood samples sent to the laboratory for cell counts. This score has been retrospectively evaluated in two pilot studies, which suggested its potential predictive value for infection. A mean ICIS value of < 3 (lower cut-off level) indicates the absence of infection. In contrast to CRP and PCT measurements, the ICIS can be determined routinely without new blood sampling and lower costs, yielding results within 15 min[53].

Recently, the monocyte distribution width, with a value > 20 U, was found to be effective for sepsis detection based on the Sepsis – 3 criteria at admission. In the presence of a raised white cell count, the value of monocyte distribution width improved diagnosing and defining early management protocols for sepsis[54]. Crawford *et al*[55] developed an automated deformability cytometric analysis using microfluidic cartridge and customized instrumentation. In this system, imaging of single cells at the rate of thousands/s with the high-speed camera can be studied as they undergo stretching in a controlled microfluidic flow. Deformability was defined as the length by width of a cell during its motion through the microfluidic chamber.

The authors found that granulocytes in patients with sepsis fluidize and elongate much more when compared to the normal population. This can help identify patients with sepsis-associated early innate immune activation. The assay time takes less than 10 min from blood collection to final output and can be used in an emergency setting to identify those who require immediate antibiotic care. This is important because an increase in mortality has been shown with every passing hour in patients with sepsis and even more so in those with septic shock, which holds in patients with cirrhosis and sepsis at a more catastrophic level (Figure 4)[55,56].

To improve on the diagnostic and prognostic accuracy of single-protein biomarkers that are currently in use, transcriptomics (the study of the whole set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell—using high-throughput methods, to identify specific gene expressions) based biomarker panels for a broader assessment of host response to infection have become novel powerful tools. Two such transcriptomic sepsis scores, the SeptiScore™ and the Sepsis MetaScore, using set algorithms, have been validated in independent cohorts. SeptiScore™ utilizes SeptiCyte™LAB technology (ImmuneExpress, Seattle, WA, United States), which consists of four messenger-RNAs (mRNA; CEACAM4, LAMP1, PLA2G7, PLAC8) that represent sepsis-related host response gene expression based mathematical algorithm that predicts sepsis earlier than traditional methods. SeptiScore™ is United States Food and Drug Administration cleared and aids in the differentiation of infection-negative sterile systemic inflammation compared to infection-positive sepsis. The Sepsis MetaScore, which utilizes the expression of 11 host mRNAs discovered from public microarray datasets, was found to have the highest prediction power for sepsis amongst all currently studied transcriptomics-based assays. mRNA based gene expression assays have also been studied to differentiate bacterial from viral sepsis (for example, the 7-mRNA bacterial or viral Metascore). All of these novel tools (Table 2) for diagnosing and identifying the severity of sepsis appear promising but lacks validation in the patients with cirrhosis[57].

UPDATE ON PROGNOSTIC SCORING SYSTEMS FOR SEPSIS IN CIRRHOSIS

The newly described qSOFA, as per the Sepsis-3 guidelines, has become an important tool that can be utilized at the bedside for the identification of sepsis and predict mortality. More recently, Rhee *et al*[58] evaluated the performance of a novel electronic SOFA (eSOFA) compared to the classical SOFA score. The eSOFA was developed by the United States Centers for Disease Control and Prevention to facilitate retrospective surveillance of sepsis events and was found to identify better, a smaller but sicker cohort of patients, than classical SOFA score system. The SOFA score defines organ dysfunction across six organ systems and assigns 0-4 points for each organ system depending on the degree of dysfunction, whereas eSOFA replaces these with binary criteria for most of the same organ systems. Currently, the diagnosis of sepsis with the SOFA score to evaluate organ dysfunction in the setting of infection and the use of qSOFA to predict the severity and outcome of sepsis have been recommended by the Sepsis-3 consensus document. Müller *et al*[59] showed that qSOFA did not predict in-hospital mortality, intensive unit admission, or length of hospitalization in patients with decompensated cirrhosis. The application of sodium level to qSOFA (called qSOFA-Na^+) improved the diagnostic ability for identifying sepsis and mortality. However, in a larger series of cirrhosis patients, Piano *et al*[60] found that the Sepsis-3 criteria were more accurate than SIRS criteria in predicting the severity of infections in patients with cirrhosis and that the qSOFA was a useful bedside tool in assessing risk for poor outcomes in hospital. Patients fulfilling Sepsis-3 criteria had a higher incidence of ACLF, septic shock, and transfer to an intensive unit that those without. In a more recent study, Augustinho *et al*[61] showed that in patients with cirrhosis hospitalized for bacterial infections, admission qSOFA was an independent predictor of survival, and for those classified as high risk for death by qSOFA, only the CLIF-SOFA predicted prognosis independently, and Sepsis-3 criteria did not play a major role in predicting risk or stratifying patients. Lan *et al*[62] in a large retrospective cohort found that CLIF-SOFA and CLIF-organ failure scores were better tools that qSOFA, MELD, or qCLIF-SOFA in the evaluation of prognosis of critically ill patients with cirrhosis with suspected infections.

A Korean study revealed that qSOFA had limited utility in predicting adverse outcomes in cirrhosis patients with sepsis at medical emergency team activation in the

Table 2 Transcriptomics based micro assays for diagnosis of sepsis[57]

Assay name (manufacturer)	Technique/sample volume	Turn-around time	Highest noted sensitivity and specificity	Detection
SeptiFast (Roche)	Real-time PCR/1.5 mL	4 h to 6 h	83%/95%	> 16 bacteria, Candida and Aspergillus fumigatus
SeptiTest (Molzyme)	Universal PCR/1 mL	8 h to 10 h	87%/96%	> 345 bacteria and 13 fungi
SeptiCyte (ImmuneExpress)	RT-qPCR with machine learning/2.5 mL	1 h to 6 h	-/ 95% (discriminates SIRS from sepsis)	All pathogens
Iridica Plex ID (Abbott)	Multiplex broad range PCR/5 mL	6 h	83%/94%	780 bacteria and Candida
MinION (Oxford Nanopore)	Nanopore sequencing/10 ng DNA	4 h to 6 h	-/100%	Few viruses and bacteria currently
U-dHRM (UCSD, United States)	Digital PCR/1 mL	3 h	-/99.9%	37 bacteria
LAMP Tech	Loop mediated isothermal amplification/30 μL	1 h	-/100%	1 pathogen per sample
Integrated droplet digital detection tech (Velox Biosystems)	DNA-zyme base sensor droplet microencapsulation 3D particle analysis	1 h to 4 h	-	1 pathogen per sample

3D: Three-dimensional; PCR: Polymerase chain reaction; RT: Reverse transcriptase; SIRS: Systemic inflammatory response syndrome.

general wards or rooms. Another scoring system, called the modified early warning score (Table 3), detected sepsis early in these patients[63]. Pan *et al*[64] showed that the "MAP, bilirubin, respiratory failure, sepsis" score, a simple prognostic model consisting of MAP, serum bilirubin level, assessment of acute respiratory failure, and sepsis [calculated using the following predictors: MAP, < 80 mmHg; serum bilirubin level, > 80 μmol/L (4.7 mg/dL); type 1 respiratory failure, and fulfilment of definition of sepsis; defined as the sum of the values of the individual predictors, each value ranging from 0 to 4], analysed on the 1st day of admission to the intensive care unit in critically ill patients with cirrhosis with acute kidney injury, was useful in predicting short term mortality in-hospital better than current complex scoring systems including the commonly used CTP and MELD scores. A novel approach to predicting sepsis and severity is by coupling electronic medical records data with machine learning algorithms. As an example of this modality, researchers have identified a novel targeted, real-time early warning score called the TREWScore that predicts the development of septic shock in adult intensive care patients 28 h before clinical onset. The HeRO score algorithm (Medical Predictive Science Corp, Charlottesville, VA, United States) utilized subtle changes and irregularities in heart rate variability to predict poor outcomes before the actual onset. However, this technology has not been fully validated and lacks power in the identification of sepsis and bloodstream infections. Such novel approaches have the potential to be of great value in diagnosing sepsis and improving outcomes in this difficult to manage cohort of patients[65].

TREATING SEPSIS IN CIRRHOSIS – CURRENT RECOMMENDATIONS AND NEWER APPROACHES

Current updated guidelines recommend that the treatment of sepsis, along with needful resuscitation, should commence immediately at the identification of sepsis and related clinical outcomes. This includes appropriate antibiotics (based on region-specific community and hospital-related pathogen patterns) and other source control measures. The Surviving Sepsis Campaign currently recommends the "Hour-1 Bundle", which includes broad-spectrum antimicrobials, intravenous fluid management, measurement of serum lactate level and inotropes, and vasopressor support in those not responding to fluid resuscitation. There is no role of early goal-directed treatment in sepsis, as was considered previously as three large multicentre trials in three major countries reported absence of benefit with such an intervention. The use of crystalloid or colloid as the initial resuscitation fluid also remains an enigma. Even though guidelines suggest that crystalloid, possibly normal saline or a buffered salt solution such as Plasmalyte need to be utilized at 30 mL/kg over 3 h, this

Table 3 The modified early warning scoring system for identification of sepsis[63]

Score	3	2	1	0	1	2	3
Respiratory rate per min		≤ 8		9-14	15-20	21-29	> 29
Heart rate per min		≤ 40	41-50	51-100	101-110	111-129	> 129
Systolic blood pressure, mmHg	≤ 70	71-80	81-100	101-199		≥ 200	
Urine output, mL/(kg·h)	Nil	< 0.5					
Temperature, °C		≤ 35	35.1-36	36.1-38	38.1-38.5	≥ 38.6	
Neurological, subjective				Alert	Reacting to voice	Reacting to pain	Unresponsive

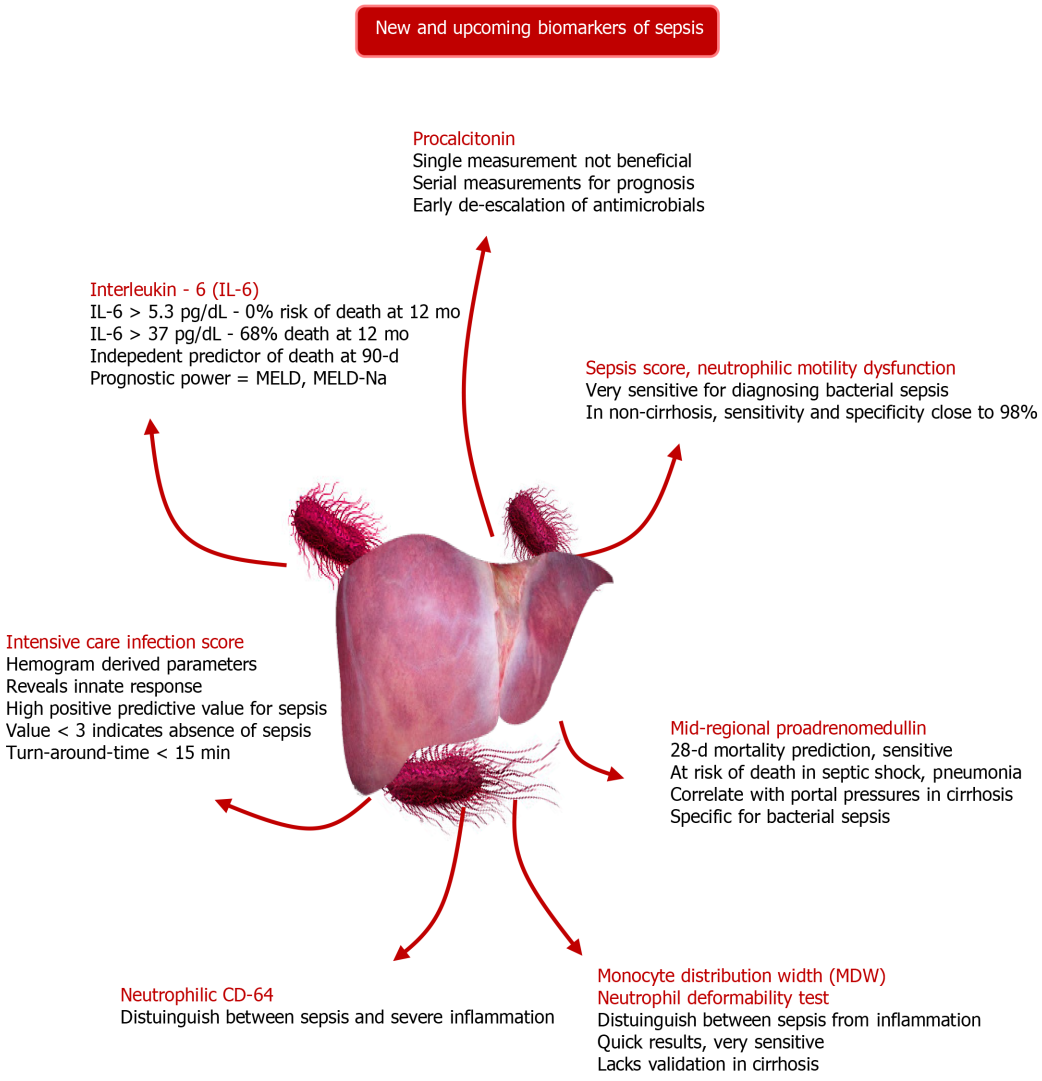

Figure 4 A summary of new and upcoming biomarkers for sepsis[48,50-57]. CD: Cluster of differentiation; IL: Interleukin; MDW: Monocyte distribution width; MELD: Model for end-stage liver disease; Na: Sodium.

practice is currently undergoing further scrutiny to improve on protocolized management. In those patients in whom crystalloids do not improve MAP, the addition of human albumin may be considered. However, no such recommendations exist, and the choice of fluid and its further modification rightfully rests on the common sense directed therapeutic decisions of the treating physician, based on close follow up of clinical parameters in the intensive care unit. Generalizability of Surviving Sepsis Campaign recommendations in the patients with cirrhosis needs validation. This is because goals of treatment may be different in patients with cirrhosis since they are frailer, have lower MAP at baseline due to the use of beta-

blockers for portal hypertension, have higher central venous oxygen saturation due to a hyperdynamic circulation with lower haematocrit, and abnormal lactate metabolism[66,67].

Philips *et al*[68] conducted an open-label trial in 308 patients with cirrhosis (published in abstract form) with sepsis-induced hypotension and randomized them to receive either 5% human albumin or normal saline. The primary endpoint was the reversal of hypotension (MAP > 65 mmHg) at 3 h, and the secondary endpoints included effects on heart rate, arterial lactate, urine output, and survival at 1 wk. The authors found that the reversal of hypotension was higher in patients receiving 5% albumin than saline at the end of 1 h [25.3% and 11.7% respectively, P = 0.03, odds ratio (95%CI): -1.9 (1.08-3.42)] and 3 h [11.7% and 3.2% respectively, P = 0.008, 3.9 (1.42-10.9)]. Sustained reduction in heart rate and lactate levels were greater in patients receiving albumin, without statistically significant changes in the urine output or adverse events between the groups. At the end of 1 wk, the proportion of patients surviving in the albumin group was higher than that in those who received saline (43.5% *vs* 38.3%, P = 0.03).

Regarding antibiotic therapy, some authors suggest that in community-acquired infections, the initial antibiotic of choice be a third-generation cephalosporin or amoxicillin-clavulanic acid and carbapenem or piperacillin and tazobactam combination in nosocomial infections in regions with a high and low prevalence of multiresistant bacteria, respectively, with or without a glycopeptide. Novel antimicrobial strategies are an area of active research. This includes targeting resistance mechanisms in pathogens. For example, the novel small molecule Inh2-B1, which targets serine-threonine protein kinase of methicillin-resistant *Staphylococcus aureus*, makes the pathogen susceptible to ceftriaxone and cefotaxime. Another example is antibiotic pairing with novel beta-lactamase or carbapenemase, as is seen with ceftazidime-avibactam and meropenem-vaborbactum, both of which were approved by the United States Food and Drug Administration for use in *Enterobacteriaceae* infections. Pathogen targeted antibody therapy is also a novel strategy to improve antimicrobial susceptibility. An example of this is the development of a monoclonal antibody against extremely drug-resistant *Acinetobacter baumannii*[43].

A haemoglobin threshold of 7 g/dL to 8 g/dL could be considered ideal in patients with cirrhosis with sepsis, as is endorsed by Baveno VI guidelines in those with acute variceal bleeding with a restrictive strategy of blood transfusion. In general, the septic shock population, the use of noradrenaline with or without vasopressin and adrenaline in a staged manner, has been recommended to maintain MAP. In a randomized controlled trial in patients with cirrhosis, Choudhury *et al*[69] demonstrated that terlipressin was as effective as noradrenaline as a vasopressor in patients with cirrhosis with septic shock and additionally provided early survival benefit with reduction in risk of variceal bleeding. In cirrhosis patients, the use of dopamine does not come highly recommended due to the high risk of inducing arrhythmias, and the administration of dobutamine is not supported because patients with cirrhosis have high cardiac output at baseline, which worsens with sepsis. Dobutamine is recommended in patients with clinically significant myocardial dysfunction. With regards to vasopressor hypo responsiveness and adrenal insufficiency in a general population of septic shock patients, the ADRENAL trial found no difference in 90-d all-cause mortality even though patients in the hydrocortisone group had faster resolution of shock, had a shorter duration of the initial episode of mechanical ventilation, and were less likely to receive blood transfusions. However, the APPROCHS (Activated Protein C and Corticosteroids for Human Septic Shock) trial showed that the 90-d all-cause mortality was lower among those who received hydrocortisone plus fludrocortisone than among those who received a placebo. A systematic review and meta-analysis in septic shock patients showed that corticosteroids possibly caused a small reduction in mortality and reduced duration of shock and intensive unit treatment but an increase in neuromuscular complications[70,71].

In patients with cirrhosis, a randomized study did not show any benefit on mortality and shock reversal with the use of intravenous hydrocortisone[72]. Since protective ventilation (low tidal volumes of 6 mL/kg of ideal body weight and plateau pressures < 30 cm H_2O) improves survival in general patients with adult respiratory distress syndrome, patients with cirrhosis who require mechanical ventilation should also be treated on the same lines. However, the sedation in such circumstances must ideally be with drugs with short half-lives such as propofol and remifentanil with avoidance of benzodiazepines[73-75]. In patients with cirrhosis, profound distributive shock leads to the development of refractoriness (a state of "vasoplegia") to inotrope,

and pressor support is higher than that seen in non-liver patients with septic shock due to increased sympathetic drive, use of beta-blockers, and more severe relative adrenal insufficiency.

Case reports and small series in general patients have shown that methylene blue (MB) (a selective inhibitor of guanylate cyclase improves vascular tone and tissue perfusion) infusion could improve refractory septic shock. Ahamed *et al*[76] recently studied the role of MB in patients with cirrhosis with refractory septic shock (published in abstract form) compared to those on a standard of care. The authors defined refractory septic shock as requirement of noradrenaline (final concentration 64 μg/mL in 250 mL 0.9% normal saline) ≥ 4 μg/min (*i.e.* 8 mL/h) + vasopressin ≥ 0.01 units/min (*i.e.* 1.5 mL/h). On retrospective analysis, they found that improvement in systolic blood pressure was significantly better in the MB group from baseline at the end of 24-, 72-, and 120 h compared to those on a standard of care. Improvement in diastolic blood pressure was notable in the MB group from baseline at the end of 24- and 72 h; between 24- and 72 h and 24- and 120 h while the increase in MAP was significantly higher in patients receiving MB from baseline at 24- and 72 h. Significant reduction in dose of noradrenaline and vasopressin dosing was also noted from baseline at the end of 24- and 72 h in the MB group. The need for additional inotropes was significantly higher at the end of 24- and 72 h in patients continued on the standard of care. The total hospital stay duration was significantly lower in the MB group (8 d *vs* 10 d, $P < 0.05$), however, without significant differences in short-term-survival (1 wk, 14 d, and 28 d) between groups[76]. A proposed algorithm for the treatment of sepsis in cirrhosis is shown in Figure 5[43,66,73,74].

Walley *et al*[77] showed that the proprotein convertase subtilisin/kexin type-9 (PCSK9) was a critical regulator of the innate immune response, and septic shock outcome and reduction in PCSK9 function were associated with increased pathogen lipid clearance through the low-density lipoprotein receptors with a decrease in the inflammatory response. Repurposing drugs for newer indications and genomic approaches to improving outcomes in septic shock is an area of active research[77]. Other promising treatment modalities in sepsis include a combination of vitamin C, hydrocortisone, thiamine, short-acting beta-blockade therapy using esmolol in patients with sepsis and persistent tachycardia, and toxin removal and inflammation control using hemadsorption techniques that utilize specialized membranes such as those with polymyxin B and finally, immune-stimulation with growth factors such as granulocyte and granulocyte-macrophage colony-stimulating factors. The role of nanoparticle-based adjuvant therapies is gaining widespread attention as a novel area with beneficial strategic output in the treatment of sepsis. Nanoparticles have small size and sizeable surface area to volume ratio and can be utilized as antibacterial agents, structure platforms for adsorbents that bind and sequester endotoxins and cytokines to restore homeostasis[78,79]. A summary of novel adjuvant therapies for sepsis is shown in Table 4.

THE ROLE OF AND MODULATION OF GUT MICROBIOTA IN SEPSIS AND CIRRHOSIS

An intact intestinal barrier and commensal balance are imperative for proper maturation and development of the immune system. In health, gut microbiota antagonize pathogens by competing with nutritional components, produce antimicrobial peptides and metabolites, and render the local milieu hostile by modifying bile salts. Not only at the local sites, but at a systemic level, immune-regulation is an important task of the healthy microbiota. Various structural components of the gut microbes called the microbe-associated molecular patterns, or MAMPs, can promote a systemic inflammatory response by activating and further maturing the innate and adaptive immune system. The microbial metabolites such as short-chain fatty acids (SCFAs) help in modulating both pro and anti-inflammatory responses to maintain immune and inflammatory balance in the host. SCFAs like butyrate and propionate activate regulatory T cells that ameliorate systemic inflammation. Other gut metabolites such as desaminotyrosine boosts type 1 interferon responses leading to improved viral pathogen clearance. In patients who develop dysbiosis, due to environmental and host factors, gut microbial health deteriorates, leading to increased risk of infections. Furthermore, the use of antibiotics increases dysbiosis that enters the host into a vicious cycle of infections and organ dysfunction. It is well known that patients with cirrhosis have dysbiosis that worsens with the severity of liver disease. In its worst form, pathobionts prevail, and the

Table 4 Novel adjuvant therapies for management of sepsis[74-76]

Therapy	Mechanism	Systemic effect in sepsis
Eritoran; resatorvid	Toll-like receptor 4 antagonist; Eritoran is structurally similar to lipopolysaccharide – A of Gram-negative bacteria. Resatorvid is a direct antagonist of toll like receptor 4	Anti-inflammatory; Immunomodulation
Polymixin B fibre column; CytoSorb	Hemoperfusion; CytoSorb has hemadsorption properties	Removal of circulating endotoxin and bacterial components
Plasma exchange; Whole blood exchange; Coupled plasma filtration adsorption; Hemofiltration	Exchange of plasma or blood with or without sorbent adsorption; either continuous or intermittent; low or high volume	Removal of endotoxins and circulating cytokines
Macrolides	Nuclear factor kB and AP-1 signalling suppression, inhibition of ERK-1 and 2 pathways	Anti-inflammatory and immunomodulating properties
Interferon-gamma	Increase in monocyte HLA-DR expression	Restores immune regulation, abolishes immunoparalysis by restoring monocyte function
Immunoglobulins	Increase in IgA and IgM levels	Boosts humoral immunity
Granulocyte macrophage colony stimulating factor	Promotes maturation and differentiation of neutrophils, monocytes, macrophages, dendritic cells, T lymphocytes and plasma cells	Improves immune regulation, reduces immunoparalysis
Anti-MIF	Antagonizes macrophage migration inhibition factor	Immunomodulation through boosting activity of endogenous glucocorticoids
Super-Antigen-Antagonist	Suppression of pro-inflammatory gene expression by inhibition of T cell activation	Th1 blockade and prevention of lethal shock
Heparin and its analogues	Anti-thrombotic, immunomodulation	Prevents early disseminated intravascular coagulation, prevents early organ failures due to diffuse system microvascular thrombosis
Naloxone	Opioid receptor antagonism	Improves hemodynamic instability
Pentoxifylline	Decreases erythrocyte aggregation and deformability, anti TNF-alpha effect	Improvement in arterial oxygen tension by improving fractionated oxygen exchange
GTS-21	Selective alpha-7-nicotinic acetylcholine receptor agonist, blocks nuclear factor – kB and cytokines downstream	Activates cholinergic anti-inflammatory pathway
Interleukin 7 and 2	Pro-inflammatory cytokines	Prevents immunoparalysis
Programmed cell death-1 (PD-1) and ligand (PD-L1) antagonist	Prevention of lymphocyte depletion, improvement in pro-inflammatory mediators and increased bacterial clearance	Immune modulation
B and T cell lymphocyte attenuator antagonism (BTLA)	Increases activity and proliferation of T cells	Increases resistance to endotoxin and prevention of endotoxin mediated shock
Antagonism of cytotoxic T lymphocyte antigen 4 (CTLA-4)	Increased activity and proliferation of T cells	Abolishes endotoxemia and associated toxic shock

Methylthiouracil	Suppresses high mobility group box – 1 (HMGB-1)	Anti-inflammatory
Structurally nanoengineered antimicrobial peptide polymers; Ceria – zirconia nanoparticles; Piceatannol-loaded albumin nanoparticles; Sialic-acid decorated nanoparticles; Exsosomes loaded with MFGE8, miR-223; Red blood cells and macrophage coated nanoparticles; Liposomes tagged to antimicrobials; Opsonin bound magnetic nanobeads	Nanoparticle technology (pre-clinical studies)	Antibacterial; Antioxidant; Anti-inflammatory; Endotoxin antagonist; Extracorporeal blood cleansing; Clearance of apoptotic cells

AP: Activator protein; BTLA: B and T lymphocyte associated; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; ERK: Extracellular signal-regulated kinases; HLA-DR: Human leukocyte antigen – DR isotype; HMGB: High-mobility group box; Ig: Immunoglobulins; MFGE8: Milk fat globule epidermal growth factor 8 protein; MIF: Macrophage migration inhibitory factor; miR: Micro-RNA; PD-L: Programmed death receptor ligand; TNF: Tumour necrosis factor.

intestinal microbiota after that functions as a repository for antimicrobial resistance (a state called "resistome"). This further endangers immune function at the local and systemic level predisposing the host to not only infectious insults but also severe inflammatory states leading to organ failures. The best proof that sepsis can be improved with modulation of gut microbiota stems from studies of healthy donor faecal microbiota transplantation (currently rechristened intestinal microbiota reinstitution therapy or intestinal microbiota re-instituition therapy) in patients with recurrent and severe *Clostridium difficile* infections[80-82]. Modulation of microbiota in a similar fashion in patients with liver disease has been shown to improve outcomes related to hepatic encephalopathy, alcoholic hepatitis with infections, ACLF, and primary sclerosing cholangitis with recurrent cholangitis[83-86]. In patients with cirrhosis, understanding the gut microbiota and its modulation are still in foetal stages. In such patients, the first step is to identify, through omics-based research, those at risk for the development of infections before sepsis development. Modulating microbiota at this stage can help prevent infections by restoring a eubiotic microbiome. In those patients with sepsis, addressing dysbiosis through active modulation of the microbiota can help improve outcomes related to sepsis and organ failures. In those surviving sepsis, modulation of microbiota to restore homeostatic balance can help prevent dysbiosis driven infectious insults in the future (Figure 6). Recently, in a randomized controlled pilot trial, Stadlbauer *et al*[87] demonstrated that dysbiosis in early sepsis could be modulated by utilizing a multispecies probiotic (Winclove 607 based on Omnibiotic® 10 AAD) with improvement in clinical outcomes.

CONCLUSION

Sepsis and septic shock are conditions associated with high mortality in the general population, more so in patients with cirrhosis due to specific hemodynamic and immune system-related changes affecting the latter. Over the past few decades, the definition of sepsis and its application in clinical practice has seen a major change, which has helped to identify better patients at risk. The sepsis care protocols have evolved over the past few years to incorporate the best clinical practices that would

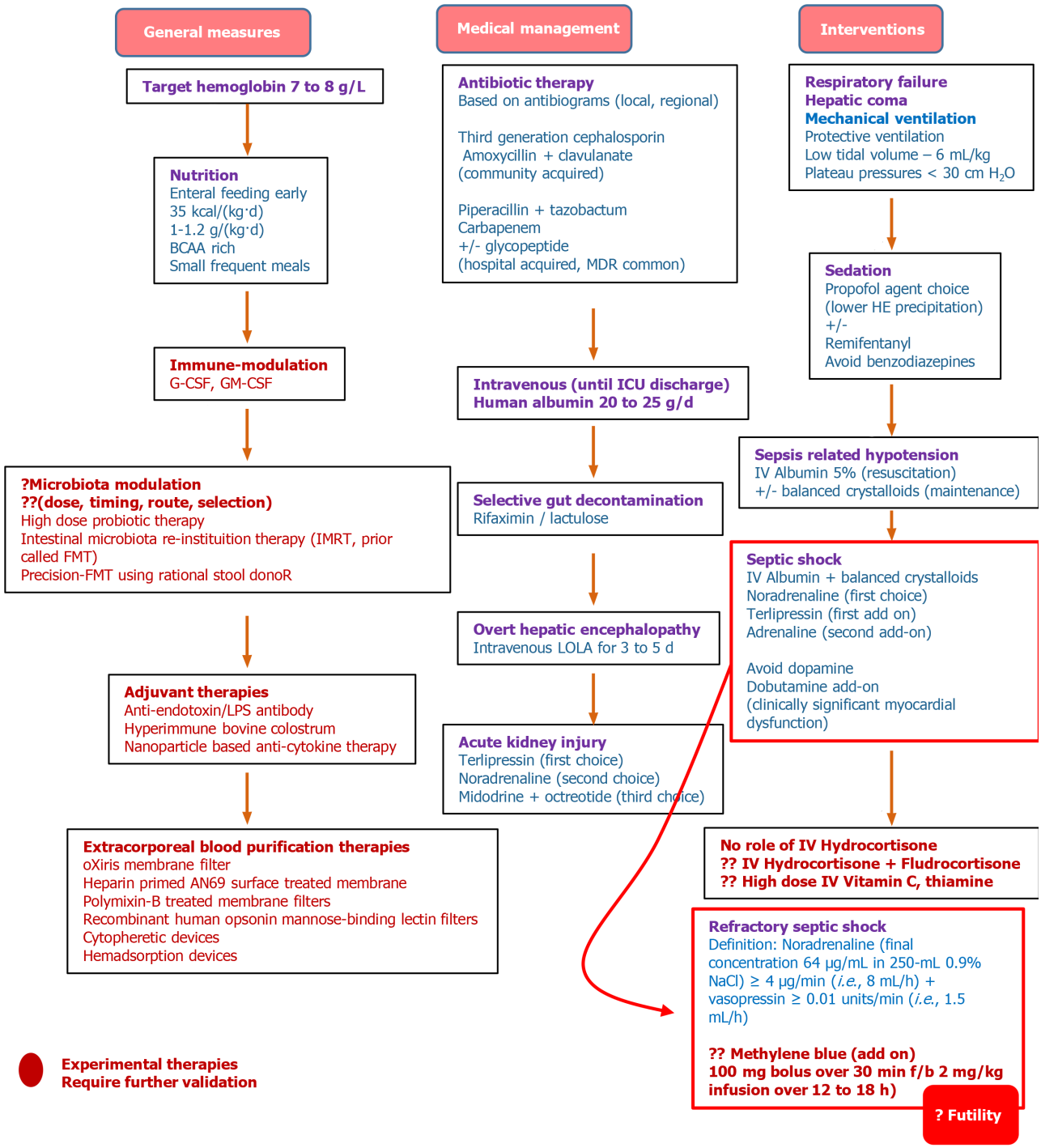

Figure 5 The proposed treatment algorithm for sepsis in cirrhosis[66,68,70-72,75]. BCAA: Branched-chain amino acids; FMT: Faecal microbiota transplantation; G-CSF: Granulocyte-colony stimulating factor; GM: Granulocyte-macrophage; ICU: Intensive care unit; IMRT: Intestinal microbiota re-instituition therapy; IV: Intravenous; LOLA: L-ornitine L-aspartate; LPS: Lipopolysaccharide; MDR: Multidrug resistant.

improve clinical outcomes in affected patients. Even though specific guidelines for sepsis identification and treatments do not exist in patients with cirrhosis, real-world evidence from the non-liver population has been of great help in managing sepsis in this difficult to treat cohort. Basic science work has identified novel areas such as the role of nutrition, immune regulation, genomics-based and nanomedicine-based approaches, as well as microbiota modulation in improving adjuvant treatments for sepsis, which could become an integral part in the management of severe infections. Novel antimicrobial strategies for combating resistance and the role of machine learning and deep data mining are also major tools currently in development as armamentarium against sepsis. In this exciting era of basic science work driven bench to bedside strategies, the improvements in challenges during the management of

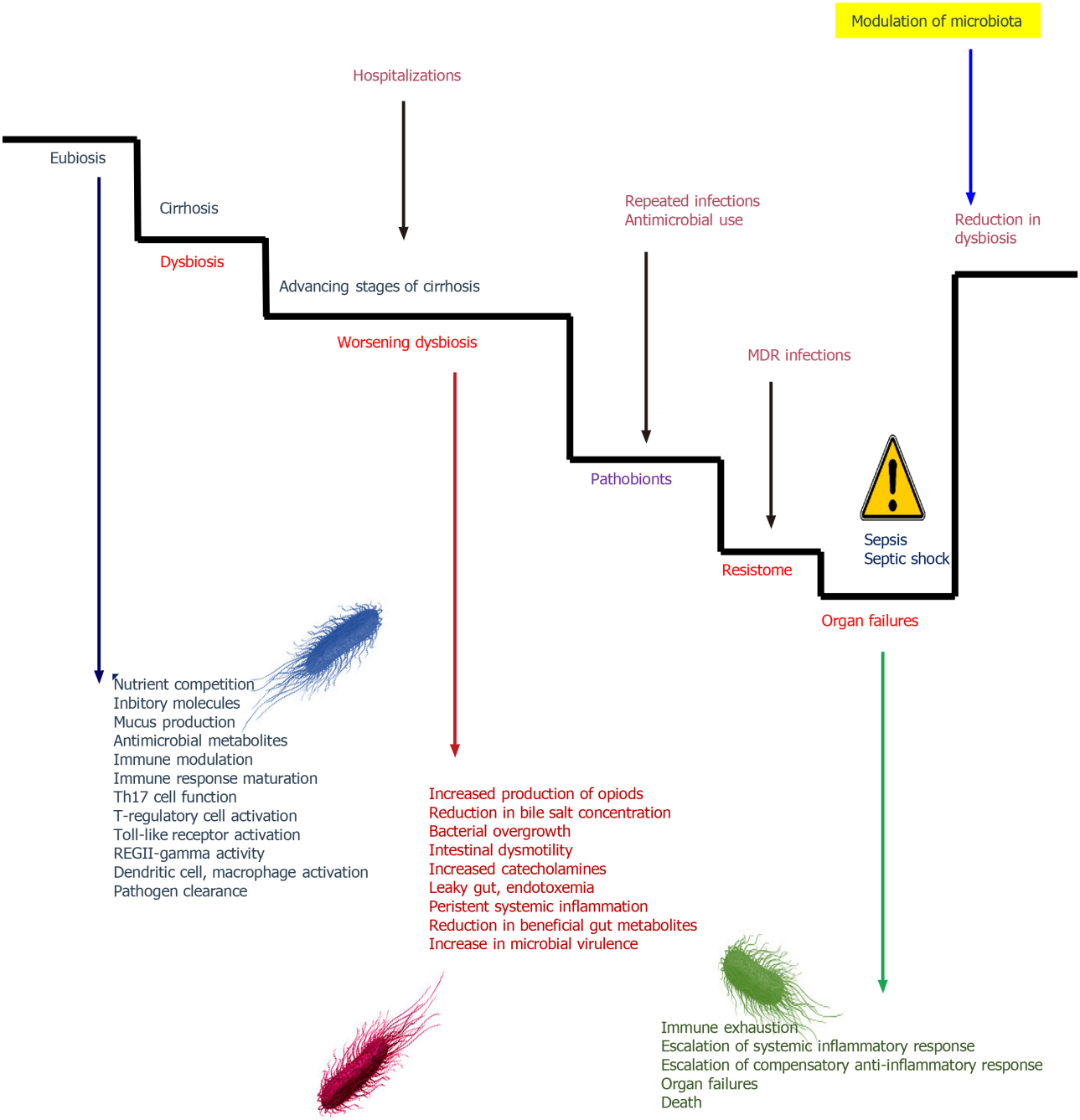

Figure 6 The role of gut microbiota in driving and worsening sepsis and cirrhosis[78,80,81]**.** Gut microbiota modulation is an interesting approach to management of sepsis in the future. Reducing dysbiotic bacterial communities and favouring commensals that improve host immune functions, promote endogenous antimicrobial metabolite formation and resist pathogenic colonization could be achieved through high dose probiotics or intestinal microbiota re-institution therapy. MDR: Multidrug resistant.

sepsis in cirrhosis in the future looks promising.

REFERENCES

1 **Dickmann P**, Bauer M. Sepsis 2019 - New Trends and Their Implications for Multiple Trauma Patients. *Z Orthop Unfall* 2020; **158**: 81-89 [PMID: 31499573 DOI: 10.1055/a-0853-2054]

2 **Barrier KM**. Summary of the 2016 International Surviving Sepsis Campaign: A Clinician's Guide. *Crit Care Nurs Clin North Am* 2018; **30**: 311-321 [PMID: 30098735 DOI: 10.1016/j.cnc.2018.04.001]

3 **Simpson N**, Lamontagne F, Shankar-Hari M. Septic shock resuscitation in the first hour. *Curr Opin Crit Care* 2017; **23**: 561-566 [PMID: 29023316 DOI: 10.1097/MCC.0000000000000460]

4 **Rahmel T**. [SSC International Guideline 2016 - Management of Sepsis and Septic Shock]. *Anasthesiol Intensivmed Notfallmed Schmerzther* 2018; **53**: 142-148 [PMID: 29426052 DOI: 10.1055/s-0043-114639]

5 **Plevin R**, Callcut R. Update in sepsis guidelines: what is really new? *Trauma Surg Acute Care Open* 2017; **2**: e000088 [PMID: 29766091 DOI: 10.1136/tsaco-2017-000088]

6 **Rhodes A**, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med* 2017; **43**: 304-377 [PMID: 28101605 DOI: 10.1007/s00134-017-4683-6]

7 **Scala R**, Schultz M, Bos LDJ, Artigas A. New Surviving Sepsis Campaign guidelines: back to the art of medicine. *Eur Respir J* 2018; **52**: 1701818 [PMID: 29997181 DOI: 10.1183/13993003.01818-2017]

8 **Gunsolus IL**, Sweeney TE, Liesenfeld O, Ledeboer NA. Diagnosing and Managing Sepsis by Probing the Host Response to Infection: Advances, Opportunities, and Challenges. *J Clin Microbiol* 2019; **57**: e00425-19 [PMID: 31043466 DOI: 10.1128/JCM.00425-19]

9 **Jalan R**, Stadlbauer V, Sen S, Cheshire L, Chang YM, Mookerjee RP. Role of predisposition, injury, response and organ failure in the prognosis of patients with acute-on-chronic liver failure: a prospective cohort study. *Crit Care* 2012; **16**: R227 [PMID: 23186071 DOI: 10.1186/cc11882]

10 **Maiwall R**, Sarin SK, Kumar S, Jain P, Kumar G, Bhadoria AS, Moreau R, Kedarisetty CK, Abbas Z, Amarapurkar D, Bhardwaj A, Bihari C, Butt AS, Chan A, Chawla YK, Chowdhury A, Dhiman R, Dokmeci AK, Ghazinyan H, Hamid SS, Kim DJ, Komolmit P, Lau GK, Lee GH, Lesmana LA, Jamwal K, Mamun-Al-Mahtab, Mathur RP, Nayak SL, Ning Q, Pamecha V, Alcantara-Payawal D, Rastogi A, Rahman S, Rela M, Saraswat VA, Shah S, Shiha G, Sharma BC, Sharma MK, Sharma K, Tan SS, Chandel SS, Vashishtha C, Wani ZA, Yuen MF, Yokosuka O, Duseja A, Jafri W, Devarbhavi H, Eapen CE, Goel A, Sood A, Ji J, Duan Z, Chen Y; of the APASL ACLF Research Consortium (AARC) working party. Development of predisposition, injury, response, organ failure model for predicting acute kidney injury in acute on chronic liver failure. *Liver Int* 2017; **37**: 1497-1507 [PMID: 28393476 DOI: 10.1111/liv.13443]

11 **Medlej K**. Calculated decisions: qSOFA (quick SOFA) score for sepsis. *Emerg Med Pract* 2018; **20**: CD2-CD4 [PMID: 30280854]

12 **Marik PE**, Taeb AM. SIRS, qSOFA and new sepsis definition. *J Thorac Dis* 2017; **9**: 943-945 [PMID: 28523143 DOI: 10.21037/jtd.2017.03.125]

13 **Engelmann C**, Thomsen KL, Zakeri N, Sheikh M, Agarwal B, Jalan R, Mookerjee RP. Validation of CLIF-C ACLF score to define a threshold for futility of intensive care support for patients with acute-on-chronic liver failure. *Crit Care* 2018; **22**: 254 [PMID: 30305132 DOI: 10.1186/s13054-018-2156-0]

14 **Li N**, Huang C, Yu KK, Lu Q, Shi GF, Zheng JM. Validation of prognostic scores to predict short-term mortality in patients with HBV-related acute-on-chronic liver failure: The CLIF-C OF is superior to MELD, CLIF SOFA, and CLIF-C ACLF. *Medicine (Baltimore)* 2017; **96**: e6802 [PMID: 28445322 DOI: 10.1097/MD.0000000000006802]

15 **Silva PE**, Fayad L, Lazzarotto C, Ronsoni MF, Bazzo ML, Colombo BS, Dantas-Correa EB, Narciso-Schiavon JL, Schiavon LL. Single-centre validation of the EASL-CLIF consortium definition of acute-on-chronic liver failure and CLIF-SOFA for prediction of mortality in cirrhosis. *Liver Int* 2015; **35**: 1516-1523 [PMID: 24840673 DOI: 10.1111/liv.12597]

16 **Jeppesen JB**, Mortensen C, Bendtsen F, Møller S. Lactate metabolism in chronic liver disease. *Scand J Clin Lab Invest* 2013; **73**: 293-299 [PMID: 23514017 DOI: 10.3109/00365513.2013.773591]

17 **De Jonghe B**, Cheval C, Misset B, Timsit JF, Garrouste M, Montuclard L, Carlet J. Relationship between blood lactate and early hepatic dysfunction in acute circulatory failure. *J Crit Care* 1999; **14**: 7-11 [PMID: 10102718 DOI: 10.1016/s0883-9441(99)90002-3]

18 **Kruse JA**, Zaidi SA, Carlson RW. Significance of blood lactate levels in critically ill patients with liver disease. *Am J Med* 1987; **83**: 77-82 [PMID: 3605185 DOI: 10.1016/0002-9343(87)90500-6]

19 **Kruse O**, Grunnet N, Barfod C. Blood lactate as a predictor for in-hospital mortality in patients admitted acutely to hospital: a systematic review. *Scand J Trauma Resusc Emerg Med* 2011; **19**: 74 [PMID: 22202128 DOI: 10.1186/1757-7241-19-74]

20 **Sterling SA**, Puskarich MA, Jones AE. The effect of liver disease on lactate normalization in severe sepsis and septic shock: a cohort study. *Clin Exp Emerg Med* 2015; **2**: 197-202 [PMID: 27752598 DOI: 10.15441/ceem.15.025]

21 **Sun DQ**, Zheng CF, Lu FB, Van Poucke S, Chen XM, Chen YP, Zhang L, Zheng MH. Serum lactate level accurately predicts mortality in critically ill patients with cirrhosis with acute kidney injury. *Eur J Gastroenterol Hepatol* 2018; **30**: 1361-1367 [PMID: 29916857 DOI: 10.1097/MEG.0000000000001189]

22 **Drolz A**, Horvatits T, Rutter K, Landahl F, Roedl K, Meersseman P, Wilmer A, Kluwe J, Lohse AW, Kluge S, Trauner M, Fuhrmann V. Lactate Improves Prediction of Short-Term Mortality in Critically Ill Patients With Cirrhosis: A Multinational Study. *Hepatology* 2019; **69**: 258-269 [PMID: 30070381 DOI: 10.1002/hep.30151]

23 **Chervonsky AV**. Just a Spoonful of Sugar Helps the Tolerance Go Up. *Cell* 2017; **169**: 1170-1172 [PMID: 28622502 DOI: 10.1016/j.cell.2017.05.040]

24 **Weis S**, Carlos AR, Moita MR, Singh S, Blankenhaus B, Cardoso S, Larsen R, Rebelo S, Schäuble S, Del Barrio L, Mithieux G, Rajas F, Lindig S, Bauer M, Soares MP. Metabolic Adaptation Establishes Disease Tolerance to Sepsis. *Cell* 2017; **169**: 1263-1275.e14 [PMID: 28622511 DOI: 10.1016/j.cell.2017.05.031]

25 **Medzhitov R**, Schneider DS, Soares MP. Disease tolerance as a defense strategy. *Science* 2012; **335**: 936-941 [PMID: 22363001 DOI: 10.1126/science.1214935]

26 **Wang A**, Huen SC, Luan HH, Yu S, Zhang C, Gallezot JD, Booth CJ, Medzhitov R. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. *Cell* 2016; **166**: 1512-1525.e12 [PMID: 27610573 DOI: 10.1016/j.cell.2016.07.026]

27 **Changani KK**, Jalan R, Cox IJ, Ala-Korpela M, Bhakoo K, Taylor-Robinson SD, Bell JD. Evidence for

altered hepatic gluconeogenesis in patients with cirrhosis using in vivo 31-phosphorus magnetic resonance spectroscopy. *Gut* 2001; **49**: 557-564 [PMID: 11559655 DOI: 10.1136/gut.49.4.557]
28 **Ebadi M**, Bhanji RA, Mazurak VC, Montano-Loza AJ. Sarcopenia in cirrhosis: from pathogenesis to interventions. *J Gastroenterol* 2019; **54**: 845-859 [PMID: 31392488 DOI: 10.1007/s00535-019-01605-6]
29 **Matsumi M**, Takahashi T, Fujii H, Ohashi I, Kaku R, Nakatsuka H, Shimizu H, Morita K, Hirakawa M, Inagaki M, Sadamori H, Yagi T, Tanaka N, Akagi R. Increased heme oxygenase-1 gene expression in the livers of patients with portal hypertension due to severe hepatic cirrhosis. *J Int Med Res* 2002; **30**: 282-288 [PMID: 12166345 DOI: 10.1177/147323000203000309]
30 **Sass G**, Barikbin R, Tiegs G. The multiple functions of heme oxygenase-1 in the liver. *Z Gastroenterol* 2012; **50**: 34-40 [PMID: 22222796 DOI: 10.1055/s-0031-1282046]
31 **Lv Y**, Yee Lau W, Wu H, Han X, Gong X, Liu N, Yue J, Li Q, Li Y, Deng J. Causes of peripheral cytopenia in hepatitic cirrhosis and portal hypertensive splenomegaly. *Exp Biol Med (Maywood)* 2017; **242**: 744-749 [PMID: 28299974 DOI: 10.1177/1535370217693113]
32 **Qamar AA**, Grace ND, Groszmann RJ, Garcia-Tsao G, Bosch J, Burroughs AK, Ripoll C, Maurer R, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Makuch R, Rendon G; Portal Hypertension Collaborative Group. Incidence, prevalence, and clinical significance of abnormal hematologic indices in compensated cirrhosis. *Clin Gastroenterol Hepatol* 2009; **7**: 689-695 [PMID: 19281860 DOI: 10.1016/j.cgh.2009.02.021]
33 **Gkamprela E**, Deutsch M, Pectasides D. Iron deficiency anemia in chronic liver disease: etiopathogenesis, diagnosis and treatment. *Ann Gastroenterol* 2017; **30**: 405-413 [PMID: 28655976 DOI: 10.20524/aog.2017.0152]
34 **Viveiros A**, Finkenstedt A, Schaefer B, Mandorfer M, Scheiner B, Lehner K, Tobiasch M, Reiberger T, Tilg H, Edlinger M, Zoller H. Transferrin as a predictor of survival in cirrhosis. *Liver Transpl* 2018; **24**: 343-351 [PMID: 29149510 DOI: 10.1002/lt.24981]
35 **Rosenbaum M**, Hall KD, Guo J, Ravussin E, Mayer LS, Reitman ML, Smith SR, Walsh BT, Leibel RL. Glucose and Lipid Homeostasis and Inflammation in Humans Following an Isocaloric Ketogenic Diet. *Obesity (Silver Spring)* 2019; **27**: 971-981 [PMID: 31067015 DOI: 10.1002/oby.22468]
36 **Ayres JS**. Disease Tolerance Trick or Treat: Give Your Brain Something Good to Eat. *Cell* 2016; **166**: 1368-1370 [PMID: 27610563 DOI: 10.1016/j.cell.2016.08.034]
37 **Schieber AM**, Lee YM, Chang MW, Leblanc M, Collins B, Downes M, Evans RM, Ayres JS. Disease tolerance mediated by microbiome E. coli involves inflammasome and IGF-1 signaling. *Science* 2015; **350**: 558-563 [PMID: 26516283 DOI: 10.1126/science.aac6468]
38 **Rui L**. Energy metabolism in the liver. *Compr Physiol* 2014; **4**: 177-197 [PMID: 24692138 DOI: 10.1002/cphy.c130024]
39 **Eghtesad S**, Poustchi H, Malekzadeh R. Malnutrition in liver cirrhosis:the influence of protein and sodium. *Middle East J Dig Dis* 2013; **5**: 65-75 [PMID: 24829672]
40 **Owen OE**, Reichle FA, Mozzoli MA, Kreulen T, Patel MS, Elfenbein IB, Golsorkhi M, Chang KH, Rao NS, Sue HS, Boden G. Hepatic, gut, and renal substrate flux rates in patients with hepatic cirrhosis. *J Clin Invest* 1981; **68**: 240-252 [PMID: 7251861 DOI: 10.1172/jci110240]
41 **García-Compeán D**, Jáquez-Quintana JO, Lavalle-González FJ, González-González JA, Muñoz-Espinosa LE, Villarreal-Pérez JZ, Maldonado-Garza HJ. Subclinical abnormal glucose tolerance is a predictor of death in liver cirrhosis. *World J Gastroenterol* 2014; **20**: 7011-7018 [PMID: 24944496 DOI: 10.3748/wjg.v20.i22.7011]
42 **Gyawali B**, Ramakrishna K, Dhamoon AS. Sepsis: The evolution in definition, pathophysiology, and management. *SAGE Open Med* 2019; **7**: 2050312119835043 [PMID: 30915218 DOI: 10.1177/2050312119835043]
43 **Varon J**, Baron RM. A current appraisal of evidence for the approach to sepsis and septic shock. *Ther Adv Infect Dis* 2019; **6**: 2049936119856517 [PMID: 31308945 DOI: 10.1177/2049936119856517]
44 **Dirchwolf M**, Ruf AE. Role of systemic inflammation in cirrhosis: From pathogenesis to prognosis. *World J Hepatol* 2015; **7**: 1974-1981 [PMID: 26261687 DOI: 10.4254/wjh.v7.i16.1974]
45 **Clària J**, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, Amorós À, Titos E, Alcaraz-Quiles J, Oettl K, Morales-Ruiz M, Angeli P, Domenicali M, Alessandria C, Gerbes A, Wendon J, Nevens F, Trebicka J, Laleman W, Saliba F, Welzel TM, Albillos A, Gustot T, Benten D, Durand F, Ginès P, Bernardi M, Arroyo V; CANONIC Study Investigators of the EASL-CLIF Consortium and the European Foundation for the Study of Chronic Liver Failure (EF-CLIF). Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. *Hepatology* 2016; **64**: 1249-1264 [PMID: 27483394 DOI: 10.1002/hep.28740]
46 **Harrison MF**. The Misunderstood Coagulopathy of Liver Disease: A Review for the Acute Setting. *West J Emerg Med* 2018; **19**: 863-871 [PMID: 30202500 DOI: 10.5811/westjem.2018.7.37893]
47 **Novotny AR**, Reim D, Assfalg V, Altmayr F, Friess HM, Emmanuel K, Holzmann B. Mixed antagonist response and sepsis severity-dependent dysbalance of pro- and anti-inflammatory responses at the onset of postoperative sepsis. *Immunobiology* 2012; **217**: 616-621 [PMID: 22204813 DOI: 10.1016/j.imbio.2011.10.019]
48 **Schuetz P**, Beishuizen A, Broyles M, Ferrer R, Gavazzi G, Gluck EH, González Del Castillo J, Jensen JU, Kanizsai PL, Kwa ALH, Krueger S, Luyt CE, Oppert M, Plebani M, Shlyapnikov SA, Toccafondi G, Townsend J, Welte T, Saeed K. Procalcitonin (PCT)-guided antibiotic stewardship: an international experts consensus on optimized clinical use. *Clin Chem Lab Med* 2019; **57**: 1308-1318 [PMID: 30721141 DOI: 10.1515/cclm-2018-1181]
49 **Branche A**, Neeser O, Mueller B, Schuetz P. Procalcitonin to guide antibiotic decision making. *Curr Opin Infect Dis* 2019; **32**: 130-135 [PMID: 30648993 DOI: 10.1097/QCO.0000000000000522]
50 **Reuken PA**, Kiehntopf M, Stallmach A, Bruns T. Mid-regional pro-adrenomedullin (MR-proADM): an even better prognostic biomarker than C-reactive protein to predict short-term survival in patients with decompensated cirrhosis at risk of infection? *J Hepatol* 2012; **57**: 1156-8; author reply 1158-9 [PMID: 22892248 DOI: 10.1016/j.jhep.2012.06.036]
51 **Remmler J**, Schneider C, Treuner-Kaueroff T, Bartels M, Seehofer D, Scholz M, Berg T, Kaiser T.

Increased Level of Interleukin 6 Associates With Increased 90-Day and 1-Year Mortality in Patients With End-Stage Liver Disease. *Clin Gastroenterol Hepatol* 2018; **16**: 730-737 [PMID: 28919544 DOI: 10.1016/j.cgh.2017.09.017]

52 **Ellett F**, Jorgensen J, Marand AL, Liu YM, Martinez MM, Sein V, Butler KL, Lee J, Irimia D. Diagnosis of sepsis from a drop of blood by measurement of spontaneous neutrophil motility in a microfluidic assay. *Nat Biomed Eng* 2018; **2**: 207-214 [PMID: 30283724 DOI: 10.1038/s41551-018-0208-z]

53 **van der Geest PJ**, Mohseni M, Linssen J, Duran S, de Jonge R, Groeneveld AB. The intensive care infection score - a novel marker for the prediction of infection and its severity. *Crit Care* 2016; **20**: 180 [PMID: 27384242 DOI: 10.1186/s13054-016-1366-6]

54 **Crouser ED**, Parrillo JE, Seymour CW, Angus DC, Bicking K, Esguerra VG, Peck-Palmer OM, Magari RT, Julian MW, Kleven JM, Raj PJ, Procopio G, Careaga D, Tejidor L. Monocyte Distribution Width: A Novel Indicator of Sepsis-2 and Sepsis-3 in High-Risk Emergency Department Patients. *Crit Care Med* 2019; **47**: 1018-1025 [PMID: 31107278 DOI: 10.1097/CCM.0000000000003799]

55 **Crawford K**, DeWitt A, Brierre S, Caffery T, Jagneaux T, Thomas C, Macdonald M, Tse H, Shah A, Di Carlo D, O'Neal HR. Rapid Biophysical Analysis of Host Immune Cell Variations Associated with Sepsis. *Am J Respir Crit Care Med* 2018; **198**: 280-282 [PMID: 29630392 DOI: 10.1164/rccm.201710-2077LE]

56 **Kumar A**, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; **34**: 1589-1596 [PMID: 16625125 DOI: 10.1097/01.CCM.0000217961.75225.E9]

57 **Mangioni D**, Peri AM, Rossolini GM, Viaggi B, Perno CF, Gori A, Bandera A. Toward Rapid Sepsis Diagnosis and Patient Stratification: What's New From Microbiology and Omics Science. *J Infect Dis* 2020; **221**: 1039-1047 [PMID: 31693109 DOI: 10.1093/infdis/jiz585]

58 **Rhee C**, Zhang Z, Kadri SS, Murphy DJ, Martin GS, Overton E, Seymour CW, Angus DC, Dantes R, Epstein L, Fram D, Schaaf R, Wang R, Klompas M; CDC Prevention Epicenters Program. Sepsis Surveillance Using Adult Sepsis Events Simplified eSOFA Criteria Versus Sepsis-3 Sequential Organ Failure Assessment Criteria. *Crit Care Med* 2019; **47**: 307-314 [PMID: 30768498 DOI: 10.1097/CCM.0000000000003521]

59 **Müller M**, Schefold JC, Leichtle AB, Srivastava D, Lindner G, Exadaktylos AK, Pfortmueller CA. qSOFA score not predictive of in-hospital mortality in emergency patients with decompensated liver cirrhosis. *Med Klin Intensivmed Notfmed* 2019; **114**: 724-732 [PMID: 30132026 DOI: 10.1007/s00063-018-0477-z]

60 **Piano S**, Bartoletti M, Tonon M, Baldassarre M, Chies G, Romano A, Viale P, Vettore E, Domenicali M, Stanco M, Pilutti C, Frigo AC, Brocca A, Bernardi M, Caraceni P, Angeli P. Assessment of Sepsis-3 criteria and quick SOFA in patients with cirrhosis and bacterial infections. *Gut* 2018; **67**: 1892-1899 [PMID: 28860348 DOI: 10.1136/gutjnl-2017-314324]

61 **Augustinho FC**, Zocche TL, Borgonovo A, Maggi DC, Rateke ECM, Matiollo C, Dantas-Correa EB, Narciso-Schiavon JL, Schiavon LL. Applicability of Sepsis-3 criteria and quick Sequential Organ Failure Assessment in patients with cirrhosis hospitalised for bacterial infections. *Liver Int* 2019; **39**: 307-315 [PMID: 30276961 DOI: 10.1111/liv.13980]

62 **Lan P**, Wang SJ, Shi QC, Fu Y, Xu QY, Chen T, Yu YX, Pan KH, Lin L, Zhou JC, Yu YS. Comparison of the predictive value of scoring systems on the prognosis of cirrhotic patients with suspected infection. *Medicine (Baltimore)* 2018; **97**: e11421 [PMID: 29995791 DOI: 10.1097/MD.0000000000011421]

63 **Son J**, Choi S, Huh JW, Lim CM, Koh Y, Kim KM, Shim JH, Lim YS, Hong SB. The quick sepsis-related organ failure score has limited value for predicting adverse outcomes in sepsis patients with liver cirrhosis. *Korean J Intern Med* 2019 [PMID: 31645093 DOI: 10.3904/kjim.2018.229]

64 **Pan HC**, Jenq CC, Tsai MH, Fan PC, Chang CH, Chang MY, Tian YC, Hung CC, Fang JT, Yang CW, Chen YC. Risk models and scoring systems for predicting the prognosis in critically ill cirrhotic patients with acute kidney injury: a prospective validation study. *PLoS One* 2012; **7**: e51094 [PMID: 23236437 DOI: 10.1371/journal.pone.0051094]

65 **Sinha M**, Jupe J, Mack H, Coleman TP, Lawrence SM, Fraley SI. Emerging Technologies for Molecular Diagnosis of Sepsis. *Clin Microbiol Rev* 2018; **31**: e00089-17 [PMID: 29490932 DOI: 10.1128/CMR.00089-17]

66 **Fernández J**, Aracil C, Solà E, Soriano G, Cinta Cardona M, Coll S, Genescà J, Hombrados M, Morillas R, Martín-Llahí M, Pardo A, Sánchez J, Vargas V, Xiol X, Ginès P. [Evaluation and treatment of the critically ill cirrhotic patient]. *Gastroenterol Hepatol* 2016; **39**: 607-626 [PMID: 26778768 DOI: 10.1016/j.gastrohep.2015.09.019]

67 **Thompson K**, Venkatesh B, Finfer S. Sepsis and septic shock: current approaches to management. *Intern Med J* 2019; **49**: 160-170 [PMID: 30754087 DOI: 10.1111/imj.14199]

68 **Philips CA**, Choudhury AK, Sahney A, Maiwall R, Mitra LG, Sarin SK. Comparison and outcomes of 5% albumin vs 0.9% normal saline fluid resuscitation in cirrhotics presenting with sepsis induced hypotension - a randomized controlled trial - fluid resuscitation in septic shock in cirrhosis (FRISC Protocol). *Hepatology* 2015; **62**: 261A [DOI: 10.1002/hep.28183]

69 **Choudhury A**, Kedarisetty CK, Vashishtha C, Saini D, Kumar S, Maiwall R, Sharma MK, Bhadoria AS, Kumar G, Joshi YK, Sarin SK. A randomized trial comparing terlipressin and noradrenaline in patients with cirrhosis and septic shock. *Liver Int* 2017; **37**: 552-561 [PMID: 27633962 DOI: 10.1111/liv.13252]

70 **Rochwerg B**, Oczkowski SJ, Siemieniuk RAC, Agoritsas T, Belley-Cote E, D'Aragon F, Duan E, English S, Gossack-Keenan K, Alghuroba M, Szczeklik W, Menon K, Alhazzani W, Sevransky J, Vandvik PO, Annane D, Guyatt G. Corticosteroids in Sepsis: An Updated Systematic Review and Meta-Analysis. *Crit Care Med* 2018; **46**: 1411-1420 [PMID: 29979221 DOI: 10.1097/CCM.0000000000003262]

71 **Fang F**, Zhang Y, Tang J, Lunsford LD, Li T, Tang R, He J, Xu P, Faramand A, Xu J, You C. Association of Corticosteroid Treatment With Outcomes in Adult Patients With Sepsis: A Systematic Review and Meta-analysis. *JAMA Intern Med* 2019; **179**: 213-223 [PMID: 30575845 DOI: 10.1001/jamainternmed.2018.5849]

72 **Arabi YM**, Aljumah A, Dabbagh O, Tamim HM, Rishu AH, Al-Abdulkareem A, Knawy BA, Hajeer AH, Tamimi W, Cherfan A. Low-dose hydrocortisone in patients with cirrhosis and septic shock: a randomized controlled trial. *CMAJ* 2010; **182**: 1971-1977 [PMID: 21059778 DOI: 10.1503/cmaj.090707]

73 **Philips CA**, Sarin SK. Sepsis in cirrhosis: emerging concepts in pathogenesis, diagnosis and management. *Hepatol Int* 2016; **10**: 871-882 [PMID: 27422251 DOI: 10.1007/s12072-016-9753-2]

74 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

75 **Simonetto DA**, Piccolo Serafim L, Gallo de Moraes A, Gajic O, Kamath PS. Management of Sepsis in Patients With Cirrhosis: Current Evidence and Practical Approach. *Hepatology* 2019; **70**: 418-428 [PMID: 30516866 DOI: 10.1002/hep.30412]

76 **Ahamed R**, Philips CA, Rajesh S, George T, Padsalgi G, Kumbar S, Augustine P. Methylene blue in critically ill cirrhosis patients with sepsis induced severe hypotension. *J Gastroenterol Hepatol* 2019; **34**: S3 [DOI: 10.1111/jgh.14879]

77 **Walley KR**, Thain KR, Russell JA, Reilly MP, Meyer NJ, Ferguson JF, Christie JD, Nakada TA, Fjell CD, Thair SA, Cirstea MS, Boyd JH. PCSK9 is a critical regulator of the innate immune response and septic shock outcome. *Sci Transl Med* 2014; **6**: 258ra143 [PMID: 25320235 DOI: 10.1126/scitranslmed.3008782]

78 **Rello J**, Valenzuela-Sánchez F, Ruiz-Rodriguez M, Moyano S. Sepsis: A Review of Advances in Management. *Adv Ther* 2017; **34**: 2393-2411 [PMID: 29022217 DOI: 10.1007/s12325-017-0622-8]

79 **Yuk SA**, Sanchez-Rodriguez DA, Tsifansky MD, Yeo Y. Recent advances in nanomedicine for sepsis treatment. *Ther Deliv* 2018; **9**: 435-450 [PMID: 29722636 DOI: 10.4155/tde-2018-0009]

80 **Philips CA**, Ahamed R, Rajesh S, Augustine P. 'You know my name, but not my story' - Deciding on an accurate nomenclature for faecal microbiota transplantation. *J Hepatol* 2020; **72**: 1212-1213 [PMID: 32197801 DOI: 10.1016/j.jhep.2020.02.004]

81 **Haak BW**, Wiersinga WJ. The role of the gut microbiota in sepsis. *Lancet Gastroenterol Hepatol* 2017; **2**: 135-143 [PMID: 28403983 DOI: 10.1016/S2468-1253(16)30119-4]

82 **Haak BW**, Prescott HC, Wiersinga WJ. Therapeutic Potential of the Gut Microbiota in the Prevention and Treatment of Sepsis. *Front Immunol* 2018; **9**: 2042 [PMID: 30250472 DOI: 10.3389/fimmu.2018.02042]

83 **Bajaj JS**. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 235-246 [PMID: 30643227 DOI: 10.1038/s41575-018-0099-1]

84 **Philips CA**, Augustine P, Yerol PK, Ramesh GN, Ahamed R, Rajesh S, George T, Kumbar S. Modulating the Intestinal Microbiota: Therapeutic Opportunities in Liver Disease. *J Clin Transl Hepatol* 2020; **8**: 87-99 [PMID: 32274349 DOI: 10.14218/JCTH.2019.00035]

85 **Philips CA**, Phadke N, Ganesan K, Ranade S, Augustine P. Corticosteroids, nutrition, pentoxifylline, or fecal microbiota transplantation for severe alcoholic hepatitis. *Indian J Gastroenterol* 2018; **37**: 215-225 [PMID: 29931479 DOI: 10.1007/s12664-018-0859-4]

86 **Philips CA**, Augustine P, Padsalgi G, Ahamed R, Jose A, Rajesh S. Only in the darkness can you see the stars: Severe alcoholic hepatitis and higher grades of acute-on-chronic liver failure. *J Hepatol* 2019; **70**: 550-551 [PMID: 30470480 DOI: 10.1016/j.jhep.2018.10.004]

87 **Stadlbauer V**, Horvath A, Komarova I, Schmerboeck B, Feldbacher N, Klymiuk I, Durdevic M, Rainer F, Blesl A, Stiegler P, Leber B. Dysbiosis in early sepsis can be modulated by a multispecies probiotic: a randomised controlled pilot trial. *Benef Microbes* 2019; **10**: 265-278 [PMID: 30694100 DOI: 10.3920/BM2018.0067]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 475-484

DOI: 10.4254/wjh.v12.i8.475

ISSN 1948-5182 (online)

MINIREVIEWS

Cell competition in liver carcinogenesis

Fabio Marongiu, Ezio Laconi

ORCID number: Fabio Marongiu 0000-0001-5860-4227; Ezio Laconi 0000-0001-9580-8333.

Author contributions: Marongiu F contributed to the planning and the writing of the MS and prepared the figures; Laconi E conceived the manuscript and contributed to its planning and writing.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Manuscript source: Invited manuscript

Received: March 3, 2020
Peer-review started: March 3, 2020
First decision: April 2, 2020

Fabio Marongiu, Ezio Laconi, Department of Biomedical Sciences, Unit of Experimental Medicine, University of Cagliari, Cagliari 09124, Italy

Corresponding author: Ezio Laconi, MD, PhD, Academic Fellow, Associate Professor, Department of Biomedical Sciences, Unit of Experimental Medicine, University of Cagliari, Via Jenner, Cagliari 09124, Italy. elaconi@unica.it

Abstract

Cell competition is now a well-established quality control strategy to optimize cell and tissue fitness in multicellular organisms. While pursuing this goal, it is also effective in selecting against altered/defective cells with putative (pre)-neoplastic potential, thereby edging the risk of cancer development. The flip side of the coin is that the molecular machinery driving cell competition can also be co-opted by neoplastic cell populations to expand unchecked, outside the boundaries of tissue homeostatic control. This review will focus on information that begins to emerge regarding the role of cell competition in liver physiology and pathology. Liver repopulation by normal transplanted hepatocytes is an interesting field of investigation in this regard. The biological coordinates of this process share many features suggesting that cell competition is a driving force for the clearance of endogenous damaged hepatocytes by normal donor-derived cells, as previously proposed. Intriguing analogies between liver repopulation and carcinogenesis will be briefly discussed and the potential dual role of cell competition, as a barrier or a spur to neoplastic development, will be considered. Cell competition is in essence a cooperative strategy organized at tissue level. One facet of such cooperative attitude is expressed in the elimination of altered cells which may represent a threat to the organismal community. On the other hand, the society of cells can be disrupted by the emergence of selfish clones, exploiting the molecular bar codes of cell competition, thereby paving their way to uncontrolled growth.

Key words: Cell competition; Liver carcinogenesis; Liver repopulation; Aging; Tissue homeostasis; Clonal expansion

Core tip: Cell competition stands as an eminently cooperative strategy which operates in coordination with mechanisms overlooking tissue mass and tissue architecture. One facet of such cooperative attitude is also expressed in the elimination of altered, putative (pre)-neoplastic cells which may potentially pose a threat to the organismal community. On the

Revised: June 22, 2020
Accepted: July 26, 2020
Article in press: July 26, 2020
Published online: August 27, 2020

P-Reviewer: Hashimoto M, Li W
S-Editor: Zhang L
L-Editor: A
P-Editor: Li JH

other hand, cell populations on the path towards neoplasia may cheat the society of cells from which they originate using the molecular bar codes of cell competition, thereby paving their way to uncontrolled growth, invasiveness and metastatic capacity.

Citation: Marongiu F, Laconi E. Cell competition in liver carcinogenesis. *World J Hepatol* 2020; 12(8): 475-484
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/475.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.475

ORGANISMAL COMMUNITIES

Cell communities in multicellular organisms are shaped by a fundamental organizing principle, instructing the relative sizes and reciprocal relationships to be enacted and maintained over time among different cell types. Such a seemingly simple fact represents the very essence of individual multicellular communities and mechanisms overlooking their correct implementation are central during development and throughout life[1-3]. For example, when part of liver tissue is lost (due to surgery or any injury), residual hepatocytes are immediately alerted and awakened from their quiescent state to enter cell cycle, divide and replenish the missing parenchyma. Conversely, following liver hyperplasia a wave of hepatocyte deletion ensues upon withdrawal of the inciting stimulus, until the original tissue mass is reinstated.

While the capacity to maintain stable tissue mass is remarkable, there is still more to it. In fact, another layer of complexity has been added to the above mechanism following the realization that quality control strategies are also at play to optimize cell fitness in tissue composition. Thus, not only the number of cells in a given tissue is constantly under control, but their functional efficiency is also monitored and actively selected for in order to limit any time-dependent decline. One such strategy is cell competition, consisting in the confrontation of homotypic cells with varying levels of fitness and resulting in the elimination of the weaker (losers) carried out by the stronger counterparts (winners). While primarily aimed at maintaining optimal functional proficiency in cell populations of normal tissues, it has been suggested that mechanisms driving cell competition can also be hijacked and exploited by pre-neoplastic and/or neoplastic cells to manifest their aggressive and dominant phenotype[4]. The aim of this review is to discuss the possible role of cell competition during hepato-carcinogenesis.

WHAT IS CELL COMPETITION

In its simplest definition, cell competition refers to a process whereby cells in a given tissue, which would be otherwise viable and functional, are instead outcompeted and cleared by the presence of a functionally more proficient population. The first report describing a similar scenario dates back to the mid the 1970s and refers to the elimination of *Minute*-mutant cells when confronted with wild type counterparts in the imaginal disk of *Drosophila melanogaster*[5]. The *Minute* mutation affects ribosomal protein genes and translates into a slower growth rate of heterozygous mutant cells. Several other mutations were subsequently identified to induce a loser phenotype in presence of wild type cells, including those involving basic cellular functions such as tissue patterning, protein translation and cell signaling[6].

In an attempt to outline the boundaries of cell competition, a series of biological features have been proposed to be associated with this phenomenon[7]. Firstly, cell competition is context-dependent, *i.e.*, the fate of each cell type, the winner and the loser, results from their reciprocal interaction, be it direct or indirect; this is arguably the single most relevant attribute of cell competition, as well as the most complex to enact. Secondly, the winner cell proliferates following induction of cell death (apoptosis) of the loser cell, *i.e.*, the two opposite events are temporally and mechanistically coordinated. As a corollary, biological forces driving cell competition tend to maintain appropriate tissue size and pattern. Furthermore, interactions conducive to cell competition occur within a relatively short range, being strongest at the interface between winner and loser cells. Last but not least, cell competition is restricted within defined developmental compartments[1], *i.e.*, it occurs within discrete

tissue boundaries that cannot be overridden by winner cells.

Within this definition, mechanism(s) governing cell competition are intertwined, at least in part, with those overlooking the fine balance between cell gain and cell loss, which in turn determine tissue size in any organ and organism[1,2]. Since alterations of the latter mechanisms represent a hallmark of neoplastic disease, it is all the more reasonable to propose that cancer cell populations may coopt strategies involving cell competition in order to selectively emerge *vis* a *vis* the surrounding counterparts[8,9].

COMPETITION FOR WHAT

Cell competition can only occur when a critical degree of phenotypic heterogeneity is present within a homotypic cell population; in addition, a limit must exist in the availability of whatever resource these cells are competing for. Molecular analysis carried out at the resolution of single cells has revealed that cell heterogeneity at genetic and epigenetic levels is indeed far more pervasive than previously thought even in normal adult tissues, raising the possibility that cell competition may not be a rare phenomenon[6]. Cells can compete for nutrients, growth factors and ultimately space, given the size constraints imposed on any tissue by homeostatic control mechanisms[3].

A paradigmatic example in which the principle of cell competition is at play is the process of antibody affinity maturation in lymphoid germinal centers[8,9]. Heterogeneity is generated through somatic hypermutation in the gene coding for the B-cell receptor. The limited resource is represented by antigen availability: The lower the antigen concentration, the higher the affinity of the resulting antibodies. Lymphocytes are in fact positively selected through the binding of their mutated receptor to antigen, which in turn is dictated by the degree of affinity of the former to the latter. Lymphocytes that are unable to reach for antigen trough their receptor die by apoptosis. Thus, the competitive fitness of B lymphocyte clones rests on their ability to bind a rescuing or "trophic" factor, which is epitomized, in this case, by the incoming antigen.

The above sequence of events is similar, in essence, to the one described in the *Minute*-mutant of *Drosophila* wing imaginal disk referred to above, which is considered as a classical model of cell competition. It was in fact proposed that in this system, slow-growing *Minute*-mutant cells have a disadvantage in competing for a survival signal, and this leads to increased expression of a pro-apoptotic cascade and final clearance of mutants by wild type cells[10]. Parenthetically, it is important to point out that the latter interpretation has been questioned by subsequent studies suggesting that differences in growth rate *per se* between mutant and wild type cells are sufficient to account for their unbalanced contribution to wing development, while cell competition *per se* would not appear to play a major role in the process[5]. These findings also indicate that a slower growth rate does not necessarily entail a loser phenotype compared to faster homotypic counterparts, *i.e.*, for cell competition to be enforced other critical differences must be present.

WHAT IS CELL FITNESS

The latter consideration brings us to the core issues pertaining cell competition: What parameters are measured between winner and loser cells to assess relative fitness and how is this accomplished? We must acknowledge that only scattered information is available so far to answer these questions. Given that evolutionary processes (including cell competition) have selected over time for cells with better and better functional proficiency, it follows that normal tissues are populated with cells with near-optimal performing capacity. This in turn implies that any damage to any cell will likely result in a decreased fitness, laying the basis for its clearance by neighboring normal cells through cell competition[11-13]. Thus, any damage above a given threshold can potentially trigger cell deletion. However, this is not a cell-autonomous process, as postulated for the classical p53-dependend apoptosis elicited in response to DNA damage[14]. Rather, it relies on the presence of surrounding cells and results from the confrontation of different levels of cellular fitness, within the biological boundaries of cell competition[15,16]. There is obviously a fundamental difference between these two strategies leading to clearance of damaged cells. While cell-autonomous mechanisms operate at single-cell level and do not take into account overall tissue function, deletion of damaged cells via cell competition is only triggered when fitter cells are

available and can possibly replace the ones that are lost. The latter consideration supports the contention that cell competition is an integral part of regulatory networks overlooking tissue maintenance and homeostasis[17,18]. Specific strategies to pursue this goal can vary even in the same tissue during different developmental phases. In mouse skin, the early embryonic epithelium is single-layered and loser cell disposal is carried out through their direct phagocytosis by surrounding winner cells; however, as the epidermis becomes stratified, loser cells are extruded from the basal layer along the differentiation conveyor and are eventually shed out[19].

Sensing relative cellular fitness is therefore an essential step in the process of cell competition[6]. One of the parameters that has emerged as relevant in this regard is the expression of Myc protein[20-23]. Higher cellular levels of this transcription factor confer a winner phenotype both during *Drosophila* development[20], in early mammalian embryo[21,24-26] and in adult, post-mitotic tissues such heart[27]. Furthermore, overexpression of Myc is associated with a super-competitor phenotype, which is able to outcompete wild type cells[20]. Similar to Myc, other genes important for cell anabolism have been implicated as triggers of cell competition, including those involved in the Hippo, Wnt/Wingless, Ras/mitogen-activated protein kinases and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways, among others[21]. Conversely, defects in genes implicated in the determination of cell polarity and tissue patterning impose a loser phenotype on the affected cells in presence of wild type counterparts[28,29].

So far, the best characterized direct sensor of cell fitness is the Flower system[30,31]. It consists of three splice isoform proteins, Flower^{ubi}, Flower^{LoseA} and Flower^{LoseB} and only the former is expressed under steady state conditions. However, when cell fitness decreases, expression of Flower^{ubi} is down-regulated, while levels of both Flower^{LoseA} and Flower^{LoseB} increase, generating what has been referred to as the "flower code", which targets cells for survival (Flower^{ubi}) or apoptosis (Flower^{LoseA} and Flower^{LoseB})[23,24]. Human Flower isoforms have recently been reported and evidence was presented that a similar winner/loser code is also operative in human cells[32].

CELL COMPETITION IN THE LIVER

Virtually no information is available on the possible role of cell competition in the liver during normal development and throughout post-natal life. However, a few studies, mostly using cell transplantation systems, have indicated that hepatic tissue appears to be susceptible to undergo this process. About 20 years ago, we proposed that a mechanism consistent with cell competition was possibly involved in a newly developed model of massive liver repopulation[33,34]. In this experimental system, animals (rats) are treated with retrorsine, a naturally occurring pyrrolizidine alkaloid that causes persistent DNA damage associated with a chronic mitotic block in targeted hepatocytes. It is noteworthy that damaged hepatocytes are able to sustain normal liver function and in fact retrorsine treated animals survive for up to 2 years[35]. However, the most intriguing finding was observed following cell transplantation. When normal syngeneic hepatocytes were orthotopically delivered after treatment with the alkaloid, they are able to massively replace endogenous parenchymal cells, with greater that 90% repopulation at the end of the process[33]. It was suggested that "the presence of normal transplanted cells may trigger selective deletion of RS-damaged resident hepatocytes, possibly through apoptosis"[33], a process that is fully consistent with cell competition (Figure 1). A few years later, a study by Oertel *et al*[36] proposed cell competition as the basis for the selective expansion of transplanted normal embryonic hepatic cells in the liver of syngeneic adult rats[36], similar to results obtained following transplantation of young adult hepatocytes in the liver of aged recipients[37].

While common molecular pathways involved in cell competition were not investigated under the above experimental conditions, biological coordinates of the described phenomena support the hypothesis that liver repopulation by transplanted hepatocytes is, at least in part, the outcome of a differential fitness comparison between resident and donor-derived cells. Accordingly, a cell-autonomous decrease in proliferative competitiveness was reported in aged *vs* young hepatocytes upon transplantation in the same microenvironment *in vivo*[38].

If cell transplantation is not performed, a slow process of repopulation sustained by endogenous hepatocytes occurs in rat liver exposed to RS, giving rise to regenerative nodules[39,40]. A similar scenario has also been reported in the liver of patients affected by type 1 tyrosinemia[41], the human counterpart of a well characterized mouse model

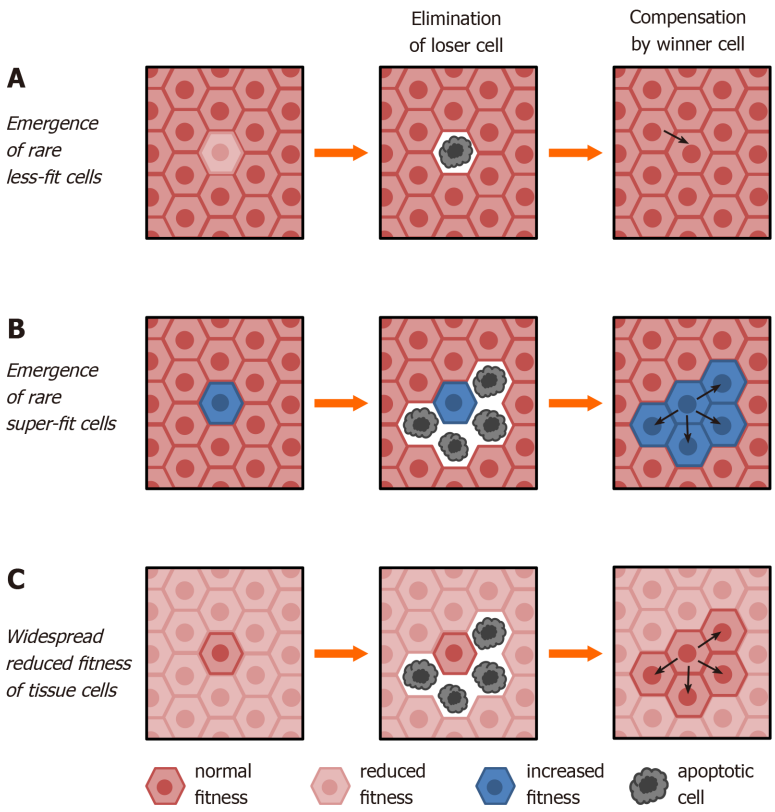

Figure 1 Modes of canonical cell competition. A: Wild type cells (dark brown) are endowed with higher fitness (winner phenotype) compared to rare altered cells (loser phenotype

of liver repopulation[42]. Whether cell competition is driving and/or contributing to either or both processes is an intriguing possibility that remains to be explored.

CELL COMPETITION IN THE PATHOGENESIS OF CANCER

We are now back to the central question of this review: Is there a role for cell completion in cancer and, more specifically, in liver carcinogenesis, From a molecular standpoint, numerous pathways that have been proposed to have a role in cell competition (summarized above) are also implicated in the pathogenesis of neoplastic disease. It would appear therefore quite reasonable to expect that alterations in these pathways might cause a defective control in the mechanisms of cell competition and, almost inevitably, a parallel increase in the risk of cancer. As an example, the Myc protein is often overexpressed in human cancer[43] and is also a known driver of cell competition[7]. Accordingly, cells with up-regulated Myc can express a super-competitor phenotype[23] and this could contribute, at least theoretically, towards a neoplastic behavior[22].

However, the potential role of cell competition in the multistep process of neoplastic development is far from being confined to situations of altered molecular control. In fact, the emerging picture is that biological mechanisms underlying cell competition under normal conditions are directly relevant to the pathogenesis of cancer from early stages to advanced disease[4]. A compelling case is the extrusion of altered, potentially pre-neoplastic cells from epithelia orchestrated by normal surrounding counterparts and referred to as epithelial defense against cancer[12]. For example, normal cells were able to induce Warburg-like metabolic changes in RasV12-transformed cells, leading to their removal from mouse intestinal epithelium[11]. Similarly, cell competition by normal cells is able to eliminate scrib mutant cells from Drosophila imaginal disk, while in the absence of wild type counterparts mutant clones do not die and progress to form tumors[29].

As one can predict, this protective strategy is not foolproof and, more to the point, it can be altered in its efficiency by environmental influences. A recent study indicated that removal of RasV12-transformed cells from mouse intestine was decreased

following feeding a high fat diet (HFD), due to altered lipid metabolism and HFD-induced inflammatory changes; treatment with aspirin was able to mitigate HFD negative effects on transformed cell clearance[44]. Similarly, hematopoietic stem and progenitor cells expressing a mutant p53 displayed a growth advantage *vs* wild type counterparts when transplanted in mice following exposure to mild irradiation, while no such advantage was evident upon transplantation of the same cell populations in untreated recipients[15]. Furthermore, we have proposed that aging is associated with a generalized decrease in the efficiency of mechanisms overlooking maintenance of cell fitness, possibly including cell competition[17,45,46]. In addition, specific genetic alterations might be positively selected, as opposed to eliminated, under environmental conditions favoring their phenotype. This could partly account for the pervasive presence of aberrant clonal expansions in aged human tissues[47] and/or in association with disease states such as ulcerative colitis[48,49]. It was shown that organoids derived from ulcerative colitis patients are populated by genetically altered cell clones that are adapted to an inflammatory microenvironment, *i.e.*, they are fitter to that environment compared with wild type intestinal cells[48,49]. Whether in these instances cell competition is at play is an important question that remains to be addressed. Relevant to this issue, a mechanistic association was reported between alterations in intestinal barrier integrity, aging, dietary regimen and the efficiency of cell competition[46].

Direct evidence that cell competition is indeed mechanistically exploited by cancer cells during growth and metastatic spread was recently presented[32,50]. Human cancer cells were shown to express the Flower code of a winner cell phenotype and inhibition of the latter resulted in reduced tumor growth and increased response to chemotherapy[32].

CELL COMPETITION IN LIVER CARCINOGENESIS

Given the limited amount of information available regarding the role of cell competition in liver under normal conditions, it is not surprising that a similar consideration also applies to the process of liver cancer development. Several years ago we have pointed out the existence of intriguing analogies between the process of liver repopulation by normal hepatocytes and carcinogenesis[34]. For example, several of the available experimental models of massive liver repopulation are also prone to develop neoplastic disease, including the RS-based model developed by our research group and referred to above. Thus, pre-neoplastic hepatocytes grow and progress to cancer upon transplantation into retrorsine treated rat liver, while the same cells are unable to expand when delivered to normal untreated host[51]. As discussed above for liver repopulation by transplanted normal hepatocytes, the biological coordinates of this phenomenon suggest that cell competition might be involved (Figure 2)[27], albeit formal proof of this linkage is not available yet.

Along these lines, an important step forward was the report by Moya *et al*[45], who have studied the role of Hippo signaling pathway in the growth of primary liver tumors and liver metastases from melanoma cells in mice. It was found that the relative level of activation of Hippo pathway in normal surrounding *vs* tumor cells was critical in determining the growth rate of the latter; specifically, inhibition of this pathway in peritumoral cells increased proliferation in nodular lesions, while tumors regressed when Hippo activity was up-regulated in surrounding normal tissue. In addition, tumor survival in wild type mice was dependent on the presence of an active Hippo pathway in cancer cells, while the activity of the pathway was dispensable when tumors were growing in a Hippo-deficient liver background[52]. These findings were interpreted to suggest that Hippo pathway-driven cell competition is an important determinant in controlling the growth of (pre)-neoplastic cell populations in the liver. It is intriguing to note that Hippo pathway activity was also shown to be essential for the maintenance of the differentiated state in hepatocytes and its inhibition correlated with the appearance of a progenitor cell phenotype[53].

CONCLUSION

Cell competition has emerged as an important quality control mechanism overlooking tissue functional proficiency during development and in post-natal life. In essence, such mechanisms entails the elimination and replacement of a less fit (loser) cell population by a fitter cell type (winner). Evidence is also accruing that this process

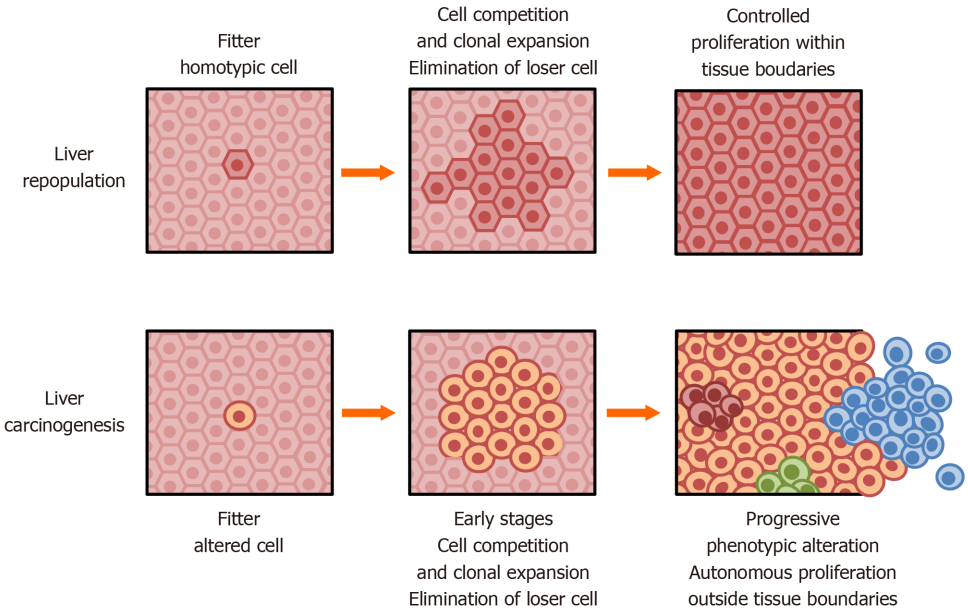

Figure 2 Liver repopulation and liver carcinogenesis. Top: In the context of persistent, widespread tissue damage, transplanted homotypic cells with normal fitness (dark brown) can outcompete resident damaged cells (light brown) and slowly repopulate nearly the entire tissue, without exceeding tissue boundaries. However, in the absence of normal transplanted hepatocytes, endogenous cells persist for at least several months; Bottom: In a similar context of persistent, widespread tissue damage, transplanted homotypic cells with normal fitness and with a pre-neoplastic phenotype (dark yellow) can outcompete resident damaged cells and form hepatic nodules that progress to cancer. The same pre-neoplastic cells do not grow following transplantation in a context of normal cell fitness in vivo (see text for details).

might be involved in the pathogenesis of neoplastic disease at different steps. On one side of the coin is the first and possibly firmest conclusion so far, *i.e.*, cell competition protects from the risk of cancer via identification and purging of single altered cells expressing a loser code, which flags a decreased fitness relative to surrounding counterparts. On the other hand, several studies suggest that the winner/loser code associated with cell competition can be exploited or hijacked by (pre)-cancerous cell populations to outcompete normal neighbors in the same tissue, thereby fueling their progression towards increasing malignancy. The prototypic example of such scenario is the super-competitor phenotype of Myc-overexpressing cells, which can be a winner phenotype over wild type cells. However, this apparently straightforward sequence of events appears difficult to reconcile with the defining biological features of cell competition, as it was aptly pointed out long ago[23]. A critical attribute of cell competition is in fact that it operates within discrete tissue boundaries that cannot be overridden by winner cells (see above). Thus, any cell type engaging in this process should stop expanding once the appropriate tissue compartment has been fully replenished, and this is also the case for the Myc super-competitor phenotype[1,21]. However, cancer cells do not obey these rules and their growth beyond set compartmental boundaries cannot be explained by cell competition *per se* (Figure 2). Stated otherwise, while canonical cell competition occurs within tissue homeostatic control mechanisms, the type of cell competition engaged by neoplastic cell populations is clearly outside such boundaries and is the expression of an eminently selfish phenotype[54,55].

A second feature of canonical cell competition which appears at odds with the phenotype of cancer cells is the type of cell interaction that is required to define relative fitness and hence the winner *vs* loser cell fate. According to the currently accepted paradigm, cell competition occurs among homotypic cells, in that it compares fitness levels within a cell population performing the same function in a tissue. By contrast, it is almost axiomatic that cancer cells have often departed significantly from the phenotype of the tissue of origin, including its functional proficiency. Therefore, a comparison of cell fitness between neoplastic cells and their normal tissue counterparts would likely favor survival of the latter, not the former.

Based on the above considerations, we propose that canonical cell competition is possibly involved in the initial stages of carcinogenesis leading, to discrete clonal expansions which are still within tissue homeostatic control[56]. However, the molecular machinery of cell competition may be co-opted by overtly neoplastic cell

populations endowed with additional phenotypic features to sustain their uncontrolled growth.

REFERENCES

1 **Leevers SJ**, McNeill H. Controlling the size of organs and organisms. *Curr Opin Cell Biol* 2005; **17**: 604-609 [PMID: 16226450 DOI: 10.1016/j.ceb.2005.09.008]
2 **Irvine KD**, Rauskolb C. Boundaries in development: formation and function. *Annu Rev Cell Dev Biol* 2001; **17**: 189-214 [PMID: 11687488 DOI: 10.1146/annurev.cellbio.17.1.189]
3 **Martín FA**, Herrera SC, Morata G. Cell competition, growth and size control in the Drosophila wing imaginal disc. *Development* 2009; **136**: 3747-3756 [PMID: 19855017 DOI: 10.1242/dev.038406]
4 **Vishwakarma M**, Piddini E. Outcompeting cancer. *Nat Rev Cancer* 2020; **20**: 187-198 [PMID: 31932757 DOI: 10.1038/s41568-019-0231-8]
5 **Morata G**, Ripoll P. Minutes: mutants of drosophila autonomously affecting cell division rate. *Dev Biol* 1975; **42**: 211-221 [PMID: 1116643 DOI: 10.1016/0012-1606(75)90330-9]
6 **Bowling S**, Lawlor K, Rodríguez TA. Cell competition: the winners and losers of fitness selection. *Development* 2019; **146** [PMID: 31278123 DOI: 10.1242/dev.167486]
7 **Johnston LA**. Socializing with MYC: cell competition in development and as a model for premalignant cancer. *Cold Spring Harb Perspect Med* 2014; **4**: a014274 [PMID: 24692189 DOI: 10.1101/cshperspect.a014274]
8 **Abbott RK**, Lee JH, Menis S, Skog P, Rossi M, Ota T, Kulp DW, Bhullar D, Kalyuzhniy O, Havenar-Daughton C, Schief WR, Nemazee D, Crotty S. Precursor Frequency and Affinity Determine B Cell Competitive Fitness in Germinal Centers, Tested with Germline-Targeting HIV Vaccine Immunogens. *Immunity* 2018; **48**: 133-146.e6 [PMID: 29287996 DOI: 10.1016/j.immuni.2017.11.023]
9 **Freitas AA**, Rosado MM, Viale AC, Grandien A. The role of cellular competition in B cell survival and selection of B cell repertoires. *Eur J Immunol* 1995; **25**: 1729-1738 [PMID: 7615000 DOI: 10.1002/eji.1830250636]
10 **Moreno E**, Basler K, Morata G. Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. *Nature* 2002; **416**: 755-759 [PMID: 11961558 DOI: 10.1038/416755a]
11 **Kon S**, Ishibashi K, Katoh H, Kitamoto S, Shirai T, Tanaka S, Kajita M, Ishikawa S, Yamauchi H, Yako Y, Kamasaki T, Matsumoto T, Watanabe H, Egami R, Sasaki A, Nishikawa A, Kameda I, Maruyama T, Narumi R, Morita T, Sasaki Y, Enoki R, Honma S, Imamura H, Oshima M, Soga T, Miyazaki JI, Duchen MR, Nam JM, Onodera Y, Yoshioka S, Kikuta J, Ishii M, Imajo M, Nishida E, Fujioka Y, Ohba Y, Sato T, Fujita Y. Cell competition with normal epithelial cells promotes apical extrusion of transformed cells through metabolic changes. *Nat Cell Biol* 2017; **19**: 530-541 [PMID: 28414314 DOI: 10.1038/ncb3509]
12 **Tanimura N**, Fujita Y. Epithelial defense against cancer (EDAC). *Semin Cancer Biol* 2020; **63**: 44-48 [PMID: 31302236 DOI: 10.1016/j.semcancer.2019.05.011]
13 **Maruyama T**, Fujita Y. Cell competition in mammals - novel homeostatic machinery for embryonic development and cancer prevention. *Curr Opin Cell Biol* 2017; **48**: 106-112 [PMID: 28719866 DOI: 10.1016/j.ceb.2017.06.007]
14 **Symonds H**, Krall L, Remington L, Saenz-Robles M, Lowe S, Jacks T, Van Dyke T. p53-dependent apoptosis suppresses tumor growth and progression in vivo. *Cell* 1994; **78**: 703-711 [PMID: 8069917 DOI: 10.1016/0092-8674(94)90534-7]
15 **Bondar T**, Medzhitov R. p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* 2010; **6**: 309-322 [PMID: 20362536 DOI: 10.1016/j.stem.2010.03.002]
16 **Otsuka K**, Suzuki K, Fujimichi Y, Tomita M, Iwasaki T. Cellular responses and gene expression profiles of colonic Lgr5+ stem cells after low-dose/low-dose-rate radiation exposure. *J Radiat Res* 2018; **59**: ii18-ii22 [PMID: 29281035 DOI: 10.1093/jrr/rrx078]
17 **Liu N**, Matsumura H, Kato T, Ichinose S, Takada A, Namiki T, Asakawa K, Morinaga H, Mohri Y, De Arcangelis A, Geroges-Labouesse E, Nanba D, Nishimura EK. Stem cell competition orchestrates skin homeostasis and ageing. *Nature* 2019; **568**: 344-350 [PMID: 30944469 DOI: 10.1038/s41586-019-1085-7]
18 **Ohsawa S**, Vaughen J, Igaki T. Cell Extrusion: A Stress-Responsive Force for Good or Evil in Epithelial Homeostasis. *Dev Cell* 2018; **44**: 284-296 [PMID: 29408235 DOI: 10.1016/j.devcel.2018.01.009]
19 **Ellis SJ**, Gomez NC, Levorse J, Mertz AF, Ge Y, Fuchs E. Distinct modes of cell competition shape mammalian tissue morphogenesis. *Nature* 2019; **569**: 497-502 [PMID: 31092920 DOI: 10.1038/s41586-019-1199-y]
20 **de la Cova C**, Abril M, Bellosta P, Gallant P, Johnston LA. Drosophila myc regulates organ size by inducing cell competition. *Cell* 2004; **117**: 107-116 [PMID: 15066286 DOI: 10.1016/s0092-8674(04)00214-4]
21 **Clavería C**, Giovinazzo G, Sierra R, Torres M. Myc-driven endogenous cell competition in the early mammalian embryo. *Nature* 2013; **500**: 39-44 [PMID: 23842495 DOI: 10.1038/nature12389]
22 **Di Giacomo S**, Sollazzo M, de Biase D, Ragazzi M, Bellosta P, Pession A, Grifoni D. Human Cancer Cells Signal Their Competitive Fitness Through MYC Activity. *Sci Rep* 2017; **7**: 12568 [PMID: 28974715 DOI: 10.1038/s41598-017-13002-1]
23 **Moreno E**, Basler K. dMyc transforms cells into super-competitors. *Cell* 2004; **117**: 117-129 [PMID: 15066287 DOI: 10.1016/s0092-8674(04)00262-4]
24 **Sancho M**, Di-Gregorio A, George N, Pozzi S, Sánchez JM, Pernaute B, Rodríguez TA. Competitive interactions eliminate unfit embryonic stem cells at the onset of differentiation. *Dev Cell* 2013; **26**: 19-30 [PMID: 23867226 DOI: 10.1016/j.devcel.2013.06.012]
25 **Díaz-Díaz C**, Fernandez de Manuel L, Jimenez-Carretero D, Montoya MC, Clavería C, Torres M. Pluripotency Surveillance by Myc-Driven Competitive Elimination of Differentiating Cells. *Dev Cell* 2017; **42**: 585-599.e4 [PMID: 28919206 DOI: 10.1016/j.devcel.2017.08.011]
26 **Hashimoto M**, Sasaki H. Epiblast Formation by TEAD-YAP-Dependent Expression of Pluripotency Factors

and Competitive Elimination of Unspecified Cells. *Dev Cell* 2019; **50**: 139-154.e5 [PMID: 31204175 DOI: 10.1016/j.devcel.2019.05.024]

27 **Villa Del Campo C**, Clavería C, Sierra R, Torres M. Cell competition promotes phenotypically silent cardiomyocyte replacement in the mammalian heart. *Cell Rep* 2014; **8**: 1741-1751 [PMID: 25199831 DOI: 10.1016/j.celrep.2014.08.005]

28 **Coelho DS**, Moreno E. Emerging links between cell competition and Alzheimer's disease. *J Cell Sci* 2019; **132** [PMID: 31263078 DOI: 10.1242/jcs.231258]

29 **Norman M**, Wisniewska KA, Lawrenson K, Garcia-Miranda P, Tada M, Kajita M, Mano H, Ishikawa S, Ikegawa M, Shimada T, Fujita Y. Loss of Scribble causes cell competition in mammalian cells. *J Cell Sci* 2012; **125**: 59-66 [PMID: 22250205 DOI: 10.1242/jcs.085803]

30 **Rhiner C**, López-Gay JM, Soldini D, Casas-Tinto S, Martín FA, Lombardía L, Moreno E. Flower forms an extracellular code that reveals the fitness of a cell to its neighbors in Drosophila. *Dev Cell* 2010; **18**: 985-998 [PMID: 20627080 DOI: 10.1016/j.devcel.2010.05.010]

31 **Swami M**. Development: A code to distinguish winners and losers. *Nat Rev Genet* 2010; **11**: 530-531 [PMID: 20585333 DOI: 10.1038/nrg2834]

32 **Madan E**, Pelham CJ, Nagane M, Parker TM, Canas-Marques R, Fazio K, Shaik K, Yuan Y, Henriques V, Galzerano A, Yamashita T, Pinto MAF, Palma AM, Camacho D, Vieira A, Soldini D, Nakshatri H, Post SR, Rhiner C, Yamashita H, Accardi D, Hansen LA, Carvalho C, Beltran AL, Kuppusamy P, Gogna R, Moreno E. Flower isoforms promote competitive growth in cancer. *Nature* 2019; **572**: 260-264 [PMID: 31341286 DOI: 10.1038/s41586-019-1429-3]

33 **Laconi S**, Pillai S, Porcu PP, Shafritz DA, Pani P, Laconi E. Massive liver replacement by transplanted hepatocytes in the absence of exogenous growth stimuli in rats treated with retrorsine. *Am J Pathol* 2001; **158**: 771-777 [PMID: 11159214 DOI: 10.1016/s0002-9440(10)64019-9]

34 **Marongiu F**, Doratiotto S, Montisci S, Pani P, Laconi E. Liver repopulation and carcinogenesis: two sides of the same coin? *Am J Pathol* 2008; **172**: 857-864 [PMID: 18321999 DOI: 10.2353/ajpath.2008.070910]

35 **Laconi S**, Montisci S, Doratiotto S, Greco M, Pasciu D, Pillai S, Pani P, Laconi E. Liver repopulation by transplanted hepatocytes and risk of hepatocellular carcinoma. *Transplantation* 2006; **82**: 1319-1323 [PMID: 17130781 DOI: 10.1097/01.tp.0000228239.78290.13]

36 **Oertel M**, Menthena A, Dabeva MD, Shafritz DA. Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. *Gastroenterology* 2006; **130**: 507-20; quiz 590 [PMID: 16472603 DOI: 10.1053/j.gastro.2005.10.049]

37 **Pasciu D**, Montisci S, Greco M, Doratiotto S, Pitzalis S, Pani P, Laconi S, Laconi E. Aging is associated with increased clonogenic potential in rat liver in vivo. *Aging Cell* 2006; **5**: 373-377 [PMID: 16911563 DOI: 10.1111/j.1474-9726.2006.00230.x]

38 **Serra MP**, Marongiu F, Marongiu M, Contini A, Laconi E. Cell-autonomous decrease in proliferative competitiveness of the aged hepatocyte. *J Hepatol* 2015; **62**: 1341-1348 [PMID: 25617502 DOI: 10.1016/j.jhep.2015.01.015]

39 **Laconi S**, Curreli F, Diana S, Pasciu D, De Filippo G, Sarma DS, Pani P, Laconi E. Liver regeneration in response to partial hepatectomy in rats treated with retrorsine: a kinetic study. *J Hepatol* 1999; **31**: 1069-1074 [PMID: 10604581 DOI: 10.1016/s0168-8278(99)80320-1]

40 **Gordon GJ**, Coleman WB, Hixson DC, Grisham JW. Liver regeneration in rats with retrorsine-induced hepatocellular injury proceeds through a novel cellular response. *Am J Pathol* 2000; **156**: 607-619 [PMID: 10666390 DOI: 10.1016/S0002-9440(10)64765-7]

41 **Kvittingen EA**, Rootwelt H, Berger R, Brandtzaeg P. Self-induced correction of the genetic defect in tyrosinemia type I. *J Clin Invest* 1994; **94**: 1657-1661 [PMID: 7929843 DOI: 10.1172/JCI117509]

42 **Overturf K**, Al-Dhalimy M, Manning K, Ou CN, Finegold M, Grompe M. Ex vivo hepatic gene therapy of a mouse model of Hereditary Tyrosinemia Type I. *Hum Gene Ther* 1998; **9**: 295-304 [PMID: 9508047 DOI: 10.1089/hum.1998.9.3-295]

43 **Paglia S**, Sollazzo M, Di Giacomo S, Strocchi S, Grifoni D. Exploring MYC relevance to cancer biology from the perspective of cell competition. *Semin Cancer Biol* 2020; **63**: 49-59 [PMID: 31102666 DOI: 10.1016/j.semcancer.2019.05.009]

44 **Sasaki A**, Nagatake T, Egami R, Gu G, Takigawa I, Ikeda W, Nakatani T, Kunisawa J, Fujita Y. Obesity Suppresses Cell-Competition-Mediated Apical Elimination of RasV12-Transformed Cells from Epithelial Tissues. *Cell Rep* 2018; **23**: 974-982 [PMID: 29694905 DOI: 10.1016/j.celrep.2018.03.104]

45 **Laconi E**, Marongiu F, DeGregori J. Cancer as a disease of old age: changing mutational and microenvironmental landscapes. *Br J Cancer* 2020; **122**: 943-952 [PMID: 32042067 DOI: 10.1038/s41416-019-0721-1]

46 **Akagi K**, Wilson KA, Katewa SD, Ortega M, Simons J, Hilsabeck TA, Kapuria S, Sharma A, Jasper H, Kapahi P. Dietary restriction improves intestinal cellular fitness to enhance gut barrier function and lifespan in D. melanogaster. *PLoS Genet* 2018; **14**: e1007777 [PMID: 30383748 DOI: 10.1371/journal.pgen.1007777]

47 **Martincorena I**. Somatic mutation and clonal expansions in human tissues. *Genome Med* 2019; **11**: 35 [PMID: 31138277 DOI: 10.1186/s13073-019-0648-4]

48 **Kakiuchi N**, Yoshida K, Uchino M, Kihara T, Akaki K, Inoue Y, Kawada K, Nagayama S, Yokoyama A, Yamamoto S, Matsuura M, Horimatsu T, Hirano T, Goto N, Takeuchi Y, Ochi Y, Shiozawa Y, Kogure Y, Watatani Y, Fujii Y, Kim SK, Kon A, Kataoka K, Yoshizato T, Nakagawa MM, Yoda A, Nanya Y, Makishima H, Shiraishi Y, Chiba K, Tanaka H, Sanada M, Sugihara E, Sato TA, Maruyama T, Miyoshi H, Taketo MM, Oishi J, Inagaki R, Ueda Y, Okamoto S, Okajima H, Sakai Y, Sakurai T, Haga H, Hirota S, Ikeuchi H, Nakase H, Marusawa H, Chiba T, Takeuchi O, Miyano S, Seno H, Ogawa S. Frequent mutations that converge on the NFKBIZ pathway in ulcerative colitis. *Nature* 2020; **577**: 260-265 [PMID: 31853061 DOI: 10.1038/s41586-019-1856-1]

49 **Nanki K**, Fujii M, Shimokawa M, Matano M, Nishikori S, Date S, Takano A, Toshimitsu K, Ohta Y, Takahashi S, Sugimoto S, Ishimaru K, Kawasaki K, Nagai Y, Ishii R, Yoshida K, Sasaki N, Hibi T, Ishihara S, Kanai T, Sato T. Somatic inflammatory gene mutations in human ulcerative colitis epithelium. *Nature* 2020; **577**: 254-259 [PMID: 31853059 DOI: 10.1038/s41586-019-1844-5]

50 **Fujita Y**. Flower power as human cancer cells compete with normal cells. *Nature* 2019; **572**: 181-182 [PMID: 31384049 DOI: 10.1038/d41586-019-02161-y]

51 **Laconi S**, Pani P, Pillai S, Pasciu D, Sarma DS, Laconi E. A growth-constrained environment drives tumor progression invivo. *Proc Natl Acad Sci USA* 2001; **98**: 7806-7811 [PMID: 11427708 DOI: 10.1073/pnas.131210498]

52 **Moya IM**, Castaldo SA, Van den Mooter L, Soheily S, Sansores-Garcia L, Jacobs J, Mannaerts I, Xie J, Verboven E, Hillen H, Algueró-Nadal A, Karaman R, Van Haele M, Kowalczyk W, De Waegeneer M, Verhulst S, Karras P, van Huffel L, Zender L, Marine JC, Roskams T, Johnson R, Aerts S, van Grunsven LA, Halder G. Peritumoral activation of the Hippo pathway effectors YAP and TAZ suppresses liver cancer in mice. *Science* 2019; **366**: 1029-1034 [PMID: 31754005 DOI: 10.1126/science.aaw9886]

53 **Yimlamai D**, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B, Shrestha K, Cahan P, Stanger BZ, Camargo FD. Hippo pathway activity influences liver cell fate. *Cell* 2014; **157**: 1324-1338 [PMID: 24906150 DOI: 10.1016/j.cell.2014.03.060]

54 **Gatenby RA**, Brown J. Mutations, evolution and the central role of a self-defined fitness function in the initiation and progression of cancer. *Biochim Biophys Acta Rev Cancer* 2017; **1867**: 162-166 [PMID: 28341421 DOI: 10.1016/j.bbcan.2017.03.005]

55 **Marongiu F**, Serra M, Laconi E. Development versus Evolution in Cancer Biology. *Trends Cancer* 2018; **4**: 342-348 [PMID: 29709258 DOI: 10.1016/j.trecan.2018.03.007]

56 **Suijkerbuijk SJ**, Kolahgar G, Kucinski I, Piddini E. Cell Competition Drives the Growth of Intestinal Adenomas in Drosophila. *Curr Biol* 2016; **26**: 428-438 [PMID: 26853366 DOI: 10.1016/j.cub.2015.12.043]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 485-492

DOI: 10.4254/wjh.v12.i8.485

ISSN 1948-5182 (online)

MINIREVIEWS

Management of hepatitis C in children and adolescents during COVID-19 pandemic

Maria Pokorska-Śpiewak, Mateusz Śpiewak

ORCID number: Maria Pokorska-Śpiewak 0000-0001-7783-6904; Mateusz Śpiewak 0000-0002-2393-4194.

Author contributions: Pokorska-Śpiewak M conducted the review of existing literature, analyzed data, and wrote the manuscript; Pokorska-Śpiewak M and Śpiewak M designed the research, revised the paper, and approved the final version of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interests for this article.

Manuscript source: Unsolicited manuscript

Received: May 28, 2020

Maria Pokorska-Śpiewak, Department of Children's Infectious Diseases, Medical University of Warsaw, Warsaw 01201, Poland

Mateusz Śpiewak, National Institute of Cardiology, Warsaw 04628, Poland

Corresponding author: Maria Pokorska-Śpiewak MD, PhD, Associate Professor, Department of Children's Infectious Diseases, Medical University of Warsaw, No. 37 Wolska Street, Warsaw 01201, Poland. mpspiewak@gmail.com

Abstract

In recent years, significant progress in the antiviral treatment of chronic hepatitis C (CHC) has been made due to the development of interferon-free therapies. Three different highly effective, oral direct-acting antiviral (DAA) regimens have been approved for use in adolescents with CHC between the ages of 12-years-old and 17-years-old in Europe. According to the current recommendations, all treatment-naïve and treatment-experienced children with CHC virus infection should be considered for DAA therapy to prevent the possible progression of hepatitis C virus-related liver disease and its complications. However, the novel coronavirus disease 2019 outbreak, which was classified as a pandemic in March 2020, is currently spreading throughout the world, resulting in a disruption of the healthcare system. This disruption is having a negative impact on the care of patients with chronic diseases, including children with CHC. Thus, several efforts have to be made by pediatric hepatologists to prioritize patient care in children with CHC. These efforts include promoting telemedicine in the outpatient setting, using local laboratory testing for follow-up visits, and engaging in the home delivery of DAAs for patients under antiviral therapy whenever possible.

Key words: Children; Chronic hepatitis C; COVID-19; Direct-acting antiviral; Hepatitis C virus

Core tip: The novel coronavirus disease 2019 outbreak, classified as a pandemic, is currently spreading throughout the world, resulting in a disruption of the healthcare system. This disruption is having a negative impact on the care of patients with chronic diseases, including children with chronic hepatitis C. In this review, we describe several

Peer-review started: May 28, 2020
First decision: June 15, 2020
Revised: June 20, 2020
Accepted: July 26, 2020
Article in press: July 26, 2020
Published online: August 27, 2020

P-Reviewer: Hu K Q, Tanaka Y
S-Editor: Zhang L
L-Editor: Filipodia
P-Editor: Wang LL

efforts that have to be made by pediatric hepatologists to prioritize patient care in children with chronic hepatitis C. They include promoting telemedicine in the outpatient setting, using local laboratory testing for follow-up visits, and engaging in the home delivery of drugs for patients under antiviral therapy whenever possible.

Citation: Pokorska-Śpiewak M, Śpiewak M. Management of hepatitis C in children and adolescents during COVID-19 pandemic. *World J Hepatol* 2020; 12(8): 485-492
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/485.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.485

INTRODUCTION

Hepatitis C virus (HCV) infection is considered a major cause of liver-related mortality and morbidity worldwide, rendering it an important public health problem[1,2]. It is estimated by the World Health Organization (WHO) that 71 million people [95% confidence interval (CI): 64-103 million] are infected with HCV globally, which represents approximately 1% of the population[1,3]. The prevalence of HCV infection in children aged 1-year-old to 19-years-old is 0.15%, corresponding to 3.5 million people (95%CI: 3.1-3.9 million)[1,4]. However, since major gaps in our current knowledge on the epidemiology of chronic hepatitis C (CHC) exist in both adults and children, most HCV-infected people are unaware of their infection[1]. Thus, the true prevalence of HCV infection in children and adolescents might be underestimated[5]. In 2016, the WHO released a global health sector strategy for eliminating viral hepatitis by 2030 that includes global and country-wide targets for the testing, treatment, and prevention of CHC[6].

Chronic HCV infection leads to a progressive disease, with 10%-20% of infected patients developing cirrhosis and approximately 7% of adult patients with cirrhosis progressing to hepatocellular carcinoma[7,8]. Data reporting liver disease progression in the pediatric population infected with HCV are limited[9]. This progression is usually described as a mild disease in children and adolescents; however, severe cases have also been described occasionally[9-12]. Liver fibrosis and inflammation in children suffering from CHC is a time-dependent process, with approximately 2% of infected children developing advanced liver disease during childhood[13-16]. In the case of vertical HCV transmission, the progression of liver disease may occur at a younger age than in children infected horizontally in the later years of life, resulting in severe liver disease in their teens or in young adulthood[9,17]. Thus, effective antiviral treatment in children with CHC could prevent the development of end-stage liver disease, cirrhosis, and hepatocellular carcinoma in young adults.

MANAGEMENT OF HCV INFECTION IN CHILDREN AND ADOLESCENTS

Since 2015, the development and approval of novel, oral, interferon-free, antiviral treatment with direct-acting antivirals (DAAs) has substantially improved the treatment of HCV infection[18,19]. With an efficacy approaching 100% and a short duration of therapy, DAAs are a highly effective, safe, and well-tolerated alternative for previously used therapies based on interferons[18,19]. Currently, approximately 10 different DAA combinations have been approved for use in adults, increasing the prospect of HCV elimination on a population level[1,18]. However, treatment options based on DAA for children are currently limited[1,18]. Only three DAA regimens have been approved for use in adolescents by the European Medicines Agency (EMA) in Europe[1,20-24] (Table 1). The first DAA regimens, a fixed-dose combination of sofosbuvir/ledipasvir and sofosbuvir with ribavirin, were approved by the EMA in 2017 for use in adolescents between 12-years-old and 17-years-old with CHC[20,25]. The first regimen with pangenotypic activity, *i.e.* glecaprevir/pibrentasvir, was approved by the EMA in 2019 for adolescents aged 12-years-old to 17-years-old[24]. In addition, in 2019, the United States Food and Drug Administration (FDA) approved sofosbuvir/ledipasvir and sofosbuvir with ribavirin for use in children between 3-years-old and 11-years-old, and in March 2020, the FDA approved another pangenotypic combination, *i.e.* sofosbuvir/velpatasvir, for the treatment of chronic HCV patients as young as 6 years of age or weighing at least 17 kg[26-28]. However, the

Table 1 Direct-acting antivirals approved for adolescents aged 12 to 17 years in Europe (May 2020)[20,22-24]

Direct-acting antivirals regimen (doses per d)	Hepatitis C virus genotype	Patients	Duration of treatment in wk
Sofosbuvir/ledipasvir (400/90 mg)	1	Treatment-naïve with or without cirrhosis or treatment-experienced without cirrhosis	12
		Treatment-experienced with cirrhosis	24
	4, 5, 6	Treatment-naïve or treatment-experienced, with or without cirrhosis	12
Sofosbuvir + ribavirin (400 mg + 15 mg/kg)	2	Treatment-naïve or treatment-experienced, with or without cirrhosis	12
	3		24
Glecaprevir/pibrentasvir (300/120 mg)	All genotypes	Without cirrhosis	8
	All genotypes	With cirrhosis	12
	3	Treatment experienced	16

FDA approvals are not applicable in Europe. According to the current recommendations, all treatment-naïve and treatment-experienced children with CHC virus infection should be considered for DAA therapy to prevent the possible progression of HCV-related liver disease and its complications[20,21]. In children younger than 12-years-old with CHC, antiviral treatment should be deferred until interferon-free regimens are available[21]. Since liver disease in HCV-infected children is usually mild, and they rarely have comorbidities or take medicines posing potential risk for drug interactions, pediatric patients seem to be ideal candidates for DAA treatment. However, treatment options for children in many regions are currently limited[1]. Due to the high costs of DAAs, very few countries have implemented recommendations for CHC treatment in adolescents in their national policies[1]. In addition, there are no approved treatment options for children younger than 12-years-old in Europe. Thus, only a small number of children and adolescents with CHC have been treated globally, especially in low- and middle-income countries[1]. Considering the positive results from the clinical trials on DAA efficacy and safety, the first real-life therapeutic programs for pediatric patients infected with HCV based on DAAs were launched in Europe in 2019.

CORONAVIRUS DISEASE 2019 AND THE LIVER

Since the end of 2019, a novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) has caused an outbreak of coronavirus disease 2019 (COVID-19), resulting in an emerging global threat rapidly spreading throughout the world[29,30]. On March 11, 2020, the WHO declared the COVID-19 a pandemic[31]. In March 2020, the epicenter of the pandemic moved from China to the United States and Europe. Children seem to be less likely to be affected by the disease. According to the available data, the proportion of children among all infected patients ranged between 0.6% and 5.2% in different regions[32-34]. The clinical course of COVID-19 in children seems to be less severe than that in adults, with fewer clinical symptoms and case-fatality rates close to 0%[29,30].

In general, patients with pre-existing morbidities are at higher risk of a severe course of COVID-19, however, liver disease was not specifically listed in the published studies so far[35]. It is possible that patients with advanced liver disease are at increased risk of SARS-CoV-2 infection due to cirrhosis-induced immunodeficiency[36]. On the other hand, immunosuppression might provide some protection against cytokine storms, which contribute to multiorgan failure associated with COVID-19[37,38]. Patients with chronic liver disease including cirrhosis may be at higher risk of death resulting from COVID-19, but risk factors in specific liver diseases have not been defined[39]. It was revealed that SARS-CoV-2, similarly to SARS-CoV, uses angiotensin-converting enzyme 2 as its entry receptor[40]. Both liver and bile duct cells express angiotensin-converting enzyme 2. Thus, the liver is a potential target for SARS-COV-2 infection[39,40]. It results in liver injury, which is observed in 15% to 58% of patients, more commonly in severe COVID-19 cases[39,40]. The incidence of liver disease in death cases of COVID-19 was as high as 58% to 78%[40]. Liver disease manifests mainly with elevated aminotransferase levels and/or slightly elevated bilirubin level[39,40]. Liver

injury is usually transient and does not require specific treatment[39].

Severe liver injury as a result of SARS-CoV-2 infection is uncommon in pediatric patients. In the rare cases of severe COVID-19 in children, increase in aminotransferase level was only mild (not exceeding 2 × upper limit of normal)[39]. There are only limited data on SARS-CoV-2 infection in patients with chronic viral hepatitis[35]. Thus, it remains unknown whether patients with chronic viral hepatitis B and/or C are more susceptible to liver injury from SARS-CoV-2[39]. Observations from China suggest that chronic hepatitis B does not affect the outcome of COVID-19[41]. No case of SARS-CoV-2 infection has been described among pediatric patients with CHC; however, the impact of the COVID-19 pandemic on the management of patients with chronic HCV infection is significant, with several aspects requiring attention[35].

MANAGEMENT OF PEDIATRIC PATIENTS WITH CHRONIC HEPATITIS DURING THE COVID-19 PANDEMIC

The COVID-19 pandemic has led to the disruption of the healthcare system. This disruption has had a negative impact on the care of patients with chronic diseases, including children with CHC, which may not only pose a risk for individual patients but also have a negative influence on viral hepatitis elimination programs[42]. The treatment and management of patients with HCV infection is influenced by closing clinics and avoiding nonemergent visits[42]. In many cases, DAA therapies in children are conducted in infectious disease departments, which are now on the front line of fighting the pandemic. This change of focus may result in a reduction in both the diagnosis and treatment rates of hepatitis patients[42]. It is essential to maintain the care of children with CHC and to find potential methods to prioritize the care of these patients despite the limited healthcare resources[35]. This may be achieved by adapting to the unique logistical and pharmacological issues caused by the pandemic[39]. Recent recommendations from the European Association for the Study of the Liver-European Society of Clinical Microbiology and Infectious Diseases and the American Association for the Study of Liver Diseases Expert Panel consensus statement on the care of patents with liver disease during the COVID-19 pandemic may also be useful for pediatricians caring for children with CHC[35,39].

The most important issue is that both patients and medical staff should avoid SARS-CoV-2 exposure and infection. The precise management of the patients depends mostly on the local COVID-19 burden[35]. It is essential to educate the patients on risk and precaution on COVID-19, especially in cases complicated by cirrhosis or end-stage liver disease, when the risk of severe course of COVID-19 exists[39]. In most regions, physical distancing and avoiding direct face-to-face contact have been officially implemented. Thus, all patients suffering from chronic liver diseases should adhere to these common rules[35]. Visits to outpatient or inpatient clinics should be avoided unless necessary. Since in most cases children with CHC present with mild disease and are in stable, good condition, visits to hepatological clinics are not essential and may be postponed. In case of patients already on DAA treatment, therapy should be continued[39]. It is reasonable to use telemedicine for follow-up visits in patients under antiviral treatment and to send them prescriptions by e-mail or organize a home delivery of DAAs, as appropriate. Routine laboratory testing may be performed in a local laboratory through primary care physicians only in cases when it is truly necessary. Collaboration between hepatologists and local health care providers and primary care physicians is essential for further management of patients during pandemics. Whenever possible, liver-related diagnostic procedures (*e.g.*, ultrasound, elastography, or liver biopsy if required) should be avoided unless they are likely to change management. In addition, an inclusion of the patients in the clinical trials should be deferred. While planning DAA treatment, its priority should be determined. In patients with stable CHC, therapy may be safely postponed to after COVID-19 pandemic. However, in selected cases with known advanced liver disease (*e.g.*, with significant fibrosis: Liver stiffness measurement > 7 kPa) or in patients with human immunodeficiency virus coinfection, decision on starting therapy despite COVID-19 pandemic should be considered. If a visit to an outpatient clinic is needed, standard operating procedures should be adopted, *e.g.*, separation from patients suspected for COVID-19, remodeling of waiting areas, keeping distance between patients, reduction of waiting times, and minimizing exposure to the medical staff[35]. The number of family members who accompany patients to their visits should be limited to one healthy parent or guardian[39]. All patients should be screened for symptoms of COVID-19 (*e.g.*, fever, cough, shortness of breath, sore throat, rhinitis), and their

temperature should be checked as they enter the clinical space[39]. There are currently no specific recommendations on screening for SARS-COV-2 infection in patients with CHC. As in individuals without HCV infection, children with CHC should be tested for COVID-19 in case of the presence of clinical symptoms suggesting the SARS-CoV-2 infection or having household contact with an infected family member. Our unpublished observations of over 100 pediatric patients with COVID-19 suggest that children usually acquire infection from infected close relatives. Thus, family history should be assessed in order to stratify the risk of the SARS-CoV-2 infection. In addition, testing should be considered in patients requiring hospitalization in order to reduce a risk of spreading the infection by an asymptomatic person in the hospital setting. Recommendations for the management of pediatric patients with CHC are summarized in Table 2.

Despite the fact that CHC does not seem to increase the risk of a severe course of COVID-19, in case of coinfection, an early admission and inclusion to the experimental antiviral therapy of COVID-19 should be considered, following local recommendations[35]. Interestingly, one of the DAAs, sofosbuvir alone or in combination with ribavirin, has been suggested for the experimental treatment of COVID-19[35,43]. In all hospitalized COVID-19 patients, regular monitoring of aminotransferase levels is recommended, particularly in cases treated with tocilizumab or remdesivir, due to their hepatotoxicity[39]. As COVID-19 is only rarely associated with elevated liver enzymes in children, all pediatric patients with high aminotransferase levels during the SARS-CoV-2 infection should be evaluated for other etiologies and underlying liver diseases, including hepatitis A, B, or C and drug-induced liver injury[39].

CONCLUSION

The open issue is how this COVID-19 pandemic will influence diagnostic and treatment strategies regarding CHC and its elimination program. Despite the special attention required by the COVID-19 pandemic, we should not forget about other diseases and chronically ill patients, including viral hepatitis. Several efforts have to be made by pediatric hepatologists to prioritize patient care in children with CHC and to avoid regression regarding programs leading to HCV elimination.

Table 2 Recommendations for the management of pediatric patients with chronic hepatitis C virus infection during the coronavirus disease 2019 pandemic[35,39]

Management	Recommendation
Physical distancing	Recommended
Patient education on risk and precaution on COVID-19	Recommended
Testing for severe acute respiratory syndrome coronavirus infection	Recommended in patients with clinical symptoms suggesting COVID-19, or with household contact with an infected family member, or requiring hospitalization
Visits to specialized centers	Should be postponed
Routine laboratory testing	Should be performed (only if truly necessary) locally/offsite
Direct-acting antiviral therapy already initiated	Should be continued
Starting direct-acting antiviral treatment	May be postponed in patients with stable chronic hepatitis C. If possible, it should be considered in patients with significant fibrosis or human immunodeficiency virus/hepatitis C virus coinfection
Telemedicine/visits by phone	Recommended instead of face-to-face visits whenever possible
Drug supply	Home delivery or sending prescriptions by e-mail
Liver-related diagnostic procedures	Should be deferred unless they are likely to change management

COVID-19: Coronavirus disease 2019.

REFERENCES

1 **Indolfi G**, Easterbrook P, Dusheiko G, El-Sayed MH, Jonas MM, Thorne C, Bulterys M, Siberry G, Walsh N, Chang MH, Meyers T, Giaquinto C, Wirth S, Chan PL, Penazzato M. Hepatitis C virus infection in children and adolescents. *Lancet Gastroenterol Hepatol* 2019; **4**: 477-487 [PMID: 30982721 DOI: 10.1016/S2468-1253(19)30046-9]

2 **Stanaway JD**, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I, Abu-Raddad LJ, Assadi R, Bhala N, Cowie B, Forouzanfour MH, Groeger J, Hanafiah KM, Jacobsen KH, James SL, MacLachlan J, Malekzadeh R, Martin NK, Mokdad AA, Mokdad AH, Murray CJL, Plass D, Rana S, Rein DB, Richardus JH, Sanabria J, Saylan M, Shahraz S, So S, Vlassov VV, Weiderpass E, Wiersma ST, Younis M, Yu C, El Sayed Zaki M, Cooke GS. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. *Lancet* 2016; **388**: 1081-1088 [PMID: 27394647 DOI: 10.1016/S0140-6736(16)30579-7]

3 **World Health Organization**. Global Hepatitis Report, 2017. Geneva: World Health Organization, 2017. https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/. Accessed May 17, 2020

4 **Schmelzer J**, Dugan E, Blach S, Coleman S, Cai Z, DePaola M, Estes C, Gamkrelidze I, Jerabek K, Ma S, Montoya S, Razavi-Shearer D, Razavi-Shearer K, Robbins-Scott S, Razavi H, El Sayed MH. Global prevalence of hepatitis C virus in children in 2018: a modelling study. *Lancet Gastroenterol Hepatol* 2020; **5**: 374-392 [PMID: 31954439 DOI: 10.1016/S2468-1253(19)30385-1]

5 **Delgado-Borrego A**, Smith L, Jonas MM, Hall CA, Negre B, Jordan SH, Ogrodowicz M, Raza R, Ludwig DA, Miller T, Lipshultz SE, Gonzalez-Peralta R, Chung RT. Expected and actual case ascertainment and treatment rates for children infected with hepatitis C in Florida and the United States: epidemiologic evidence from statewide and nationwide surveys. *J Pediatr* 2012; **161**: 915-921 [PMID: 22765955 DOI: 10.1016/j.jpeds.2012.05.002]

6 **World Health Organization**. Global health sector strategy on viral hepatitis 2016-2021. https://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/. Accessed May 17, 2020

7 **Blachier M**, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol* 2013; **58**: 593-608 [PMID: 23419824 DOI: 10.1016/j.jhep.2012.12.005]

8 **Missiha SB**, Ostrowski M, Heathcote EJ. Disease progression in chronic hepatitis C: modifiable and nonmodifiable factors. *Gastroenterology* 2008; **134**: 1699-1714 [PMID: 18471548 DOI: 10.1053/j.gastro.2008.02.069]

9 **Pokorska-Śpiewak M**, Kowalik-Mikołajewska B, Aniszewska M, Pluta M, Walewska-Zielecka B, Marczyńska M. Determinants of liver disease progression in children with chronic hepatitis C virus infection. *Pol J Pathol* 2015; **66**: 368-375 [PMID: 27003768 DOI: 10.5114/pjp.2015.57248]

10 **Mohan P**, Colvin C, Glymph C, Chandra RR, Kleiner DE, Patel KM, Luban NL, Alter HJ. Clinical spectrum and histopathologic features of chronic hepatitis C infection in children. *J Pediatr* 2007; **150**: 168-174, 174.e1 [PMID: 17236895 DOI: 10.1016/j.jpeds.2006.11.037]

11 **European Paediatric Hepatitis C Virus Network**. Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. *Clin Infect Dis* 2005; **41**: 45-51 [PMID: 15937762 DOI: 10.1086/430601]

12 **Mohan P**, Barton BA, Narkewicz MR, Molleston JP, Gonzalez-Peralta RP, Rosenthal P, Murray KF, Haber B, Schwarz KB, Goodman ZD. Evaluating progression of liver disease from repeat liver biopsies in children with chronic hepatitis C: a retrospective study. *Hepatology* 2013; **58**: 1580-1586 [PMID: 23703847 DOI:

10.1002/hep.26519]
13 **Goodman ZD**, Makhlouf HR, Liu L, Balistreri W, Gonzalez-Peralta RP, Haber B, Jonas MM, Mohan P, Molleston JP, Murray KF, Narkewicz MR, Rosenthal P, Smith LJ, Robuck PR, Schwarz KB. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. *Hepatology* 2008; **47**: 836-843 [PMID: 18167062 DOI: 10.1002/hep.22094]
14 **Guido M**, Bortolotti F, Leandro G, Jara P, Hierro L, Larrauri J, Barbera C, Giacchino R, Zancan L, Balli F, Crivellaro C, Cristina E, Pucci A, Rugge M. Fibrosis in chronic hepatitis C acquired in infancy: is it only a matter of time? *Am J Gastroenterol* 2003; **98**: 660-663 [PMID: 12650803 DOI: 10.1111/j.1572-0241.2003.07293.x]
15 **Bortolotti F**, Verucchi G, Cammà C, Cabibbo G, Zancan L, Indolfi G, Giacchino R, Marcellini M, Marazzi MG, Barbera C, Maggiore G, Vajro P, Bartolacci S, Balli F, Maccabruni A, Guido M; Italian Observatory for HCV Infection and Hepatitis C in Children. Long-term course of chronic hepatitis C in children: from viral clearance to end-stage liver disease. *Gastroenterology* 2008; **134**: 1900-1907 [PMID: 18439604 DOI: 10.1053/j.gastro.2008.02.082]
16 **Indolfi G**, Guido M, Azzari C, Resti M. Histopathology of hepatitis C in children, a systematic review: implications for treatment. *Expert Rev Anti Infect Ther* 2015; **13**: 1225-1235 [PMID: 26202832 DOI: 10.1586/14787210.2015.1070668]
17 **Pembrey L**, Newell ML, Tovo PA; EPHN Collaborators. The management of HCV infected pregnant women and their children European paediatric HCV network. *J Hepatol* 2005; **43**: 515-525 [PMID: 16144064 DOI: 10.1016/j.jhep.2005.06.002]
18 **Indolfi G**, Bailey H, Serranti D, Giaquinto C, Thorne C; PENTAHep Study Group. Treatment and monitoring of children with chronic hepatitis C in the Pre-DAA era: A European survey of 38 paediatric specialists. *J Viral Hepat* 2019; **26**: 961-968 [PMID: 30980773 DOI: 10.1111/jvh.13111]
19 **Pawlowska M**, Sobolewska-Pilarczyk M, Domagalski K. Hepatitis C virus infection in children in the era of direct-acting antiviral. *World J Gastroenterol* 2018; **24**: 2555-2566 [PMID: 29962813 DOI: 10.3748/wjg.v24.i24.2555]
20 **Indolfi G**, Hierro L, Dezsofi A, Jahnel J, Debray D, Hadzic N, Czubkowski P, Gupte G, Mozer-Glassberg Y, van der Woerd W, Smets F, Verkade HJ, Fischler B. Treatment of Chronic Hepatitis C Virus Infection in Children: A Position Paper by the Hepatology Committee of European Society of Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2018; **66**: 505-515 [PMID: 29287014 DOI: 10.1097/MPG.0000000000001872]
21 **Indolfi G**, Fischler B, Gonzalez-Peralta RP, Ciocca M, Porta G, Neelam M, El-Guindi M, Kelly D, Ni YH, Sibal A, Leung DH, Chang MH; Hepatitis Expert Team of the Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition (FISPGHAN). Comparison of Recommendations for Treatment of Chronic Hepatitis C Virus Infection in Children and Adolescents: A Position Paper of the Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2020; **70**: 711-717 [PMID: 32205770 DOI: 10.1097/MPG.0000000000002710]
22 **Balistreri WF**, Murray KF, Rosenthal P, Bansal S, Lin CH, Kersey K, Massetto B, Zhu Y, Kanwar B, German P, Svarovskaia E, Brainard DM, Wen J, Gonzalez-Peralta RP, Jonas MM, Schwarz K. The safety and effectiveness of ledipasvir-sofosbuvir in adolescents 12-17 years old with hepatitis C virus genotype 1 infection. *Hepatology* 2017; **66**: 371-378 [PMID: 27997679 DOI: 10.1002/hep.28995]
23 **Wirth S**, Rosenthal P, Gonzalez-Peralta RP, Jonas MM, Balistreri WF, Lin CH, Hardikar W, Kersey K, Massetto B, Kanwar B, Brainard DM, Shao J, Svarovskaia E, Kirby B, Arnon R, Murray KF, Schwarz KB. Sofosbuvir and ribavirin in adolescents 12-17 years old with hepatitis C virus genotype 2 or 3 infection. *Hepatology* 2017; **66**: 1102-1110 [PMID: 28543053 DOI: 10.1002/hep.29278]
24 **Jonas MM**, Squires RH, Rhee SM, Lin CW, Bessho K, Feiterna-Sperling C, Hierro L, Kelly D, Ling SC, Strokova T, Del Valle-Segarra A, Lovell S, Liu W, Ng TI, Porcalla A, Gonzalez YS, Burroughs M, Sokal E. Pharmacokinetics, Safety, and Efficacy of Glecaprevir/Pibrentasvir in Adolescents With Chronic Hepatitis C Virus: Part 1 of the DORA Study. *Hepatology* 2020; **71**: 456-462 [PMID: 31254392 DOI: 10.1002/hep.30840]
25 **Thorne C**, Indolfi G, Turkova A, Giaquinto C, Nastouli E. Treating hepatitis C virus in children: time for a new paradigm. *J Virus Erad* 2015; **1**: 203-205 [PMID: 27482412 DOI: 10.1016/S2055-6640(20)30500-8]
26 **Murray KF**, Balistreri WF, Bansal S, Whitworth S, Evans HM, Gonzalez-Peralta RP, Wen J, Massetto B, Kersey K, Shao J, Garrison KL, Parhy B, Brainard DM, Arnon R, Gillis LA, Jonas MM, Lin CH, Narkewicz MR, Schwarz K, Rosenthal P. Safety and Efficacy of Ledipasvir-Sofosbuvir With or Without Ribavirin for Chronic Hepatitis C in Children Ages 6-11. *Hepatology* 2018; **68**: 2158-2166 [PMID: 30070726 DOI: 10.1002/hep.30123]
27 **Rosenthal P**, Schwarz KB, Gonzalez-Peralta RP, Lin CH, Kelly DA, Nightingale S, Balistreri WF, Bansal S, Jonas MM, Massetto B, Brainard DM, Hsueh CH, Shao J, Parhy B, Davison S, Feiterna-Sperling C, Gillis LA, Indolfi G, Sokal EM, Murray KF, Wirth S. Sofosbuvir and Ribavirin Therapy for Children Aged 3 to <12 Years With Hepatitis C Virus Genotype 2 or 3 Infection. *Hepatology* 2020; **71**: 31-43 [PMID: 31222783 DOI: 10.1002/hep.30821]
28 **Schwarz KB**, Rosenthal P, Murray KF, Honegger JR, Hardikar W, Hague R, Mittal N, Massetto B, Brainard DM, Hsueh CH, Shao J, Parhy B, Narkewicz MR, Rao GS, Whitworth S, Bansal S, Balistreri WF. Ledipasvir-Sofosbuvir for 12 Weeks in Children 3 to <6 Years Old With Chronic Hepatitis C. *Hepatology* 2020; **71**: 422-430 [PMID: 31220349 DOI: 10.1002/hep.30830]
29 **Rasmussen SA**, Thompson LA. Coronavirus Disease 2019 and Children: What Pediatric Health Care Clinicians Need to Know. *JAMA Pediatr* 2020 [PMID: 32242896 DOI: 10.1001/jamapediatrics.2020.1224]
30 **Zimmermann P**, Curtis N. Coronavirus Infections in Children Including COVID-19: An Overview of the Epidemiology, Clinical Features, Diagnosis, Treatment and Prevention Options in Children. *Pediatr Infect Dis J* 2020; **39**: 355-368 [PMID: 32310621 DOI: 10.1097/INF.0000000000002660]
31 **World Health Organization**. Coronavirus disease 2019 (COVID-19). Situation report-51. Geneva (Switzerland), World Health Organization. 2020. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-covid-19.pdf
32 **Tagarro A**, Epalza C, Santos M, Sanz-Santaeufemia FJ, Otheo E, Moraleda C, Calvo C. Screening and

Severity of Coronavirus Disease 2019 (COVID-19) in Children in Madrid, Spain. *JAMA Pediatr* 2020 [PMID: 32267485 DOI: 10.1001/jamapediatrics.2020.1346]

33 **Choi SH**, Kim HW, Kang JM, Kim DH, Cho EY. Epidemiology and clinical features of coronavirus disease 2019 in children. *Clin Exp Pediatr* 2020; **63**: 125-132 [PMID: 32252139 DOI: 10.3345/cep.2020.00535]

34 **CDC COVID-19 Response Team**. Coronavirus Disease 2019 in Children - United States, February 12-April 2, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 422-426 [PMID: 32271728 DOI: 10.15585/mmwr.mm6914e4]

35 **Boettler T**, Newsome PN, Mondelli MU, Maticic M, Cordero E, Cornberg M, Berg T. Care of patients with liver disease during the COVID-19 pandemic: EASL-ESCMID position paper. *JHEP Rep* 2020; **2**: 100113 [PMID: 32289115 DOI: 10.1016/j.jhepr.2020.100113]

36 **Albillos A**, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol* 2014; **61**: 1385-1396 [PMID: 25135860 DOI: 10.1016/j.jhep.2014.08.010]

37 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

38 **Li X**, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal* 2020; **10**: 102-108 [PMID: 32282863 DOI: 10.1016/j.jpha.2020.03.001]

39 **Fix OK**, Hameed B, Fontana RJ, Kwok RM, McGuire BM, Mulligan DC, Pratt DS, Russo MW, Schilsky ML, Verna EC, Loomba R, Cohen DE, Bezerra JA, Reddy KR, Chung RT. Clinical Best Practice Advice for Hepatology and Liver Transplant Providers During the COVID-19 Pandemic: AASLD Expert Panel Consensus Statement. *Hepatology* 2020; **72**: 287-304 [PMID: 32298473 DOI: 10.1002/hep.31281]

40 **Xu L**, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. *Liver Int* 2020; **40**: 998-1004 [PMID: 32170806 DOI: 10.1111/liv.14435]

41 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

42 **Karimi-Sari H**, Rezaee-Zavareh MS. COVID-19 and viral hepatitis elimination programs: Are we stepping backward? *Liver Int* 2020 [PMID: 32319207 DOI: 10.1111/liv.14486]

43 **Sayad B**, Sobhani M, Khodarahmi R. Sofosbuvir as Repurposed Antiviral Drug Against COVID-19: Why Were We Convinced to Evaluate the Drug in a Registered/Approved Clinical Trial? *Arch Med Res* 2020 [PMID: 32387040 DOI: 10.1016/j.arcmed.2020.04.018]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 493-505

DOI: 10.4254/wjh.v12.i8.493

ISSN 1948-5182 (online)

MINIREVIEWS

Glucagon-like peptide-1 receptor agonists in non-alcoholic fatty liver disease: An update

Areti Sofogianni, Athanasios Filippidis, Lampros Chrysavgis, Konstantinos Tziomalos, Evangelos Cholongitas

ORCID number: Areti Sofogianni 0000-0003-4958-2913; Athanasios Filippidis 0000-0001-9343-2854; Lampros Chrysavgis 0000-0003-2632-0742; Konstantinos Tziomalos 0000-0002-3172-1594; Evangelos Cholongitas 0000-0002-3645-582X.

Author contributions: Sofogianni A, Filippidis A and Chrysavgis L drafted the review; Tziomalos K and Cholongitas E critically revised the draft.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Manuscript source: Invited

Areti Sofogianni, Athanasios Filippidis, Konstantinos Tziomalos, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, Thessaloniki 54636, Greece

Lampros Chrysavgis, Evangelos Cholongitas, First Department of Internal Medicine, Laiko General Hospital, Medical School of National and Kapodistrian University of Athens, Athens 11527, Greece

Corresponding author: Evangelos Cholongitas, MD, PhD, Associate Professor, First Department of Internal Medicine, Laiko General Hospital, Medical School of National and Kapodistrian University of Athens, Agiou Thoma 17, Athens 11527, Greece. cholongitas@yahoo.gr

Abstract

Non-alcoholic fatty liver disease (NAFLD) is the predominant cause of chronic liver disease worldwide. NAFLD progresses in some cases to non-alcoholic steatohepatitis (NASH), which is characterized, in addition to liver fat deposition, by hepatocyte ballooning, inflammation and liver fibrosis, and in some cases may lead to hepatocellular carcinoma. NAFLD prevalence increases along with the rising incidence of type 2 diabetes mellitus (T2DM). Currently, lifestyle interventions and weight loss are used as the major therapeutic strategy in the vast majority of patients with NAFLD. Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are used in the management of T2DM and do not have major side effects like hypoglycemia. In patients with NAFLD, the GLP-1 receptor production is down-regulated. Recently, several animal and human studies have emphasized the role of GLP-1RAs in ameliorating liver fat accumulation, alleviating the inflammatory environment and preventing NAFLD progression to NASH. In this review, we summarize the updated literature data on the beneficial effects of GLP-1RAs in NAFLD/NASH. Finally, as GLP-1RAs seem to be an attractive therapeutic option for T2DM patients with concomitant NAFLD, we discuss whether GLP-1RAs should represent the first line pharmacotherapy for these patients.

Key words: Glucagon-like peptide-1 receptor agonists; Non-alcoholic fatty liver disease; Type 2 diabetes mellitus; Clinical studies; Fatty liver; Animal studies

manuscript

Received: March 16, 2020
Peer-review started: May 16, 2020
First decision: April 26, 2020
Revised: May 2, 2020
Accepted: June 20, 2020
Article in press: June 20, 2020
Published online: August 27, 2020

P-Reviewer: Qi XS, Yoshioka K
S-Editor: Dou Y
L-Editor: Filipodia
P-Editor: Wang LL

Core tip: The strong relationship between non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus points to a need to evaluate the therapeutic potential of antidiabetic drugs in patients with NAFLD. Accordingly, glucagon-like peptide-1 receptor agonists, which are well-tolerated antidiabetic agents with no risk of hypoglycemia, seem to be a very appealing therapeutic option for type 2 diabetes mellitus patients with NAFLD. Herein, based on data from animal studies and clinical trials, we discuss the beneficial impact of glucagon-like peptide-1 receptor agonists on NAFLD.

Citation: Sofogianni A, Filippidis A, Chrysavgis L, Tziomalos K, Cholongitas E. Glucagon-like peptide-1 receptor agonists in non-alcoholic fatty liver disease: An update. *World J Hepatol* 2020; 12(8): 493-505
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/493.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.493

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases, affecting approximately one third of the population globally[1]. It includes a wide spectrum of clinical presentations, from isolated fat accumulation in the liver to inflammation and fibrosis [*i.e.*, non-alcoholic steatohepatitis (NASH)], cirrhosis and hepatocellular carcinoma[2]. NAFLD is inextricably linked to major comorbidities of the metabolic syndrome, including obesity, insulin resistance, type 2 diabetes mellitus (T2DM) and dyslipidemia[3]. In addition, various metabolism disorders, including thyroid dysfunction, are associated with the occurrence of NAFLD. Of note, thyroid hormones are of cardinal importance in regulating fat deposition and insulin resistance as well as lipid and carbohydrate metabolism, thereby contributing to NAFLD/NASH modification[4,5].

Hypothyroidism has been suggested as an independent risk factor for NAFLD/NASH development in both adult and children/adolescent population. Moreover, the inconsistent findings on current literature regarding the association between NAFLD and free thyroid hormones (free triiodothyronine and free thyroxine) may indicate a key role for thyroid-stimulating hormone in NAFLD onset and progression, independently of free triiodothyronine and free thyroxine[4]. The increasing prevalence of NAFLD in combination with its severe complications underlines the need for effective and safe treatments. Presently, diet and lifestyle changes are the main treatment options for NAFLD, whereas vitamin E and pioglitazone have limited application, mostly in non-diabetic patients[6].

Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are used for the treatment of T2DM[7]. This class includes exenatide, lixisenatide, liraglutide, albiglutide, semaglutide and dulaglutide[8]. GLP-1RAs are divided into short- and long-acting[8]. The former include exenatide and lixisenatide, whereas the latter include liraglutide, albiglutide, dulaglutide, semaglutide and once weekly exenatide[8]. GLP-1 receptors are expressed mainly in the pancreas but are also present in the brain, adipose tissue, muscles, heart, kidney, lung, stomach and hepatocytes[7,9,10]. Their primary actions are the stimulation of insulin secretion and the reduction of glucagon secretion[7]. In patients with T2DM, they reduce hemoglobin A1c (HbA_{1c}) levels by approximately 1.5% without the risk of hypoglycemia[8]. Their main side effects are nausea and vomiting[8]. Interestingly, the production of GLP-1 is reduced in patients with NAFLD[11].

Accumulating data suggest that GLP-1RAs improve liver histology in patients with NAFLD. In the present review, we discuss the role of these agents in the management of NAFLD.

LITERATURE RESEARCH

We systematically reviewed the literature in the PubMed database up to December 2019. The following search terms were used: "(GLP-1 receptor agonists OR glucagon-like peptide-1 agonists OR glucagon-like peptide-1 analogues OR GLP-1 analogues OR liraglutide OR exenatide OR semaglutide OR dulaglutide OR lixisenatide OR

albiglutide) AND (NASH OR NAFLD OR non-alcoholic fatty liver disease OR fatty liver disease OR non-alcoholic steatohepatitis).

EXENATIDE

Effects of exenatide in animal models of NAFLD

Several studies reported beneficial effects of exenatide in animal models of NAFLD, and a variety of mechanisms appear to underpin these effects. First, exenatide appears to reduce intrahepatic oxidative stress. Indeed, in rats, administration of exendin-4 resulted in an increase in glutathione levels, which in turn reduced oxidative stress[12]. A reduction of the hepatic expression of receptors for advanced glycation end-products also appears to contribute to the antioxidant effects of exenatide[13].

An improvement in insulin resistance also may play a role in the improvement of hepatic steatosis during exenatide treatment. In rats, administration of exendin-4 resulted in an increase in cystathionine beta synthase, which resulted in a reduction in insulin resistance[12]. Treatment with exendin-4 also results in an increase of adiponectin levels, which again improves insulin sensitivity[14,15]. In contrast, levels of visfatin, which appears to play a role in insulin resistance, were reduced after treatment with exenatide[15]. Exenatide also improves insulin sensitivity by increasing peroxisome proliferator-activated receptor (PPAR)-γ activity[16].

Exenatide exerts anti-inflammatory effects, which contribute to the improvement in hepatic histology in NAFLD. Accordingly, exenatide was shown to inhibit the NLRP3 inflammasome by enhancing the autophagy/mitophagy pathway[17]. In another study in mice, administration of exendin-4 for 4 wk reduced inflammation both in the liver and in the vascular, wall as shown by a decreased accumulation of monocytes and macrophages and a reduced recruitment of oxidized LDL, which correlated with reduced formation of foam cells[18]. Kawaguchi *et al*[19] reported that mice treated with exendin-4 had a lower NAFLD activity score compared with mice that received saline[19]. This beneficial effect was mediated by an inhibition of the action of Δ-5-desaturase, which resulted in a reduction of pro-inflammatory eicosanoids and an increase in dihomo-γ-linolenic acid and anti-inflammatory eicosanoids[19].

Another important mechanism implicated in the reduction of hepatic fat accumulation during exenatide treatment is the amelioration of hepatic lipid metabolism. Exenatide was shown to suppress hepatic very-low density lipoprotein (VLDL) production, resulting in improvement of hepatic steatosis[20]. Mice treated with exendin-4 showed an increased expression of acyl-CoA oxidase and medium chain acyl-coenzyme A dehydrogenase, which are both related to β-oxidation[9]. In addition, the expression of several enzymes participating in hepatic lipid metabolism, including sirtuin-1, phospho-5′ adenosine monophosphate-activated protein kinase, phospho-Foxo1 and glucose transporter 2, was also up-regulated during exendin-4 treatment[9]. On the other hand, the levels of modulators of hepatic lipogenesis such as sterol regulatory element-binding protein-1c (SREBP-1c) and stearoyl CoA desaturase-1 mRNA were decreased[9]. Exenatide-induced up-regulation of sirtuin also increases fibroblast growth factor-1 activity, which is another important regulator of hepatic lipid metabolism[21]. During treatment with exenatide, enzymes related to hepatic lipogenesis, including ACC, stearoyl CoA desaturase-1 and SREBP-1c are down-regulated whereas enzymes participating in β-oxidation, including PPARa and fatty acyl-CoA oxidase, are up-regulated[14].

Yamamoto *et al*[22] randomly assigned male db/db non-obese NASH mice to methionine-choline sufficient diet or methionine-choline deficient diet (MCD), a well-established inductor of hepatic steatosis and inflammation, plus exendin-4 (20 μg/kg per day intraperitoneally) or MCD plus saline for 4 or 8 wk[22]. Exendin-4 administration significantly ameliorated both the MCD-induced oil red O-positive area, an index of hepatic fat deposition, and the liver triglyceride content in the MCD plus exendin-4 group compared to the saline group at 4 and 8 wk through suppression of FATP4, which plays a role in hepatic free fatty acid uptake[22]. In addition, exendin-4 administration led to significant decreased mRNA expression of SREBP-1c, a gene responsible for free fatty acid production in MCD-fed mice at 4 and 8 wk, along with markedly reduced serum alanine transaminase (ALT) levels at 8, but not at 4 wk, in the same group[22]. Of note, exendin-4 therapy attenuated the increased, by MCD, hepatic mRNA expression levels of inflammation-related genes such as tumor necrosis factor-α, monocyte chemotactic protein-1 and cc-chemokine receptor 2 and also decreased insulin levels and homeostasis model assessment of insulin resistance[22].

In rabbits, treatment with exenatide decreased the expression of fat mass and

obesity-associated gene (*FTO*), which is associated with both lipogenesis and oxidative stress[23]. In another study, treatment with exenatide improved histological features of NAFLD through enhancing the action of PPARa, which is another key regulator of fatty acid β-oxidation[16]. In mice, exendin-4 reduced VLDL-triglycerides and VLDL-ApoB, inhibited the expression of Srebp-1c, Fasn and Dgat1, which participate in hepatic lipogenesis, and down-regulated the genes *Acox1* and *Cpt1a*, which play a role in fatty acid oxidation[20].

Endoplasmic reticulum (ER) stress appears to play an important role in the pathogenesis of hepatic steatosis, and exenatide was shown to alleviate ER stress by enhancing the sirtuin 1/heat shock factor 1/heat shock protein pathway[24,25]. Treatment of mice with exenatide was also shown to improve mitochondrial lipid metabolism, which in turn resulted in decreased steatosis[26]. Exenatide also activates the phophoinositide 3-kinase/Akt pathway, which might improve liver histology in NAFLD through the regeneration of hepatocytes[23].

In addition to these effects of exenatide on the liver, an enhancement of lipid catabolism in the adipose tissue during treatment with this agent also appears to ameliorate hepatic steatosis by decreasing free fatty acid influx into the liver[27]. Interestingly, it has been recently reported that co-agonists of GLP-1 and glucagon receptor ameliorate hepatic steatosis and inflammation more than GLP-1 agonists alone[28]. The concomitant administration of exendin-4 and glucokinase activator in mice also resulted in a reduction of liver steatosis, liver weight, intrahepatic triglyceride levels and serum ALT levels[29].

Effects of exenatide in clinical studies of NAFLD

It is of interest whether data from animal studies can be translated into humans, in order to clarify the beneficial impact of GLP-1RAs on human NAFLD (Table 1). In particular, in a study by Gastaldelli *et al*[30], 15 males with newly diagnosed T2DM or impaired glucose tolerance were randomized to receive exenatide or placebo, each therapy on two sessions with random order, 30 min before the performance of an oral glucose tolerance test (OGTT)[30]. Adipose tissue glucose uptake, hepatic glucose uptake, hepatic glucose production and oral glucose absorption were assessed by positron emission tomography scan[30]. Exenatide, compared to placebo, markedly reduced oral glycose absorption and hepatic glycose production, resulting in minimal change in glucose serum concentration during the 2-h OGTT. In addition, treatment with exenatide reduced hepatic and adipose tissue insulin resistance and increased hepatic glucose uptake compared with placebo resulting in postprandial euglycemia[30]. The aforementioned findings were observed despite a decrease in insulin levels by exenatide compared with placebo[30].

In addition, a prospective randomized trial was conducted in order to clarify the effect of exenatide on ectopic fat accumulation[31]. Forty four obese patients with inadequately controlled T2DM were randomized to receive either exenatide (5 μg twice daily for 4 wk, followed by 10 μg twice daily for 22 wk) or reference antidiabetic treatment according to French guidelines[31]. Patients' hepatic, myocardial and pancreatic triglyceride content as well as epicardial adipose tissue were assessed by magnetic resonance imaging and magnetic resonance spectrometry (MRS) at baseline and after 26 wk of treatment[31]. In the exenatide group compared with the reference group, anthropometric parameters such as body weight, waist, thigh and hip circumference and laboratory values, such as fasting plasma insulin, total cholesterol and palmitoleic acid plasma levels, were decreased[31]. Moreover, a significant reduction in epicardial adipose tissue mass and liver fat content was observed in the exenatide compared with the reference group, and both correlated with weight loss[31].

In an open-label, parallel-group, uncontrolled, 6-mo study, the effect of exenatide on hepatic fat accumulation and liver enzymes was evaluated[32]. One hundred and twenty five patients with T2DM were divided into two groups: The first group received exenatide (10 μg twice daily) alone or in combination with other oral antidiabetic drugs while the second group received oral antidiabetic drugs without exenatide for 6 mos[32]. At the end of follow-up, group 1, compared with group 2, had reduced values of body mass index, waist circumference, alkaline phosphatase, ALT and intrahepatic fat accumulation (calculated by the fatty liver index)[32].

Of note, in a small prospective study, 25 obese patients with NAFLD and inadequately-controlled T2DM despite treatment with metformin and sulphonylureas or dipeptidyl-peptidase-IV inhibitors, received GLP-1RA (exenatide in 19 patients and liraglutide in six patients) for a period of 6 mo[33]. At the end of the study, GLP-1RA treatment resulted in a 7%-11% reduction in abdominal visceral adipose tissue and subcutaneous adipose tissue, while HbA_{1c}, ALT and γ-glutamyl-transferase (γGT) levels improved along with a marked increase in serum adiponectin levels[33]. In

Table 1 Characteristics and outcomes of clinical studies that evaluated the effects of exenatide, lisixenatide and dulaglutide on non-alcoholic fatty liver disease

Ref.	Type of study, country	Number of patients	Treatment	Effects on NAFLD
Gastaldelli *et al*[30], 2016	Randomized double-blind *vs* placebo/Pisa, Italy	15	Exenatide 5 μg *vs* placebo 30 min before a 75-g OGTT	Exenatide significantly ameliorated oral glucose absorption, hepatic glucose production, hepatic and adipose tissue insulin resistance, reduced insulin levels and increased hepatic glucose uptake
Dutour *et al*[31], 2016	Prospective randomized trial, France	44	Exenatide 5 μg twice daily for 4 wk, followed by 10 μg twice daily for additional 22 wk *vs* reference antidiabetic treatment according to French guidelines	Exenatide markedly reduced body weight, waist, thigh, hip circumference, fasting plasma insulin, total cholesterol and palmitoleic acid plasma levels
Blaslov *et al*[32], 2014	Open label parallel-group, uncontrolled study, Croatia	125	Exenatide (10 μg twice daily) on its own or in combination with other oral antidiabetic drugs *vs* other oral antidiabetic drugs without exenatide for 6 mo	Exenatide remarkably attenuated body mass index, waist circumference, ALP, ALT, intrahepatic fat accumulation assessed by fatty liver index
Cuthbertson *et al*[33], 2012	Prospective study, United Kingdom	25 [exenatide (n = 19), liraglutide (n = 6)]	Exenatide 5 μg twice daily titrated to 10 μg twice daily after one month; liraglutide 0.6 mg once daily, titrated to 1.2 mg once daily for 6 mo	GLP-1RA reduced, compared to baseline, abdominal visceral and subcutaneous adipose tissue, HbA1c, ALT, γ-GT and intrahepatic lipid content and increased adiponectin serum levels
Fan *et al*[34], 2013	Randomized clinical trial, China	117	Exenatide (5 μg for four weeks followed by 10 μg for additional 8 wk, two times daily) *vs* metformin (0.5-2 g/d)	Exenatide decreased body weight, waist-to-hip ratio, ALT, AST, AST/ALT ratio, γ-GT, 2-h postprandial glucose serum levels, CRP and increased adiponectin serum levels
Savvidou *et al*[35], 2016	Open label, randomized controlled intervention trial, Greece	120	Exenatide 5 μg twice daily for 4 wk and 10 μg twice daily as supplementation on glargine insulin *vs* intense self-regulated insulin therapy for 6 mo	Both therapies significantly increased adiponectin serum levels compared to baseline, but no significant change between the groups; Exenatide, compared to insulin group, reduced more robustly body weight but not HbA1c
Shao *et al*[37], 2014	Randomized controlled trial, China	60	Exenatide 5 μg twice daily, followed by 10 μg twice daily for additional 8 wk plus insulin glargine *vs* intensive insulin therapy with insulin glargine and insulin as part for a time period of 12 wk	Body weight, waist circumference, ALT, AST, γ-GT were markedly reduced in exenatide compared to insulin group, while levels of fasting blood glucose, postprandial blood glucose, HbA1c, triglyceride and total bilirubin were significantly reduced at both groups at 12 wk, compared to baseline
Bi *et al*[38], 2014	Randomized controlled trial, China	33	Exenatide 5 μg twice daily for 4 wk, followed by maximum 10 μg twice daily for 20 wk *vs* insulin *vs* pioglitazone 30 mg daily, titrated to 45 mg at fourth week, 6 mo study	Exenatide reduced, compared to baseline, intrahepatic fat, visceral and subcutaneous fat volumes, body weight, waist circumference, serum triglycerides, HbA1c, TNF-a
Sathyanarayana *et al*[39], 2011	Randomized controlled study, United States	21	Exenatide 10 μg twice daily plus pioglitazone 45 mg/d *vs* pioglitazone 45 mg/d for 12 mo	Combination pharmacotherapy with exenatide, compared to pioglitazone, significantly decreased serum ALT and triglyceride levels as well as intrahepatic fat content and increased adiponectin plasma levels
Gluud *et al*[41], 2014	Review, Denmark	15 studies included in this meta-analysis	12 randomized clinical trials on lisixenatide *vs* placebo and 3 randomized clinical trials on lisixenatide *vs* liraglutide, exenatide or sitagliptin	Lisixenatide markedly increased the proportion of overweight or obese patients with T2DM who achieved ALT levels normalization
Seko *et al*[42], 2017	Retrospective study, Japan	15	Dulaglutide 0.75 mg once weekly for 12 wk	Dulaglutide, compared to baseline, reduced body weight, ALT, AST, HbA1c and liver stiffness
Ghosh *et al*[43], 2019	Retrospective study, India	85 T2DM overweight patients	Dulaglutide 1.5 mg once weekly for 20 wk	Dulaglutide led to significant reductions in HbA1c, body weight, ALT and AST levels
Cusi *et al*[44], 2018	Post hoc analysis, multicenter	4 randomized, placebo-controlled trials with 1499 T2DM patients	Dulaglutide 1.5 mg once weekly for 6 mo	Dulaglutide, compared to placebo, significantly decreased ALT, AST, γ-GT, particularly in patients with elevated transaminase levels at the onset of the study

NAFLD: Non-alcoholic fatty liver disease; OGTT: Oral glucose tolerance test; ALP: Alkaline phosphatase; ALT: Alanine transaminase; GLP-1RA: Glucagon-like peptide-1 receptor agonists; AST: Aspartate aminotransferase; CRP: C-reactive protein; T2DM: Type 2 diabetes mellitus; γ-GT: γ-glutamyl-transferase.

parallel, intrahepatic lipid content, evaluated by MRS, was reduced by 42% at 24 wk compared with baseline, a change that correlated with HbA_{1c} reduction during the same time period[33].

In a larger study including 117 patients with T2DM and NAFLD, Fan *et al*[34] assessed the impact of exenatide on anthropometric and laboratory values. The patients were randomly assigned to receive either exenatide (5 μg for 4 wk followed by 10 μg for 8 wk, two times daily) or metformin (0.5-2 g/d)[34]. At the end of follow-up (12 wk), in the exenatide group, compared with the metformin group, body weight, waist-to-hip ratio, ALT, aspartate aminotransferase (AST), AST to ALT ratio, γGT and 2-h postprandial glucose serum levels were markedly reduced[34]. Interestingly, high-sensitivity C-reactive protein (hsCRP) levels, a marker of subclinical inflammation, were improved and adiponectin serum levels were significantly increased in the exenatide group compared to the metformin group, and these changes might have played a role in the reduction in transaminase levels[34].

Indeed, adiponectin appears to exert a hepato-protective effect in patients with NAFLD[35]. Exendin-4 also appears to protect hepatocytes from steatosis through autophagy and reduction of apoptosis associated with ER stress[36]. The latter is associated with intrahepatic fat accumulation, but autophagy has a protective role on cell survival[36]. Accumulation of fatty acids is related to ER stress, cell death, apoptosis and elevated caspase-3 levels, while administration of exendin-4 reduces caspase-3 levels[36]. In another study, patients with NAFLD who were treated with exenatide had lower levels of AST, ALT and γGT, compared with patients treated with insulin[37]. Exenatide also induced a reduction of intrahepatic fat, visceral fat and subcutaneous fat[38]. In a small study in 21 patients, the combination of exenatide and pioglitazone resulted in a reduction in intrahepatic fat content, serum ALT and triglyceride levels and in an increase in plasma adiponectin levels[39].

LIXISENATIDE AND DULAGLUTIDE

There are limited data regarding the effects of lixisenatide and dulaglutide on NAFLD (Table 1). In a study in conscious dogs, lixisenatide did not affect hepatic glucose uptake[40]. In a meta-analysis of 12 randomized controlled trials, lixisenatide increased the proportion of obese or overweight patients with T2DM who achieved normalization of ALT levels[41]. On the other hand, the administration of dulaglutide for 12 wk at a dose of 0.75 mg once weekly in patients with NAFLD reduced HbA_{1c} levels, body weight, transaminases and liver stiffness[42]. In another study in 85 overweight patients with inadequately controlled T2DM conducted in India, treatment

with dulaglutide 1.5 mg once weekly for 20 wk resulted in significant reductions in HbA_{1c}, body weight, ALT and AST levels[43]. Also, in a post hoc analysis of four randomized, placebo-controlled trials in patients with T2DM (n = 1499), dulaglutide decreased transaminase and γGT levels compared with placebo, particularly in patients with elevated transaminase levels at baseline[44].

LIRAGLUTIDE

In a prospective study, liraglutide was administered for 6 mo in 19 women with polycystic ovary syndrome and controls[45]. Serum procollagen type 3 amino-terminal peptide levels, a marker of hepatic fibrosis, decreased in patients with polycystic ovary syndrome but not in controls[45]. In another study in 26 patients with glucose intolerance and biopsy-proven NASH, treatment with liraglutide for 24 wk reduced ALT levels[46]. Ten patients were treated with liraglutide for 96 wk, and liver biopsy at the end of treatment showed an improvement in liver histology in six of them[46]. In a retrospective study that included 46 patients, the liver to kidney attenuation ratio in computed tomography (an index of hepatic steatosis) increased after treatment with liraglutide 0.9 mg/d for 6 mo[47]. Another retrospective analysis of 82 patients with NAFLD who were treated with sitagliptin, liraglutide or pioglitazone revealed that patients who received sitagliptin showed a decrease in ALT activity whereas the AST to platelet count ratio index (APRI score), a marker of liver fibrosis, did not change[48]. In contrast, patients treated with liraglutide or pioglitazone experienced a decrease in both ALT activity and APRI[48]. In a subgroup analysis of the Liraglutide Effect and Action in Diabetes-2 trial, 103 patients were treated with liraglutide 0.6, 1.2 and 1.8 mg/d, 37 patients received glimepiride and 20 were given placebo for 26 wk[49]. Liver to spleen attenuation ratio increased in patients treated with liraglutide 1.8 mg but did not change in those treated with lower doses of liraglutide or with glimepiride[49]. ALT activity showed comparable decreases with both agents[49]. In a more recent study, 30 non-diabetic patients with abdominal obesity and NAFLD were managed with liraglutide or with lifestyle modification[50]. Liraglutide was effective in decreasing weight, hepatic steatosis and hepatocellular apoptosis, but benefits were not sustained after discontinuation of treatment, in contrast with lifestyle modification[50].

In another study, 87 patients with T2DM and NAFLD were randomized to receive liraglutide, metformin or gliclazide for 24 wk[51]. Gliclazide resulted in smaller improvement in liver function and less reduction in intrahepatic fat content, HbA_{1c} levels and body weight compared with liraglutide and metformin[51]. Slightly greater improvements were achieved with liraglutide than with metformin[51]. In a single-center, randomized, open-label study in 19 patients with T2DM, liraglutide reduced visceral fat at 24 wk[52]. Urinary albumin-to-creatinine ratio and hsCRP levels were also significantly reduced by liraglutide at 12 and 24 wk[52]. HbA_{1c} levels, body weight and hepatic fat also decreased in patients treated with liraglutide[52]. In a prospective trial in 68 patients with uncontrolled T2DM, treatment with liraglutide for 6 mo was associated with a decrease in body weight and HbA_{1c} and a reduction in liver fat content[53].

A multicenter, double-blind, randomized, placebo-controlled, phase 2 trial was conducted in four United Kingdom medical centers to compare liraglutide with placebo in overweight patients who showed clinical evidence of NASH[54]. Nine (39%) of 23 patients who received liraglutide and underwent end-of-treatment liver biopsy had resolution of definite NASH compared with two (9%) of 22 patients in the placebo group[54]. In another study in patients with T2DM, treatment with liraglutide or sitagliptin for 12 wk did not reduce hepatic steatosis, which was estimated using MRS[55]. In a study in China, which enrolled 835 patients with T2DM, liraglutide improved blood glucose levels, lipid levels and liver function[56]. In a similar study, which compared treatment of T2DM with liraglutide or metformin, liraglutide was more effective in alleviating liver inflammation and improving liver function[57]. Finally, in a prospective study (n = 25), treatment with either exenatide or liraglutide for 6 mo decreased ALT activity and hepatic fat content (evaluated with MRS)[33] (Table 2).

SEMAGLUTIDE

Recently, Newsome *et al*[58] evaluated the effects of semaglutide on liver biochemistry (ALT) and hsCRP levels in patients at risk for NAFLD. The authors analyzed data

Table 2 Characteristics and outcomes of clinical studies that evaluated the effects of liraglutide and semaglutide on non-alcoholic fatty liver disease

Ref.	Type of study; country	Number of patients	Treatment	Effects on NAFLD
Kahal *et al*[45], 2014	Prospective; United Kingdom	36	Liraglutide 0.9 mg/d for 6 mo	Serum procollagen type 3 amino-terminal peptide levels, a marker of hepatic fibrosis, decreased in women with PCOS
Eguchi *et al*[46], 2014	Prospective; Japan	26	Liraglutide 0.9 mg/d for 24-96 wk	ALT activity decreased. NASH decreased in 6/10 patients who underwent repeat biopsy at 96 wk
Suzuki *et al*[47], 2013	Retrospective; Japan	46	Liraglutide 0.9 mg/d for 6 mo	Liver to kidney attenuation ratio in CT (an index of hepatic steatosis) increased
Ohki *et al*[48], 2012	Retrospective; Japan	82	Liraglutide 0.9 mg/d for 340 d or sitagliptin 50-100 mg/d for 250 d or pioglitazone 15 mg/d for 1200 d	ALT activity was reduced with all agents. Liraglutide and pioglitazone but not sitagliptin reduced the APRI score
Jendle *et al*[49], 2009	Randomized controlled; multicenter	160	Liraglutide 0.6, 1.2 or 1.8 mg/d or glimepiride 4 mg/d or placebo for 26 wk	Liver to spleen attenuation ratio in CT (a marker of hepatic steatosis) increased in patients treated with liraglutide 1.8 mg/d and did not change in those treated with lower doses of liraglutide or glimepiride. ALT activity showed comparable decreases with both agents
Khoo *et al*[50], 2009	Randomized controlled; Singapore	30	Liraglutide 3 mg/d for 16 wk or lifestyle modification	Liraglutide was effective for decreasing weight, hepatic steatosis and hepatocellular apoptosis, but benefits were not sustained after discontinuation, in contrast with lifestyle modification
Feng *et al*[51], 2017	Randomized controlled; China	87	Liraglutide, metformin, or gliclazide for 24 wk	Liraglutide has better results in improving liver function, reductions in intrahepatic fat content and HbA1c level, and weight loss than metformin and gliclazide
Bouchi *et al*[52], 2017	Randomized controlled; Japan	19	Liraglutide 0.9 mg/d plus insulin or insulin alone for 14 wk	Liraglutide reduces visceral fat, hepatic fat accumulation, albuminuria and micro-inflammation and improves QOL
Petit *et al*[53], 2017	Prospective; France	68	Liraglutide 1.2 mg/d for 6 mo	Liraglutide significantly reduced liver fat content
Armstrong *et al*[54], 2016	Double-blind, randomized, controlled; multicenter United Kingdom	52	Liraglutide 1.8 mg/d or placebo for 48 wk	Liraglutide led to histological resolution of NASH
Smits *et al*[55], 2016	Randomized placebo-controlled; Holland	52	Liraglutide 1.8 mg/d, sitagliptin 100 mg/d or placebo	Liraglutide or sitagliptin treatment does not reduce hepatic steatosis or fibrosis
Zhang *et al*[56], 2016	Randomized controlled; China	835	Liraglutide 1.2 mg/d or metformin 500 mg/3 times per day	Liraglutide improves the blood glucose and lipid levels as well as liver function
Tian *et al*[57], 2018	Randomized controlled; China	127	Liraglutide 0.6-1.2 mg/d or metformin 1000-1500 mg/d for 12 wk	Liraglutide decreases ALT levels and is more effective than metformin at alleviating liver inflammation and improving liver function
Cuthbertson *et al*[33], 2012	Prospective; United Kingdom	25	Exenatide 10 mg twice daily or liraglutide 1.2 mg/d	Both liraglutide and exenatide reduce body weight, HbA1c and intrahepatic lipid accumulation
Newsome *et al*[58], 2019	Retrospective (data from 2 trials); United Kingdom	957 (trial 1) and 3297 (trial 2)	Semaglutide 0.05, 0.1, 0.2, 0.3 or 0.4 mg/d for 52 wk (trial 1) and semaglutide 0.5 or 1.0 mg/wk for 104 wk (trial 2)	Semaglutide significantly reduced ALT and hsCRP in clinical trials in subjects with obesity and/or type 2 diabetes

PCOS: Polycystic ovary syndrome; NAFLD: Non-alcoholic fatty liver disease; ALT: Alanine transaminase; hsCRP: High-sensitivity C-reactive protein; NASH: Non-alcoholic steatohepatitis; CT: Computed tomography; APRI: AST to platelet count ratio index.

from two randomized, double-blind, multinational, placebo-controlled trials: A) A 104-wk cardiovascular outcomes trial, in which semaglutide 0.5 or 1.0 mg was given once weekly subcutaneously in T2DM patients with HbA_{1c} levels ≥ 7% (SUSTAIN-6 trial) and B) A 52-wk weight management trial, in which semaglutide 0.05-0.4 mg was given daily subcutaneously in obese patients without T2DM[58]. Among patients (n = 499, 52%) with abnormal ALT levels (men > 30 IU/L, women > 19 IU/L) at baseline, ALT reductions were observed in 6%-21% of patients at doses ≥ 0.2 mg/d) (P < 0.05 *vs* placebo) in the weight management trial. Similarly, hsCRP reductions were recorded in 25%-43% of patients receiving semaglutide 0.2 and 0.4 mg/d (P < 0.05 *vs* placebo)[58]. Among those who had abnormal ALT levels and received semaglutide 0.4 mg in the weight management trial, the prevalence of metabolic syndrome was reduced (25.6% at week 28 *vs* 50.0% at baseline)[58]. Normalization of elevated baseline ALT occurred in 25%-46% of patients in the weight management trial in a dose dependent manner (*vs* 18% in placebo group), while in the SUSTAIN-6 trial, reductions in ALT levels were recorded only at the 1.0 mg dose (9% *vs* placebo, P = 0.0024) at week 104[58]. However, changes in ALT and hsCRP levels were not significant after adjustment for weight change. Histological data are awaited from an ongoing phase 2 trial of semaglutide in biopsy-proven NASH (NCT02970942)[58] (Table 2).

CONCLUSION

Both animal and clinical studies are highly promising for the beneficial effect of GLP-1RAs in patients with NAFLD. Importantly, GLP-1RAs have good safety profile, since the most common adverse events are nausea and diarrhea, while the risk of pancreatitis is very small and not confirmed in a recent meta-analysis[59]. Among GLP-1RAs, liraglutide has been studied more extensively in the setting of NAFLD, leading to amelioration in both hepatic and visceral fat accumulation as well as improvement in liver function tests and histological lesions in patients with NAFLD[46,51]. Nonetheless, the need for daily injection is a major limitation, presumably affecting patients' medication compliance. Long-acting GLP1-RAs, such as dulaglutide and semaglutide, seem an appealing therapeutic option. Dulaglutide pharmacotherapy combines the beneficial effects of short-acting liraglutide on ameliorating anthropometric and laboratory parameters, such as body mass and ALT serum levels respectively, with the significant advantage of weekly injection administration[42,60]. Beyond the latter advantage, disposable and prefilled devises for dulaglutide medication are also available[60]. Regarding semaglutide, appears to have some

additional advantages to other GLP-1RA agents. Based on data from SUSTAIN-6, a placebo-controlled trial, semaglutide medication led to marked prevention of cardiovascular events, the predominant cause of mortality among NAFLD patients, while SUSTAIN-7 trial demonstrated the superiority of semaglutide over dulaglutide regarding glucose control and body weight reduction among T2DM patients[61,62]. Of great interest, the recent Food and Drug Administration approval of oral semaglutide taken once a day for T2DM, might become the first-line approach for patients with both T2DM and NAFLD. Of note, a plethora of data concerning the efficacy of GLP-1RAs on NAFLD is based on exenatide. Indeed, exenatide exerts hepato-protective as well as glucose-lowering actions combined with remarkable amelioration of anthropometric parameters and liver dysfunction markers[30,34]. However, similarly to liraglutide pharmacotherapy, the need for twice daily administration therapy appears as a significant limitation[63]. On the other hand, pharmacotherapy with lisixenatide requires once daily administration and beyond that, its tolerability profile seems to be better than exenatide, since T2DM patients treated with lisixenatde experienced markedly less nausea than the corresponding exenatide treated group of T2DM patients[64]. Nevertheless, more data with lisixenatide efficacy on NAFLD modification are required in order to consider the aforementioned drug as a propitious therapeutic opportunity. In conclusion, it seems that GLP-1RA administration in patients with T2DM is an attractive therapeutic option associated with weight loss, glycemic control and potentially reversal of biochemical and/or histological features of NAFLD in patients with concomitant NAFLD. However, larger, long-term, randomized, controlled trials should be conducted to better define the role of these agents in the management of NAFLD.

REFERENCES

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

2 **Neuschwander-Tetri BA**. Non-alcoholic fatty liver disease. *BMC Med* 2017; **15**: 45 [PMID: 28241825 DOI: 10.1186/s12916-017-0806-8]

3 **Abdallah LR**, de Matos RC, E Souza YPDM, Vieira-Soares D, Muller-Machado G, Pollo-Flores P. Non-alcoholic Fatty Liver Disease and Its Links with Inflammation and Atherosclerosis. *Curr Atheroscler Rep* 2020; **22**: 7 [PMID: 32020371 DOI: 10.1007/s11883-020-0820-8]

4 **Guo Z**, Li M, Han B, Qi X. Association of non-alcoholic fatty liver disease with thyroid function: A systematic review and meta-analysis. *Dig Liver Dis* 2018; **50**: 1153-1162 [PMID: 30224316 DOI: 10.1016/j.dld.2018.08.012]

5 **Eshraghian A**, Hamidian Jahromi A. Non-alcoholic fatty liver disease and thyroid dysfunction: a systematic review. *World J Gastroenterol* 2014; **20**: 8102-8109 [PMID: 25009382 DOI: 10.3748/wjg.v20.i25.8102]

6 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]

7 **Trevaskis JL**, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, Erickson MR, Napora J, Parkes DG, Roth JD. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G762-G772 [PMID: 22268099 DOI: 10.1152/ajpgi.00476.2011]

8 **Nauck M**. Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. *Diabetes Obes Metab* 2016; **18**: 203-216 [PMID: 26489970 DOI: 10.1111/dom.12591]

9 **Lee J**, Hong SW, Chae SW, Kim DH, Choi JH, Bae JC, Park SE, Rhee EJ, Park CY, Oh KW, Park SW, Kim SW, Lee WY. Exendin-4 improves steatohepatitis by increasing Sirt1 expression in high-fat diet-induced obese C57BL/6J mice. *PLoS One* 2012; **7**: e31394 [PMID: 22363635 DOI: 10.1371/journal.pone.0031394]

10 **Gupta NA**, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, Anania FA. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. *Hepatology* 2010; **51**: 1584-1592 [PMID: 20225248 DOI: 10.1002/hep.23569]

11 **Bernsmeier C**, Meyer-Gerspach AC, Blaser LS, Jeker L, Steinert RE, Heim MH, Beglinger C. Glucose-induced glucagon-like Peptide 1 secretion is deficient in patients with non-alcoholic fatty liver disease. *PLoS One* 2014; **9**: e87488 [PMID: 24489924 DOI: 10.1371/journal.pone.0087488]

12 **Niu S**, Wang L, He M, Peng Y, Li S. Exendin-4 regulates redox homeostasis in rats fed with high-fat diet. *Acta Biochim Biophys Sin (Shanghai)* 2015; **47**: 397-403 [PMID: 25910576 DOI: 10.1093/abbs/gmv027]

13 **Allam MM**, El Gazzar WB. Exendin-4, a glucagon-like peptide-1 receptor agonist downregulates hepatic receptor for advanced glycation end products in non-alcoholic steatohepatitis rat model. *Arch Physiol Biochem* 2018; **124**: 10-17 [PMID: 28696785 DOI: 10.1080/13813455.2017.1348362]

14 **Ding X**, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 2006; **43**: 173-181 [PMID: 16374859 DOI: 10.1002/hep.21006]

15 **Li L**, Yang G, Li Q, Tan X, Liu H, Tang Y, Boden G. Exenatide prevents fat-induced insulin resistance and raises adiponectin expression and plasma levels. *Diabetes Obes Metab* 2008; **10**: 921-930 [PMID: 18093209 DOI: 10.1111/j.1463-1326.2007.00832.x]

16 **Svegliati-Baroni G**, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, Faraci G, Pacetti D, Vivarelli M, Nicolini D, Garelli P, Casini A, Manco M, Mingrone G, Risaliti A, Frega GN, Benedetti A, Gastaldelli A. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver Int* 2011; **31**: 1285-1297 [PMID: 21745271 DOI: 10.1111/j.1478-3231.2011.02462.x]

17 **Shao N**, Yu XY, Ma XF, Lin WJ, Hao M, Kuang HY. Exenatide Delays the Progression of Nonalcoholic Fatty Liver Disease in C57BL/6 Mice, Which May Involve Inhibition of the NLRP3 Inflammasome through the Mitophagy Pathway. *Gastroenterol Res Pract* 2018; **2018**: 1864307 [PMID: 29849583 DOI: 10.1155/2018/1864307]

18 **Wang Y**, Parlevliet ET, Geerling JJ, van der Tuin SJ, Zhang H, Bieghs V, Jawad AH, Shiri-Sverdlov R, Bot I, de Jager SC, Havekes LM, Romijn JA, Willems van Dijk K, Rensen PC. Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. *Br J Pharmacol* 2014; **171**: 723-734 [PMID: 24490861 DOI: 10.1111/bph.12490]

19 **Kawaguchi T**, Itou M, Taniguchi E, Sata M. Exendin-4, a glucagon-like peptide-1 receptor agonist, modulates hepatic fatty acid composition and Δ-5-desaturase index in a murine model of non-alcoholic steatohepatitis. *Int J Mol Med* 2014; **34**: 782-787 [PMID: 24993337 DOI: 10.3892/ijmm.2014.1826]

20 **Parlevliet ET**, Wang Y, Geerling JJ, Schröder-Van der Elst JP, Picha K, O'Neil K, Stojanovic-Susulic V, Ort T, Havekes LM, Romijn JA, Pijl H, Rensen PC. GLP-1 receptor activation inhibits VLDL production and reverses hepatic steatosis by decreasing hepatic lipogenesis in high-fat-fed APOE*3-Leiden mice. *PLoS One* 2012; **7**: e49152 [PMID: 23133675 DOI: 10.1371/journal.pone.0049152]

21 **Lee J**, Hong SW, Park SE, Rhee EJ, Park CY, Oh KW, Park SW, Lee WY. Exendin-4 regulates lipid metabolism and fibroblast growth factor 21 in hepatic steatosis. *Metabolism* 2014; **63**: 1041-1048 [PMID: 24933399 DOI: 10.1016/j.metabol.2014.04.011]

22 **Yamamoto T**, Nakade Y, Yamauchi T, Kobayashi Y, Ishii N, Ohashi T, Ito K, Sato K, Fukuzawa Y, Yoneda M. Glucagon-like peptide-1 analogue prevents nonalcoholic steatohepatitis in non-obese mice. *World J Gastroenterol* 2016; **22**: 2512-2523 [PMID: 26937139 DOI: 10.3748/wjg.v22.i8.2512]

23 **Li S**, Wang X, Zhang J, Li J, Liu X, Ma Y, Han C, Zhang L, Zheng L. Exenatide ameliorates hepatic steatosis and attenuates fat mass and FTO gene expression through PI3K signaling pathway in nonalcoholic fatty liver disease. *Braz J Med Biol Res* 2018; **51**: e7299 [PMID: 29924135 DOI: 10.1590/1414-431x20187299]

24 **Zheng X**, Xu F, Liang H, Cao H, Cai M, Xu W, Weng J. SIRT1/HSF1/HSP pathway is essential for exenatide-alleviated, lipid-induced hepatic endoplasmic reticulum stress. *Hepatology* 2017; **66**: 809-824 [PMID: 28439947 DOI: 10.1002/hep.29238]

25 **Xu F**, Li Z, Zheng X, Liu H, Liang H, Xu H, Chen Z, Zeng K, Weng J. SIRT1 mediates the effect of GLP-1 receptor agonist exenatide on ameliorating hepatic steatosis. *Diabetes* 2014; **63**: 3637-3646 [PMID: 24947350 DOI: 10.2337/db14-0263]

26 **Kalavalapalli S**, Bril F, Guingab J, Vergara A, Garrett TJ, Sunny NE, Cusi K. Impact of exenatide on mitochondrial lipid metabolism in mice with nonalcoholic steatohepatitis. *J Endocrinol* 2019; **241**: 293-305 [PMID: 31082799 DOI: 10.1530/JOE-19-0007]

27 **Tanaka K**, Masaki Y, Tanaka M, Miyazaki M, Enjoji M, Nakamuta M, Kato M, Nomura M, Inoguchi T, Kotoh K, Takayanagi R. Exenatide improves hepatic steatosis by enhancing lipid use in adipose tissue in nondiabetic rats. *World J Gastroenterol* 2014; **20**: 2653-2663 [PMID: 24627601 DOI: 10.3748/wjg.v20.i10.2653]

28 **Patel V**, Joharapurkar A, Kshirsagar S, Patel M, Sutariya B, Patel H, Pandey D, Patel D, Ranvir R, Kadam S, Bahekar R, Jain M. Coagonist of glucagon-like peptide-1 and glucagon receptors ameliorates nonalcoholic fatty liver disease. *Can J Physiol Pharmacol* 2018; **96**: 587-596 [PMID: 29406832 DOI: 10.1139/cjpp-2017-0683]

29 **Dhanesha N**, Joharapurkar A, Shah G, Kshirsagar S, Patel V, Patel K, Bahekar R, Jain M. Treatment with exendin-4 improves the antidiabetic efficacy and reverses hepatic steatosis in glucokinase activator treated db/db mice. *Eur J Pharmacol* 2013; **714**: 188-192 [PMID: 23810686 DOI: 10.1016/j.ejphar.2013.06.015]

30 **Gastaldelli A**, Gaggini M, Daniele G, Ciociaro D, Cersosimo E, Tripathy D, Triplitt C, Fox P, Musi N, DeFronzo R, Iozzo P. Exenatide improves both hepatic and adipose tissue insulin resistance: A dynamic positron emission tomography study. *Hepatology* 2016; **64**: 2028-2037 [PMID: 27639082 DOI: 10.1002/hep.28827]

31 **Dutour A**, Abdesselam I, Ancel P, Kober F, Mrad G, Darmon P, Ronsin O, Pradel V, Lesavre N, Martin JC, Jacquier A, Lefur Y, Bernard M, Gaborit B. Exenatide decreases liver fat content and epicardial adipose tissue in patients with obesity and type 2 diabetes: a prospective randomized clinical trial using magnetic resonance imaging and spectroscopy. *Diabetes Obes Metab* 2016; **18**: 882-891 [PMID: 27106272 DOI: 10.1111/dom.12680]

32 **Blaslov K**, Zibar K, Bulum T, Duvnjak L. Effect of exenatide therapy on hepatic fat quantity and hepatic biomarkers in type 2 diabetic patients. *Clin Res Hepatol Gastroenterol* 2014; **38**: e61-e63 [PMID: 24315013 DOI: 10.1016/j.clinre.2013.10.013]

33 **Cuthbertson DJ**, Irwin A, Gardner CJ, Daousi C, Purewal T, Furlong N, Goenka N, Thomas EL, Adams VL, Pushpakom SP, Pirmohamed M, Kemp GJ. Improved glycaemia correlates with liver fat reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor agonists. *PLoS One* 2012; **7**: e50117 [PMID: 23236362 DOI: 10.1371/journal.pone.0050117]

34 **Fan H**, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. *Arq Bras Endocrinol Metabol* 2013; **57**: 702-708 [PMID: 24402015 DOI: 10.1590/s0004-27302013000900005]

35 **Savvidou S**, Karatzidou K, Tsakiri K, Gagalis A, Hytiroglou P, Goulis J. Circulating adiponectin levels in type 2 diabetes mellitus patients with or without non-alcoholic fatty liver disease: Results of a small, open-label, randomized controlled intervention trial in a subgroup receiving short-term exenatide. *Diabetes Res*

Clin Pract 2016; **113**: 125-134 [PMID: 26803355 DOI: 10.1016/j.diabres.2015.12.003]
36 **Sharma S**, Mells JE, Fu PP, Saxena NK, Anania FA. GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. *PLoS One* 2011; **6**: e25269 [PMID: 21957486 DOI: 10.1371/journal.pone.0025269]
37 **Shao N**, Kuang HY, Hao M, Gao XY, Lin WJ, Zou W. Benefits of exenatide on obesity and non-alcoholic fatty liver disease with elevated liver enzymes in patients with type 2 diabetes. *Diabetes Metab Res Rev* 2014; **30**: 521-529 [PMID: 24823873 DOI: 10.1002/dmrr.2561]
38 **Bi Y**, Zhang B, Xu W, Yang H, Feng W, Li C, Tong G, Li M, Wang X, Shen S, Zhu B, Weng J, Zhu D. Effects of exenatide, insulin, and pioglitazone on liver fat content and body fat distributions in drug-naive subjects with type 2 diabetes. *Acta Diabetol* 2014; **51**: 865-873 [PMID: 25118999 DOI: 10.1007/s00592-014-0638-3]
39 **Sathyanarayana P**, Jogi M, Muthupillai R, Krishnamurthy R, Samson SL, Bajaj M. Effects of combined exenatide and pioglitazone therapy on hepatic fat content in type 2 diabetes. *Obesity (Silver Spring)* 2011; **19**: 2310-2315 [PMID: 21660077 DOI: 10.1038/oby.2011.152]
40 **Moore MC**, Werner U, Smith MS, Farmer TD, Cherrington AD. Effect of the glucagon-like peptide-1 receptor agonist lixisenatide on postprandial hepatic glucose metabolism in the conscious dog. *Am J Physiol Endocrinol Metab* 2013; **305**: E1473-E1482 [PMID: 24148347 DOI: 10.1152/ajpendo.00354.2013]
41 **Gluud LL**, Knop FK, Vilsbøll T. Effects of lixisenatide on elevated liver transaminases: systematic review with individual patient data meta-analysis of randomised controlled trials on patients with type 2 diabetes. *BMJ Open* 2014; **4**: e005325 [PMID: 25526792 DOI: 10.1136/bmjopen-2014-005325]
42 **Seko Y**, Sumida Y, Tanaka S, Mori K, Taketani H, Ishiba H, Hara T, Okajima A, Umemura A, Nishikawa T, Yamaguchi K, Moriguchi M, Kanemasa K, Yasui K, Imai S, Shimada K, Itoh Y. Effect of 12-week dulaglutide therapy in Japanese patients with biopsy-proven non-alcoholic fatty liver disease and type 2 diabetes mellitus. *Hepatol Res* 2017; **47**: 1206-1211 [PMID: 27917557 DOI: 10.1111/hepr.12837]
43 **Ghosh A**, Nair R. Improved Clinical Outcomes with Dulaglutide as Add-on Medication to Oral Antidiabetic Drugs with or Without Insulin in Overweight Indian Patients with Type 2 Diabetes Mellitus: Retrospective Study in a Real-World Setting. *Curr Diabetes Rev* 2020; **16**: 490-496 [PMID: 31686642 DOI: 10.2174/1573399815666191104115449]
44 **Cusi K**, Sattar N, García-Pérez LE, Pavo I, Yu M, Robertson KE, Karanikas CA, Haupt A. Dulaglutide decreases plasma aminotransferases in people with Type 2 diabetes in a pattern consistent with liver fat reduction: a post hoc analysis of the AWARD programme. *Diabet Med* 2018; **35**: 1434-1439 [PMID: 29869810 DOI: 10.1111/dme.13697]
45 **Kahal H**, Abouda G, Rigby AS, Coady AM, Kilpatrick ES, Atkin SL. Glucagon-like peptide-1 analogue, liraglutide, improves liver fibrosis markers in obese women with polycystic ovary syndrome and nonalcoholic fatty liver disease. *Clin Endocrinol (Oxf)* 2014; **81**: 523-528 [PMID: 24256515 DOI: 10.1111/cen.12369]
46 **Eguchi Y**, Kitajima Y, Hyogo H, Takahashi H, Kojima M, Ono M, Araki N, Tanaka K, Yamaguchi M, Matsuda Y, Ide Y, Otsuka T, Ozaki I, Ono N, Eguchi T, Anzai K; Japan Study Group for NAFLD (JSG-NAFLD). Pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients (LEAN-J). *Hepatol Res* 2015; **45**: 269-278 [PMID: 24796231 DOI: 10.1111/hepr.12351]
47 **Suzuki D**, Toyoda M, Kimura M, Miyauchi M, Yamamoto N, Sato H, Tanaka E, Kuriyama Y, Miyatake H, Abe M, Umezono T, Fukagawa M. Effects of liraglutide, a human glucagon-like peptide-1 analogue, on body weight, body fat area and body fat-related markers in patients with type 2 diabetes mellitus. *Intern Med* 2013; **52**: 1029-1034 [PMID: 23676586 DOI: 10.2169/internalmedicine.52.8961]
48 **Ohki T**, Isogawa A, Iwamoto M, Ohsugi M, Yoshida H, Toda N, Tagawa K, Omata M, Koike K. The effectiveness of liraglutide in nonalcoholic fatty liver disease patients with type 2 diabetes mellitus compared to sitagliptin and pioglitazone. *ScientificWorldJournal* 2012; **2012**: 496453 [PMID: 22927782 DOI: 10.1100/2012/496453]
49 **Jendle J**, Nauck MA, Matthews DR, Frid A, Hermansen K, Düring M, Zdravkovic M, Strauss BJ, Garber AJ; LEAD-2 and LEAD-3 Study Groups. Weight loss with liraglutide, a once-daily human glucagon-like peptide-1 analogue for type 2 diabetes treatment as monotherapy or added to metformin, is primarily as a result of a reduction in fat tissue. *Diabetes Obes Metab* 2009; **11**: 1163-1172 [PMID: 19930006 DOI: 10.1111/j.1463-1326.2009.01158.x]
50 **Khoo J**, Hsiang JC, Taneja R, Koo SH, Soon GH, Kam CJ, Law NM, Ang TL. Randomized trial comparing effects of weight loss by liraglutide with lifestyle modification in non-alcoholic fatty liver disease. *Liver Int* 2019; **39**: 941-949 [PMID: 30721572 DOI: 10.1111/liv.14065]
51 **Feng W**, Gao C, Bi Y, Wu M, Li P, Shen S, Chen W, Yin T, Zhu D. Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease. *J Diabetes* 2017; **9**: 800-809 [PMID: 28332301 DOI: 10.1111/1753-0407.12555]
52 **Bouchi R**, Nakano Y, Fukuda T, Takeuchi T, Murakami M, Minami I, Izumiyama H, Hashimoto K, Yoshimoto T, Ogawa Y. Reduction of visceral fat by liraglutide is associated with ameliorations of hepatic steatosis, albuminuria, and micro-inflammation in type 2 diabetic patients with insulin treatment: a randomized control trial. *Endocr J* 2017; **64**: 269-281 [PMID: 27916783 DOI: 10.1507/endocrj.EJ16-0449]
53 **Petit JM**, Cercueil JP, Loffroy R, Denimal D, Bouillet B, Fourmont C, Chevallier O, Duvillard L, Vergès B. Effect of Liraglutide Therapy on Liver Fat Content in Patients With Inadequately Controlled Type 2 Diabetes: The Lira-NAFLD Study. *J Clin Endocrinol Metab* 2017; **102**: 407-415 [PMID: 27732328 DOI: 10.1210/jc.2016-2775]
54 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]
55 **Smits MM**, Tonneijck L, Muskiet MH, Kramer MH, Pouwels PJ, Pieters-van den Bos IC, Hoekstra T, Diamant M, van Raalte DH, Cahen DL. Twelve week liraglutide or sitagliptin does not affect hepatic fat in

type 2 diabetes: a randomised placebo-controlled trial. *Diabetologia* 2016; **59**: 2588-2593 [PMID: 27627981 DOI: 10.1007/s00125-016-4100-7]

56 **Zhang Z**, Qi Y, Kong W, Jin Q, Wang X, Dong Y, Wang Y, Li H. Efficacy and Clinical Value of Liraglutide for Treatment of Diabetes Mellitus Complicated by Non-Alcoholic Fatty Liver Disease. *Med Sci Monit* 2018; **24**: 7399-7404 [PMID: 30325900 DOI: 10.12659/MSM.911062]

57 **Tian F**, Zheng Z, Zhang D, He S, Shen J. Efficacy of liraglutide in treating type 2 diabetes mellitus complicated with non-alcoholic fatty liver disease. *Biosci Rep* 2018; 38 [PMID: 30473540 DOI: 10.1042/BSR20181304]

58 **Newsome P**, Francque S, Harrison S, Ratziu V, Van Gaal L, Calanna S, Hansen M, Linder M, Sanyal A. Effect of semaglutide on liver enzymes and markers of inflammation in subjects with type 2 diabetes and/or obesity. *Aliment Pharmacol Ther* 2019; **50**: 193-203 [PMID: 31246368 DOI: 10.1111/apt.15316]

59 **Liu Y**, Tian Q, Yang J, Wang H, Hong T. No pancreatic safety concern following glucagon-like peptide-1 receptor agonist therapies: A pooled analysis of cardiovascular outcome trials. *Diabetes Metab Res Rev* 2018; **34**: e3061 [PMID: 30109766 DOI: 10.1002/dmrr.3061]

60 **Sumida Y**, Yoneda M, Tokushige K, Kawanaka M, Fujii H, Yoneda M, Imajo K, Takahashi H, Eguchi Y, Ono M, Nozaki Y, Hyogo H, Koseki M, Yoshida Y, Kawaguchi T, Kamada Y, Okanoue T, Nakajima A, Jsg-Nafld JSGON. Antidiabetic Therapy in the Treatment of Nonalcoholic Steatohepatitis. *Int J Mol Sci* 2020; 21 [PMID: 32168769 DOI: 10.3390/ijms21061907]

61 **Marso SP**, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsbøll T; SUSTAIN-6 Investigators. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2016; **375**: 1834-1844 [PMID: 27633186 DOI: 10.1056/NEJMoa1607141]

62 **Pratley RE**, Aroda VR, Lingvay I, Lüdemann J, Andreassen C, Navarria A, Viljoen A; SUSTAIN 7 investigators. Semaglutide versus dulaglutide once weekly in patients with type 2 diabetes (SUSTAIN 7): a randomised, open-label, phase 3b trial. *Lancet Diabetes Endocrinol* 2018; **6**: 275-286 [PMID: 29397376 DOI: 10.1016/S2213-8587(18)30024-X]

63 **George C**, Byun A, Howard-Thompson A. New Injectable Agents for the Treatment of Type 2 Diabetes Part 2-Glucagon-Like Peptide-1 (GLP-1) Agonists. *Am J Med* 2018; **131**: 1304-1306 [PMID: 29969616 DOI: 10.1016/j.amjmed.2018.05.043]

64 **Rosenstock J**, Raccah D, Korányi L, Maffei L, Boka G, Miossec P, Gerich JE. Efficacy and safety of lixisenatide once daily versus exenatide twice daily in type 2 diabetes inadequately controlled on metformin: a 24-week, randomized, open-label, active-controlled study (GetGoal-X). *Diabetes Care* 2013; **36**: 2945-2951 [PMID: 23698396 DOI: 10.2337/dc12-2709]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 506-518

DOI: 10.4254/wjh.v12.i8.506

ISSN 1948-5182 (online)

META-ANALYSIS

Racial disparities in nonalcoholic fatty liver disease clinical trial enrollment: A systematic review and meta-analysis

Parita Patel, Charles Muller, Sonali Paul

ORCID number: Parita Patel 0000-0003-3969-9026; Charles Muller 0000-0002-3254-3806; Sonali Paul 0000-0002-0136-5496.

Author contributions: Patel P and Muller C contributed to this manuscript equally; Paul S designed the study; Patel P and Muller C collected clinical data; Muller C conducted statistical analysis; Patel P, Muller C, and Paul S prepared and approved the final version of the manuscript.

Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Parita Patel, Charles Muller, Sonali Paul, Section of Gastroenterology, Hepatology, and Nutrition, University of Chicago Medical Center, Chicago, IL 60637, United States

Corresponding author: Sonali Paul, MD, MSc, Assistant Professor, Section of Gastroenterology, Hepatology, and Nutrition, University of Chicago Medical Center, 5841 S Maryland Avenue, MC 4076, Chicago, IL 60637, United States. spaul@medicine.bsd.uchicago.edu

Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) has a heterogeneous distribution across racial and ethnic groups, with a disproportionate burden among Hispanics. Although there are currently no approved therapies for treatment of NAFLD, several therapies have been investigated in clinical trials.

AIM

To analyze the inclusion of racial and ethnic minority groups in clinical trials for NAFLD.

METHODS

We performed a systematic review of North American, English-language, prospective studies for NAFLD therapies published from 2005 to 2019. Racial and ethnic enrollment data were recorded for each eligible study. Meta-analysis was performed to compute pooled prevalence of different racial and ethnic groups, followed by further subgroup analyses. These analyses were based on diagnosis of non-alcoholic steatohepatitis (NASH) and timing of study on enrollment by ethnicity. Descriptive statistics were performed to compare racial and ethnic study enrollment to previously reported NAFLD population prevalence.

RESULTS

Thirty-eight studies met criteria for inclusion in the systematic review. When reported, median age of enrolled subjects was 49 years (range 41.5-58) with 56% female participants. NAFLD was defined through biopsy findings in 79% (n = 30) of the studies. Of the included articles, treatment modalities ranged from medications (n = 28, 74%), lifestyle interventions (n = 5, 13%), bariatric surgery (n = 4, 11%) and phlebotomy (n = 1, 2%). Twenty-eight studies (73%) included racial and/or ethnic demographic information, while only 17 (45%) included information regarding Hispanic participation. Of the 2983 patients enrolled in all eligible trials, a total of only 346 (11.6%) Hispanic participants was reported. Meta-analysis revealed a pooled Hispanic prevalence of 24.3% (95% confidence

Manuscript source: Unsolicited manuscript

Received: April 13, 2020
Peer-review started: April 13, 2020
First decision: April 29, 2020
Revised: July 9, 2020
Accepted: July 26, 2020
Article in press: July 26, 2020
Published online: August 27, 2020

P-Reviewer: Chen HW
S-Editor: Liu M
L-Editor: A
P-Editor: Wang LL

interval 16.6-32.0, I^2 94.6%) among studies documenting Hispanic enrollment. Hispanic enrollment increased over time from 15% from 2005-2014 to 37% from 2015-2019.

CONCLUSION
In a meta-analysis of NAFLD trials, documentation of racial/ethnic demographic data occurred in less than half of studies. Standardization of reporting of race/ethnicity and targeted interventions toward minority recruitment are needed to improve diversity of enrollment.

Key words: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Hispanic; Racial disparities; Meta-analysis

Core tip: The Hispanic population in the United States is disproportionately affected by non-alcoholic fatty liver disease (NAFLD). Currently, there is no Food and Drug Administration approved treatment for this disease, but several clinical trials are investigating new potential therapies. This study evaluates the inclusion of race and ethnicity in the enrollment of these trials. In a systemic review and meta-analysis of clinical trials for treatment of NAFLD, 44% of eligible trials reported data on race and ethnicity. Despite a high burden of disease, Hispanic participation remained low. Future targeted interventions must take place to increase the enrollment of diverse and representative study populations in clinical trials.

Citation: Patel P, Muller C, Paul S. Racial disparities in nonalcoholic fatty liver disease clinical trial enrollment: A systematic review and meta-analysis. *World J Hepatol* 2020; 12(8): 506-518
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/506.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.506

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States, affecting up to 25% of the global adult population[1]. NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), with some patients experiencing eventual cirrhosis. Given the rising incidence, NAFLD is poised to become the leading indication for liver transplantation in the coming years[2].

Risk factors for the development of NAFLD include insulin resistance and metabolic syndrome (encompassing elevated fasting glucose levels, hypertension, dyslipidemia, and central obesity). However, not all individuals with these risk factors develop NAFLD. In a recent systematic review and meta-analysis, heterogeneity in NAFLD burden between racial and ethnic groups was noted, with the highest prevalence seen in Hispanic populations (pooled prevalence 22.9%)[3].

Although it remains unclear why Hispanics are at a higher risk of developing NAFLD and NASH, there is likely an interplay of multifactorial causes. Genetic risk factors play a large role in the pathogenesis of NAFLD. Studies have shown the single nucleotide polymorphisms in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), transmembrane 6 superfamily member 2 (*TM6SF2*), and membrane bound O-acyl transferase (*MBOAT*) play various roles in different races[4]. For example, the isoleucine to methionine substitution at position 148 (*I148M*) variant in *PNPLA3*, has been strongly linked to hepatic fat content. This variant occurs more frequently in Hispanics (49%) compared to non-Hispanic whites (23%) or African Americans (17%)[4]. Additionally, other factors such as culture, environment, and socioeconomic status, play an important role.

Although weight loss through lifestyle interventions or bariatric surgery can reverse the effects of NAFLD, there are currently no Food and Drug Administration (FDA) approved therapies for the treatment of NAFLD. Several promising therapies are currently being investigated in clinical trials. Although the burden of NAFLD on Hispanics is significant, it is unknown if this population is represented in these clinical trials. Identifying possible racial disparities is the first step in improving targeted

interventions for patient subgroups. The aim of this study was to evaluate the enrollment of Hispanics in NAFLD trials conducted in the United States and Canada. We hypothesized that the expected rate of Hispanics in NAFLD therapy trials should be proportionate to the burden of disease among Hispanics within the NAFLD population.

MATERIALS AND METHODS

Literature search strategy

The literature search was performed using the PubMed (United States National Institutes of Health, Bethesda, MD, United States) database from January 1, 2005 to March 31, 2019. Three index search terms for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and fatty liver were combined. Other potential studies were identified from reference lists of previously published review articles. The search was restricted to English-language articles. Conference abstracts were excluded. Three investigators (Patel P, Muller C and Paul S) reviewed articles for study inclusion. Discrepancies were resolved by consensus.

Study selection and data collection

Published studies of patients with NAFLD or NASH receiving any therapeutic intervention were included. NAFLD and NASH were independently defined by each study, usually either by imaging or histology.

Randomized controlled trials (RCTs) or prospective cohort studies conducted in the United States and Canada with human subjects aged 18 years or older were included. Retrospective studies, case-control, case series, case reports, reviews, and studies with non-human subjects or non-English language were excluded. Three investigators (Patel P, Muller C and Paul S) reviewed articles for study inclusion with discrepancies resolved by consensus. All data were extracted by 1 researcher and verified by another independent researcher and included study author, country, publication date, study design, intervention, sex, age, and race and/or ethnicity. Enrollment demographic information regarding race and ethnicity, when available, was recorded as defined in each individual study. For the purposes of analysis, ethnicity referred to designations of "Hispanic" or "non-Hispanic", reported along with an independent racial designation for each participant.

An assessment of risk of bias was not performed as we had a heterogenous inclusion criteria, and a risk assessment is not applicable to our study design. Additionally, given the framework of our research question, we have demonstrated that these studies are, in fact, biased towards patient selection.

Data synthesis and analysis

NAFLD prevalence data was obtained using a recent systematic review and meta-analysis that examined racial and ethnic disparities in NAFLD prevalence among adult patients in the United States through August 2, 2016[3]. In this study, the prevalence of NAFLD in the Hispanic population was 22.9% compared to 14.4% in white persons and 13.0% in black persons[3]. Additionally, the prevalence of NASH followed similar trends in this analysis with Hispanics disproportionately affected with a prevalence of 45.4%[3].

Descriptive statistics were performed with frequencies and proportions reported. Two-tailed *z*-test was performed to compare differences in proportions. All meta-analyses were performed using random effects models and results were pooled using the maximum likelihood estimation. The arcsine transformation was used to estimate the absolute proportion of Hispanics participating in each study. Study heterogeneity was assessed using the Cochrane I^2 statistic. All statistical analyses were performed using OpenMeta software. The statistical methods of this study were reviewed by Dr. Sonali Paul.

Prespecified subgroup analyses explored differences in Hispanic trial participation by specifically a diagnosis of NASH, mode of NAFLD diagnosis, and type of therapeutic intervention. Further subgroup analyses examined the effect of study design (RCT versus prospective cohort) on enrollment by ethnicity.

RESULTS

The search strategy yielded 14406 citations using the relevant search terms, with 38 meeting eligibility criteria (Figure 1). Thirty-two studies (84%) were conducted in the United States, 4 studies (11%) performed in Canada, and 2 studies (5%) were multinational (Table 1). Twenty-six (68%) studies were randomized controlled trials and 12 (32%) were prospective cohort or open label studies. When reported, median age of enrolled subjects was 49 years old (range 41.5-58) with 56% female participants. NAFLD was defined through biopsy findings in 79% (n = 30) of the studies. Of the included articles, treatment modalities ranged from medications (n = 28, 74%), lifestyle interventions (n = 5, 13%), bariatric surgery (n = 4, 11%) and phlebotomy (n = 1, 2%).

Reporting of racial data

Of the 38 identified trials, 25 (66%) included racial data with a total of 2531 total enrolled patients. Twenty-one (84%) trials were conducted in the United States, 2 (8%) trials were performed in Canada and 2 (8%) were multinational trials. The median age of enrolled patients was 49.5 years (range 41-58). NAFLD was diagnosed by biopsy in 80% (n = 20) of the trials, with 20% (n = 5) diagnosed by imaging. Interventions included medications (n = 23, 92%) or bariatric surgery (n = 2, 8%) (Table 1).

Enrollment of Hispanic patients

Among the 38 eligible trials, only 17 (44.7%) included information regarding patient ethnicity. Of the 2983 patients enrolled in all eligible trials, a total of only 346 (11.6%) Hispanic participants was reported. Among the 25 studies that included data on race, 14 included data on Hispanic participation. Of note, 3 studies that did not have racial data did provide data on Hispanic participation (Table 1).

Among the 17 trials that reported Hispanic participation, there were 346 Hispanic patients out of 1577 total enrolled patients with a participation rate of 21.9% compared to 74.8% of Caucasian participants among those including data on Caucasian participation. The 21.9% unadjusted pooled prevalence of Hispanic trial participants was similar to the 22.8% unadjusted pooled NAFLD Hispanic prevalence (990/4332 total patients) in the recent systematic review by Rich *et al*[3] (P = 0.365).

A meta-analysis was then performed to estimate pooled prevalence while taking heterogeneity of included studies into consideration. The pooled prevalence was found to be 24.3% [95% confidence interval (CI) 16.6-32.0] with significant heterogeneity (I^2 = 94.6%) (Figure 2).

Further sub-group meta-analyses were performed in patients with biopsy proven NASH and found Hispanic participation to be 24.7% (Table 2), considerably lower than the 45.4% prevalence of NASH in the Hispanic population found in the recent meta-analysis by Rich *et al*[3]. Caucasian and African American participation in studies using NASH as inclusion criteria, was slightly lower than those of NAFLD studies (67.3% *vs* 63.9% and 8.0% *vs* 2.7%, respectively).

To determine if rates of Hispanic enrollment changed over time, studies conducted before and after 2015 were compared. The pooled prevalence of Hispanic patients in studies from 2005-2014 was 15%, compared to 37% for studies from 2015-2019. Trends in Hispanic study participation over time are displayed in Figure 3.

DISCUSSION

The purpose of this systematic review and meta-analysis was to characterize the participation rate of Hispanic patients in clinical trials investigating therapies for NAFLD. Despite the importance of genetics and race in the prevalence of NAFLD, our results show that racial/ethnic demographic data are under-reported, with only 25 of 38 (66%) eligible clinical trials reporting race or ethnicity. Both the FDA and the National Institutes of Health (NIH) have published recommendations on how to report race and ethnicity data in clinical trials, however in practice these guidelines are not strictly followed or enforced[5,6]. Previous studies have demonstrated reporting of race/ethnicity to be similarly suboptimal in clinical trials across several specialties[7,8]. An analysis of clinical trial enrollment for several disease processes (spanning general medicine, oncology, cardiovascular disease, and infectious diseases) from 2009, found that 21% of studies failed to include racial or ethnic demographic data[7].

In this review, of the 38 trials that met eligibility criteria, 25 reported racial information. Among these only 17 (68%) provided data on ethnicity (participation of Hispanic patients). It is well established that the prevalence of NAFLD and NASH is

Table 1 Summary of non-alcoholic fatty liver disease studies included in systematic review

Year	Author	Study design	NAFLD or NASH	How NAFLD defined (ultrasound/biopsy)	Intervention	Total enrolled	% Men	Median age (yr)	Reporting of Race	Reporting of ethnicity	% White	% Black	% Hispanic	% Asian	% Other	Unknown
2005	Huang *et al*	Uncontrolled, open-label trial	NASH	Biopsy	Dietary intervention/ counseling	23	47.8	48	N	Y	87%	0%	13%	0%	0%	0%
2005	Clark *et al*	Prospective cohort	NAFLD	Biopsy	Roux-en-Y	16	50.0	43	Y	N	88%	NR	NR	NR	NR	12%[3]
2006	Barker *et al*	Retrospective cohort	NASH	Biopsy	Roux-en-Y	19	10.5	49	N	N	NR	NR	NR	NR	NR	NR
2006	Browning *et al*	Prospective cohort	NAFLD	Imaging (MRI)[2]	Statins	268	44.0	54	Y	Y	38%	50%	10%	0%	2%	0%
2006	Belfort *et al*	RCT	NASH	Biopsy	Pioglitazone	47	44.7	51	N	N	NR	NR	NR	NR	NR	NR
2007	Balas *et al*	RCT	NASH	Biopsy	Pioglitazone	35	54.3	48	N	N	NR	NR	NR	NR	NR	NR
2007	Lutchman *et al*	Uncontrolled, open-label trial	NASH	Biopsy	Discontinuation of pioglitazone	13	54.0	41.5	Y	Y	84%	0%	8%	8%	0%	0%
2009	Loomba *et al*	Uncontrolled, open-label trial	NASH	Biopsy	Metformin	26	50.0	44	Y	Y	65%	0%	15%	19%	0%	0%
2010	Chalasani *et al*	RCT	NASH	Biopsy	Pioglitazone/ Vitamin E	247	40.0	46	Y	Y	NR	NR	15%	NR	NR	85%[4]
2011	Foster *et al*	RCT	NAFLD	CT	Atorvastatin	80	71.0, 77.5 with NAFLD	59	Y	Y	93%	2%	2%	2%	0.5%	0%
2011	Van Wagner *et al*	RCT	NASH	Biopsy	Pentoxyfylline	30	43.3	50.5	Y	Y	80%	0%	17%	3%	0%	0%
2011	Zein *et al*	RCT	NASH	Biopsy	Pentoxyfylline	55	69.1	50	Y	N	93%	NR	NR	NR	NR	7%[3]
2011	Torres *et al*	RCT	NASH	Biopsy	Rosiglitazone/ metformin	108	50.4	49	Y	Y	65%	4%	22%		4%	5%
2012	Le *et al*	RCT	NASH	MRI	Colesevelam	50	46.0	47	Y	Y	38	0%	28%	22	8	0%
2012	Zein *et al*	RCT	NASH	Biopsy	Pentoxyfylline	47	70.2	50	Y	N	92%	NR	NR	NR	NR	8%[3]
2012	Sullivan *et al*	RCT	NAFLD	Biopsy	Exercise	18	27.8	48	N	N	NR	NR	NR	NR	NR	NR
2012	Fealy *et al*	RCT	NAFLD	Imaging (MRI)	Exercise	13	NR	58	N	N	NR	NR	NR	NR	NR	NR
2013	Mudaliar *et al*	RCT	NAFLD	Biopsy	Obeticholic acid	64	51.6	52	Y	Y[1]	42%	28%	25%	5%	0%	0%
2013	Beaton *et al*	Uncontrolled, open-label trial	NAFLD or NASH	Biopsy	Phlebotomy	31	61.3	49	N	N	NR	NR	NR	NR	NR	NR

2014	Sanyal *et al*	RCT	NASH	Biopsy	EPA-E	243	39.1	48	Y	N	91%	3%	0%	0%	6%	0%
2015	Dasarthy *et al*	RCT	NASH	Biopsy	Omega 3 fatty acids	37	21.6	50	Y	Y	92%	3%	5%	0%	0%	0%
2015	Argo *et al*	RCT	NASH	Biopsy	N-3 fish oil	34	38.2	46	Y	N	97%	NR	NR	NR	NR	3%[3]
2015	Loomba *et al*	RCT	NASH	Biopsy	Ezetemibe	50	38.0	49	Y	Y	NR	NR	34%	NR	NR	66%[4]
2015	Neuschwander *et al*	RCT	NAFLD	Biopsy	Obeticholic acid	283	33.9	51	Y	Y	83%	2%	15%	6%	10%	0%
2015	Vilar-Gomez *et al*	Prospective cohort	NASH	Biopsy	Bariatric surgery	293	41.0	48	Y	N	98%	NR	NR	NR	NR	2%[3]
2015	Glass *et al*	Prospective cohort	NASH	Biopsy	Weight loss	45	28.9	46	N	N	NR	NR	NR	NR	NR	NR
2016	Harrison *et al*	RCT	NASH	Biopsy	GT020 (galectin 3 protein inhibitor)	31	54.8	54	N	NR	NR	NR	NR	NR	NR	NR
2016	Cusi *et al*	RCT	NASH	Biopsy	Pioglitazone	101	70.3	51	Y	Y	25%	NR	67%	NR	8%	0%
2016	Cui *et al*	RCT	NAFLD	MRI	Sitagliptin	84	41.7	53.5	N	Y	32%	NR	36%	NR	NR	NR
2016	Ratziu *et al*	RCT	NASH	Biopsy	Elafibranor (PPAR agonist)	274	55	52	Y	N	89%	NR	NR	NR	NR	NR
2017	Winn *et al*	RCT	NAFLD	MRI	Exercise	21	NR	46	N	N	NR	NR	NR	NR	NR	NR
2017	Joy *et al*	RCT	NASH	Biopsy	Sitagliptin	12	41.7	56	Y	N	92%	NR	NR	NR	NR	8.3%[3]
2017	Loomba *et al*	RCT	NASH	Biopsy	Selonsertib (ASK1 inhibitor)	72	31	54.2	Y	N	90%	NR	NR	NR	NR	10%[3]
2017	Lawitz *et al*	Prospective cohort	NAFLD	MRI	Acetyl-CoA carboxylase inhibitor (GS-0976)	20	55	45	Y	N	100%	0%	NR	0%	0%	NR
2018	Shiffman *et al*	RCT	NAFLD	Biopsy or MRI	Emricasan	38	63.2	NR	Y	N	89%	NR	NR	NR	NR	11%[3]
2018	Schwenger *et al*	Prospective cohort	NAFLD	Biopsy	Bariatric surgery	42	23.8	48	N	N	NR	NR	NR	NR	NR	NR
2018	Chalasani *et al*	RCT	NAFLD	MRI	Leucine/metformin/sildenafil	70	44.3	46	Y	Y	63%	4.2%	27%	2.9%	1.4%	2.9%
2019	Harrison *et al*	Prospective cohort	NASH	Biopsy	FGF19 analog (NGM282)	43	20.9	50	N	Y	NR	NR	76.7%	NR	NR	23.3[4]

[1]Race and ethnicity categorized together.
[2]Definition of NAFLD included presence of steatosis on MRI.
[3]Patients were categorized as either white or non-white.
[4]Patients were categorized as either Hispanic or non-Hispanic. NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; Y: Yes; N: No; MRI: Magnetic resonance imaging; NR: Not recorded; RCT: Randomized control trial.

higher among Hispanic patients than among either non-Hispanic whites or other minority groups, with a prevalence of 25%-40% and 25%, respectively[9-13]. Although

Table 2 Proportion of trial patients with non-alcoholic steatohepatitis by race/ethnicity

	NASH	
	Prevalence (%)	**95%CI**
Hispanic	24.7	9.1-40.4
White persons	63.9	42.4-85.5
Black persons	2.7	0.5-4.9

NASH: Non-alcoholic steatohepatitis; CI: Confidence interval.

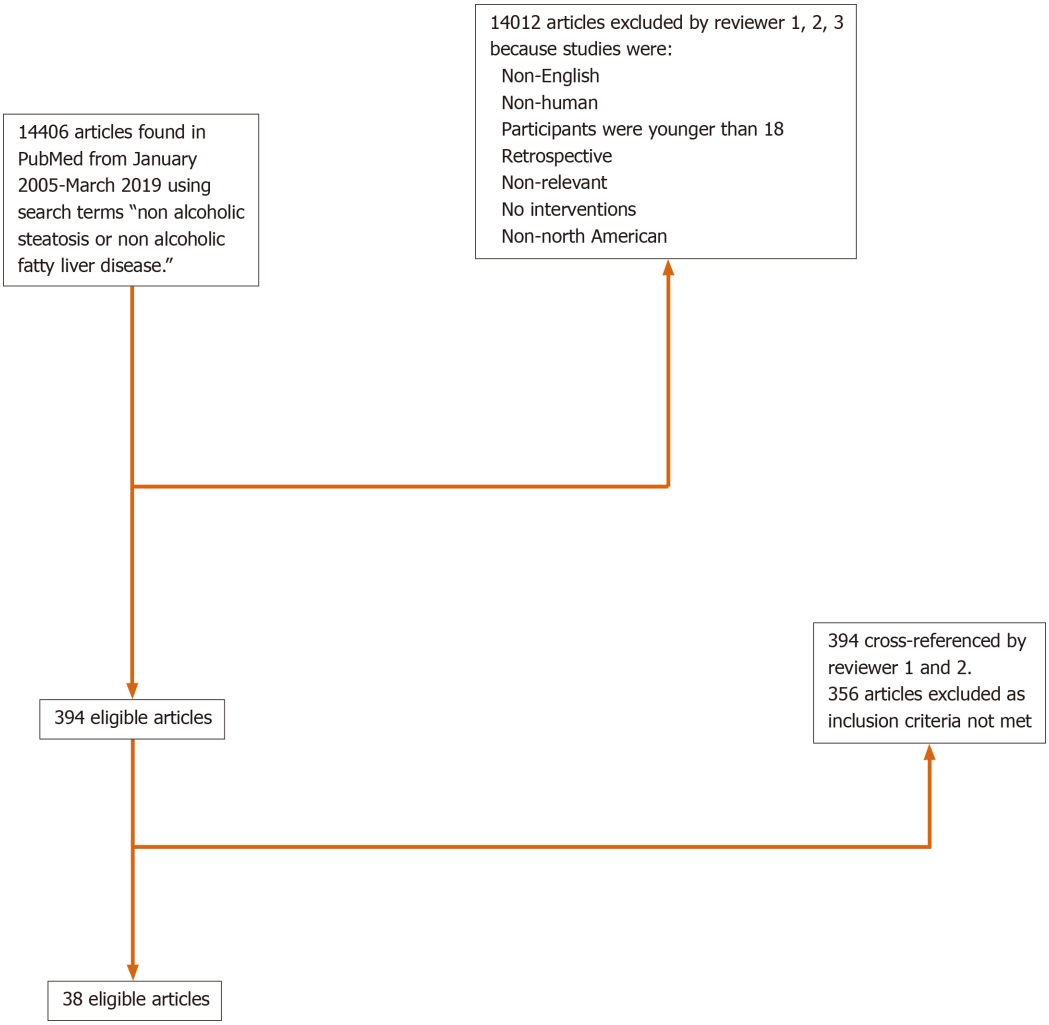

Figure 1 Summary of review process of all PubMed articles using search terms.

Hispanic participation among trials that included information about Hispanic enrollment (24.3%) was close to that of the United States Hispanic population (18.3%)[14], it does not provide an accurate reflection of the racial and ethnic makeup of the NAFLD population, which is closer to 30% Hispanic[12]. There was also significant discrepancy between Hispanic participation in NASH trials (24.7%) and the prevalence of NASH (45.5%) reported in a recent meta-analysis[3]. It is not known whether the low rate of Hispanic participation in these trials is due to lack of collection of ethnic demographic data on behalf of the investigators, failure to report ethnicity by subjects, or true under-enrollment.

Under-reporting of Hispanic trial participants could be in part due to heterogeneity in self-reported ethnicity among Hispanic patients and diversity of their country of origin and race[15]. Health sciences typically follow the United States Census practice of categorizing "Hispanic" as an ethnicity that is distinct from race. This practice can

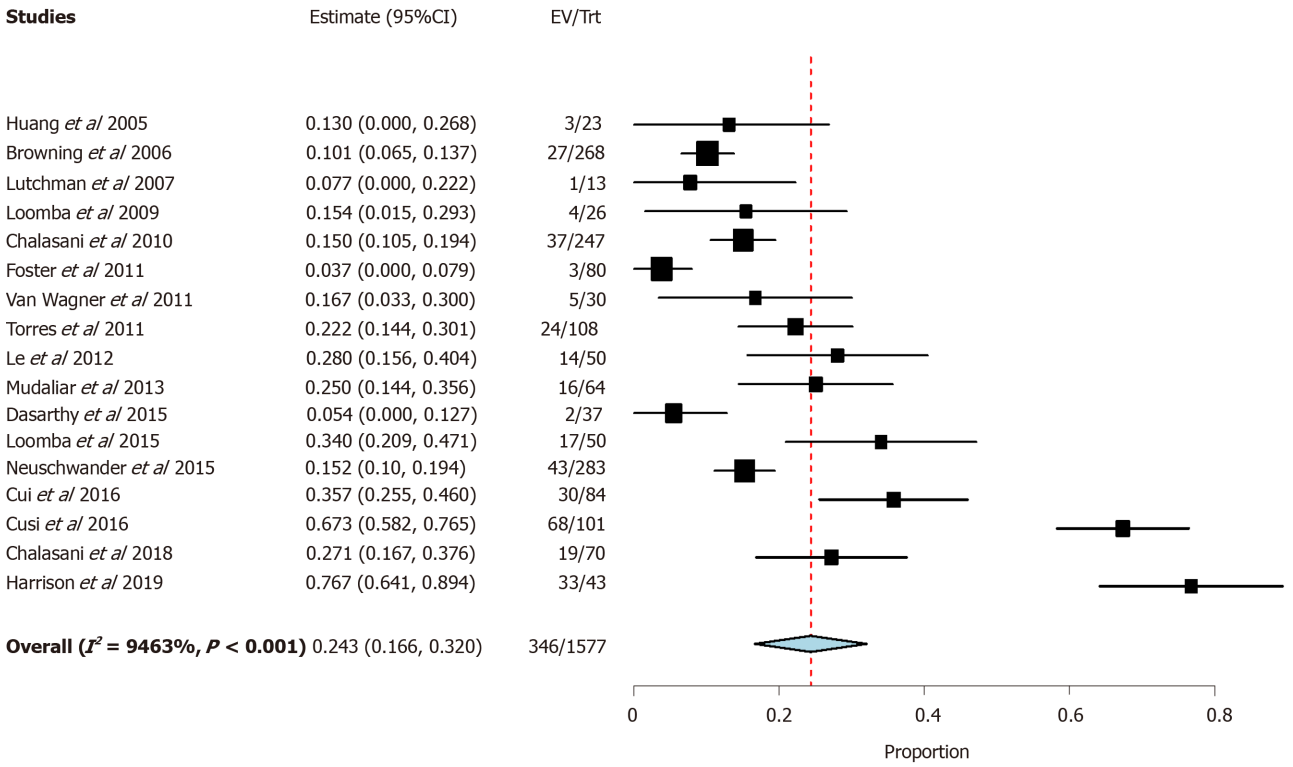

Figure 2 Pooled prevalence of Hispanic patients among studies reporting ethnicity.

result in discordance between patients' self-perceived race/ethnicity and their associated categorization in health systems in addition simplification of a diverse, heterogenous group, and inaccuracy in reporting[15,16].

Heterogeneity of enrollment practices for the included trials is also likely contributing significantly to Hispanic under-enrollment. Hispanic enrollment (among those reporting any Hispanic participants) ranged from 4%-67%. The I^2 statistic of 94% highlights the significant heterogeneity of Hispanic enrollment among studies included in this meta-analysis. The finding that 10 of the 17 trials (59%) including data on Hispanic enrollment were conducted in states that shared a border with Mexico[17-26] highlights the opportunistic, rather than systematic, nature of trial recruitment and enrollment. The generalizability of such clinical trials is significantly compromised when they fail to include information about key demographics.

When comparing racial and ethnic enrollment between studies using NAFLD and NASH as inclusion criteria, we found that Hispanic enrollment in NASH trials increased relative to enrollment of Caucasian and African American participants. These findings are consistent with those of prior studies demonstrating that Hispanic NAFLD patients are more likely to progress to steatohepatitis than Caucasians or African Americans[3,27]. However, given that 45% of Hispanic NAFLD patients experience progression to steatohepatitis, compared to 32% and 20% of Caucasian and African American NAFLD patients, respectively[3], Hispanics are likely even more under-represented in studies of NASH relative to the disease burden in that population. The observed proportion of African American NASH participants in our study was particularly low (2.7%), but is likely a reflection of the low rate of African American enrollment in included studies in general.

Acknowledging the importance of diverse trial participation to generalizability of findings in the development of new therapies, the NIH has stipulated that all sponsored clinical trials include women and minority patients since 1993[7]. Aside from ethical issues related to equity and justice, racial differences in response to pharmacologic therapy identified in diverse trials have been used to guide current clinical practice[28,29]. Recent work suggesting differences in the underlying genetic contributions to NAFLD in patients of different racial/ethnic backgrounds[30,31] highlight the importance of diversity in clinical trial participation. Genetic variants on *PNPLA3, TM6SF2*, and neurcan (*NCAN)* can increase the heritability of NAFLD by up to 27% within families. A missense mutation of *PNPLA3* has a strong association with hepatic fat accumulation and with a higher susceptibility to develop more severe histologic liver damage, irrespective of the degree of obesity or presence of

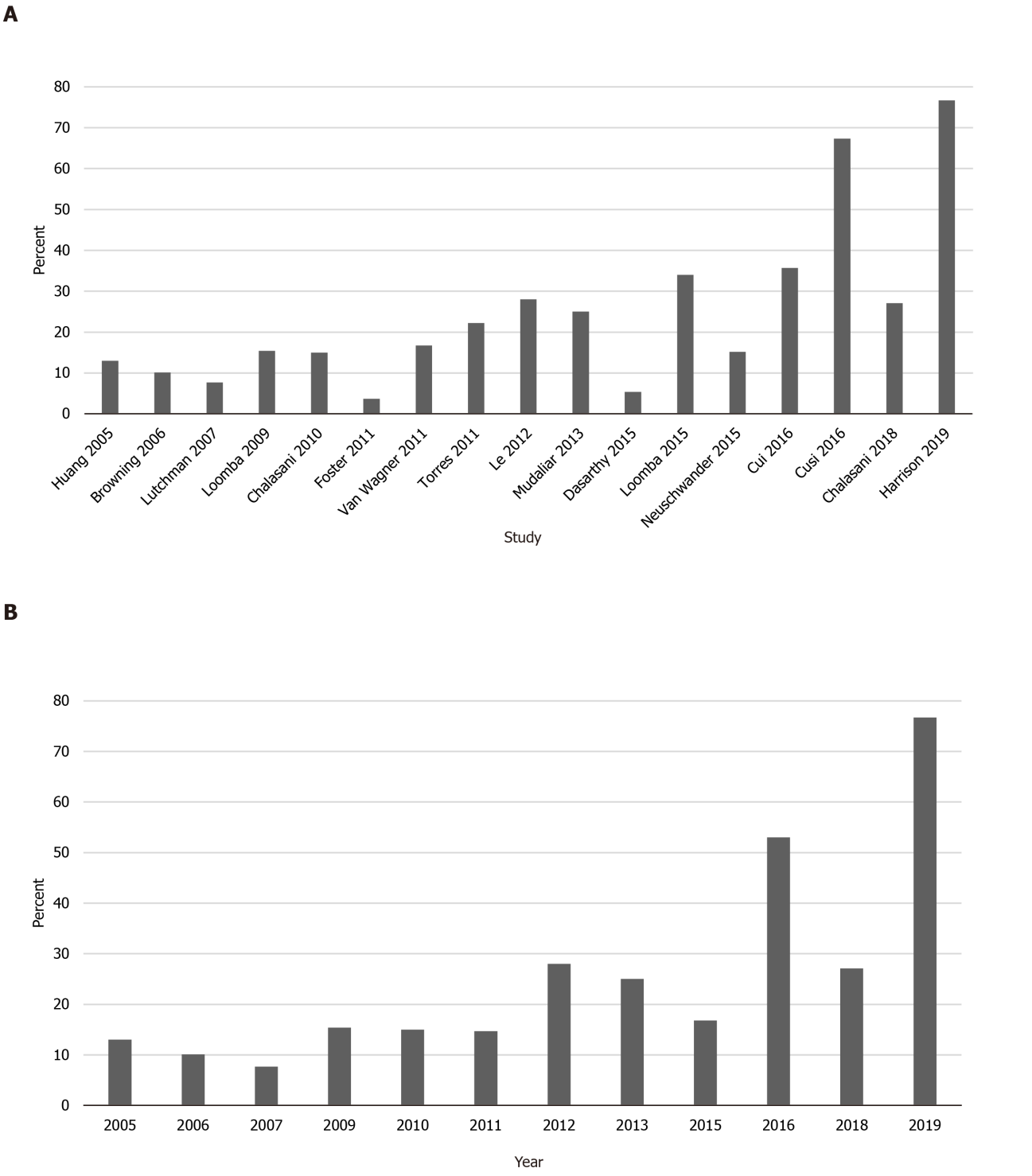

Figure 3 Percentage of Hispanic enrollment in trials. A: Percentage of Hispanic enrollment in trials; B: Percentage of Hispanic enrollment in trials grouped by year.

diabetes[32-34]. This variant in *PNPLA3* gene has been observed in highest frequency in Hispanics[4].

Despite the benefits of diversity in trial enrollment, minority patients have historically been underrepresented in clinical trials. Barriers to minority participation in clinical trials include mistrust of providers/research, reduced access to healthcare, financial and time constraints, lack of education about clinical trials, and cultural or language differences impairing communication with trial recruiters or providers[35]. Hispanic patients, in particular, have been underrepresented in clinical trials for multiple conditions. In a 2004 analysis of colorectal, lung and prostate cancer studies, Murthy *et al*[36] found that Hispanic patients only constituted 3.1% trial participants.

Compared to African American patients, Hispanic patients are less likely to be aware of or recruited to participate in clinical research[37-39]. Although lack of access to healthcare resources and lower socioeconomic status are shared among multiple minority groups, language barriers create a burden for Hispanic patients in particular. Interventions such as provision of Spanish-speaking recruitment materials or personnel have been shown to improve enrollment of Hispanic patients in clinical trials[40,41] and serve as potential targets for increasing diversity of study populations for NAFLD. In spite of these historic barriers to Hispanic participation in clinical trials, a trend toward increasing Hispanic enrollment over time was observed in our study, with Hispanic enrollment in studies conducted after 2015 nearly triple that of studies from 2005-2014. While these results are encouraging, future efforts are needed to standardize reporting of race/ethnicity in clinical trials and encourage diverse, representative enrollment.

A major limitation of this study is the low rate of reporting demographic data on Hispanic participation among the trials analyzed. Although many trials did not include any racial/ethnic demographic data, the rate of inclusion of data on Hispanic participation was particularly poor (44% of eligible trials). From the information available, it is not known if these trials did not actually recruit any Hispanic participants or if they simply failed to collect or report data on their inclusion.

In conclusion, North American clinical trials of NAFLD from 2015-2019 did not consistently include data on Hispanic participation. Among trials that did include racial/ethnic demographic data, Hispanic patients may be underrepresented relative to the burden of NAFLD and NASH among this population. Future efforts aimed at improving or standardizing reporting of race in clinical trials and at increasing enrollment of diverse and representative study populations are needed to address this disparity.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States and has a heterogeneous distribution across racial and ethnic groups, with a disproportionate burden among Hispanics. Although it remains unclear why Hispanics are at a higher risk of developing NAFLD and nonalcoholic steatohepatitis (NASH), there is likely an interplay of multifactorial causes including genetics, culture, socioeconomic status and environment. Despite this high burden of disease, there are currently no approved therapies for the treatment of NAFLD. Several promising therapies are currently being investigated in clinical trials but it is unknown if Hispanics are appropriately represented in these clinical trials.

Research motivation

Identifying possible racial disparities is the first step in improving targeted interventions for patient subgroups. The purpose of this systematic review and meta-analysis was to characterize the participation rate of different races and ethnicities in clinical trials investigating therapies for NAFLD.

Research objectives

The aim of this study was to evaluate the enrollment of Hispanics in NAFLD trials conducted in the United States and Canada. We hypothesized that the expected rate of Hispanics in NAFLD therapy trials should be proportionate to the burden of disease among Hispanics within the NAFLD population.

Research methods

The literature search was performed using the PubMed (US National Institutes of Health, Bethesda, MD, United States) database from January 1, 2005 to March 31, 2019 using the following search terms: Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and fatty liver. Randomized controlled trials (RCTs) or prospective cohort studies conducted in the United States and Canada with human subjects aged 18 years or older were included. Descriptive statistics were performed with frequencies and proportions reported. Two-tailed *z*-test was performed to compare differences in proportions. All meta-analyses were performed using random effects models and results were pooled using the maximum likelihood estimation.

Research results

Of the 38 trials that met eligibility criteria, twenty-five reported racial information. Among these only 17 (68%) provided data on ethnicity (participation of Hispanic patients). Among the 2983 patients enrolled in all eligible trials, a total of only 346 (11.6%) Hispanic participants was reported. Among the 17 trials that reported Hispanic participation, there were 346 Hispanic patients out of 1577 total enrolled patients with a participation rate of 21.9% compared to 74.8% of Caucasian participants among those including data on Caucasian participation. A meta-analysis was then performed to estimate pooled prevalence while taking heterogeneity of included studies into consideration. The pooled prevalence was found to be 24.3% (95%CI: 16.6-32.0) with significant heterogeneity (I^2 = 94.6%). To determine if rates of Hispanic enrollment changed over time, studies conducted before and after 2015 were compared. The pooled prevalence of Hispanic patients in studies from 2005-2014 was 15%, compared to 37% for studies from 2015-2019.

Research conclusions

North American clinical trials of NAFLD from 2015-2019 did not consistently include data on Hispanic participation. Among trials that did include racial/ethnic demographic data, Hispanic patients may be underrepresented relative to the burden of NAFLD and NASH among this population.

Research perspectives

Future efforts aimed at improving or standardizing reporting of race in clinical trials and at increasing enrollment of diverse and representative study populations are needed to address this disparity. It is not known whether the low rate of Hispanic participation in these trials is due to lack of collection of ethnic demographic data on behalf of the investigators, failure to report ethnicity by subjects, or true under-enrollment. Despite the benefits of diversity in trial enrollment, minority patients have historically been underrepresented in clinical trials. Barriers to minority participation in clinical trials include mistrust of providers/research, reduced access to healthcare, financial and time constraints, lack of education about clinical trials, and cultural or language differences impairing communication with trial recruiters or providers. Interventions such as provision of Spanish-speaking recruitment materials or personnel have been shown to improve enrollment of Hispanic patients in clinical trials and serve as potential targets for increasing diversity of study populations for NAFLD.

REFERENCES

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

2 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

3 **Rich NE**, Oji S, Mufti AR, Browning JD, Parikh ND, Odewole M, Mayo H, Singal AG. Racial and Ethnic Disparities in Nonalcoholic Fatty Liver Disease Prevalence, Severity, and Outcomes in the United States: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2018; **16**: 198-210.e2 [PMID: 28970148 DOI: 10.1016/j.cgh.2017.09.041]

4 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

5 **US Dept. of Health and Human Services**, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, Center for Devices and Radiologic Health. Collection of Race and Ethnicity Data in Clinical Trials. [cited 2018 December 1]. In Food and Drug Administration 2005. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/collection-race-and-ethnicity-data-clinical-trials

6 **National Institute of Health**. NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research. [cited 2018 December 1]. In NIH Central Resource for Grand and Funding Information. Available from: http://grants.nih.gov/grants/funding/women_min/guidelines_amended_10_2001.htm 2017

7 **Geller SE**, Koch A, Pellettieri B, Carnes M. Inclusion, analysis, and reporting of sex and race/ethnicity in clinical trials: have we made progress? *J Womens Health (Larchmt)* 2011; **20**: 315-320 [PMID: 21351877 DOI: 10.1089/jwh.2010.2469]

8 **Chen MS Jr**, Lara PN, Dang JH, Paterniti DA, Kelly K. Twenty years post-NIH Revitalization Act: enhancing minority participation in clinical trials (EMPaCT): laying the groundwork for improving minority clinical trial accrual: renewing the case for enhancing minority participation in cancer clinical trials. *Cancer*

2014; **120** Suppl 7: 1091-1096 [PMID: 24643646 DOI: 10.1002/cncr.28575]
9 **Ruhl CE**, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2003; **124**: 71-79 [PMID: 12512031 DOI: 10.1053/gast.2003.50004]
10 **Kallwitz ER**, Guzman G, TenCate V, Vitello J, Layden-Almer J, Berkes J, Patel R, Layden TJ, Cotler SJ. The histologic spectrum of liver disease in African-American, non-Hispanic white, and Hispanic obesity surgery patients. *Am J Gastroenterol* 2009; **104**: 64-69 [PMID: 19098851 DOI: 10.1038/ajg.2008.12]
11 **Ong JP**, Younossi ZM. Epidemiology and natural history of NAFLD and NASH. *Clin Liver Dis* 2007; **11**: 1-16, vii [PMID: 17544968 DOI: 10.1016/j.cld.2007.02.009]
12 **Weston SR**, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, Terrault NA. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005; **41**: 372-379 [PMID: 15723436 DOI: 10.1002/hep.20554]
13 **Sherif ZA**, Saeed A, Ghavimi S, Nouraie SM, Laiyemo AO, Brim H, Ashktorab H. Global Epidemiology of Nonalcoholic Fatty Liver Disease and Perspectives on US Minority Populations. *Dig Dis Sci* 2016; **61**: 1214-1225 [PMID: 27038448 DOI: 10.1007/s10620-016-4143-0]
14 **United States Census Bureau**. QuickFacts. [cited 2018 December 1]. Available from: http://www.census.gov/quickfacts/table/PST045215/00
15 **Caballero AE**. Understanding the Hispanic/Latino patient. *Am J Med* 2011; **124**: S10-S15 [PMID: 21939793 DOI: 10.1016/j.amjmed.2011.07.018]
16 **Hasnain-Wynia R**, Baker DW. Obtaining data on patient race, ethnicity, and primary language in health care organizations: current challenges and proposed solutions. *Health Serv Res* 2006; **41**: 1501-1518 [PMID: 16899021 DOI: 10.1111/j.1475-6773.2006.00552.x]
17 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR, NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]
18 **Torres DM**, Jones FJ, Shaw JC, Williams CD, Ward JA, Harrison SA. Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open- label trial. *Hepatology* 2011; **54**: 1631-1639 [PMID: 21748770 DOI: 10.1002/hep.24558]
19 **Le TA**, Chen J, Changchien C, Peterson MR, Kono Y, Patton H, Cohen BL, Brenner D, Sirlin C, Loomba R; San Diego Integrated NAFLD Research Consortium (SINC). Effect of colesevelam on liver fat quantified by magnetic resonance in nonalcoholic steatohepatitis: a randomized controlled trial. *Hepatology* 2012; **56**: 922-932 [PMID: 22431131 DOI: 10.1002/hep.25731]
20 **Mudaliar S**, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, Adorini L, Sciacca CI, Clopton P, Castelloe E, Dillon P, Pruzanski M, Shapiro D. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 574-82.e1 [PMID: 23727264 DOI: 10.1053/j.gastro.2013.05.042]
21 **Loomba R**, Sirlin CB, Ang B, Bettencourt R, Jain R, Salotti J, Soaft L, Hooker J, Kono Y, Bhatt A, Hernandez L, Nguyen P, Noureddin M, Haufe W, Hooker C, Yin M, Ehman R, Lin GY, Valasek MA, Brenner DA, Richards L; San Diego Integrated NAFLD Research Consortium (SINC). Ezetimibe for the treatment of nonalcoholic steatohepatitis: assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial). *Hepatology* 2015; **61**: 1239-1250 [PMID: 25482832 DOI: 10.1002/hep.27647]
22 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]
23 **Cusi K**, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A, Portillo-Sanchez P. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med* 2016; **165**: 305-315 [PMID: 27322798 DOI: 10.7326/M15-1774]
24 **Chalasani N**, Vuppalanchi R, Rinella M, Middleton MS, Siddiqui MS, Barritt AS 4th, Kolterman O, Flores O, Alonso C, Iruarrizaga-Lejarreta M, Gil-Redondo R, Sirlin CB, Zemel MB. Randomised clinical trial: a leucine-metformin-sildenafil combination (NS-0200) vs placebo in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2018; **47**: 1639-1651 [PMID: 29696666 DOI: 10.1111/apt.14674]
25 **Harrison SA**, Rossi SJ, Paredes AH, Trotter JF, Bashir MR, Guy CD, Banerjee R, Jaros MJ, Owers S, Baxter BA, Ling L, DePaoli AM. NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients With Nonalcoholic Steatohepatitis. *Hepatology* 2020; **71**: 1198-1212 [PMID: 30805949 DOI: 10.1002/hep.30590]
26 **Cui J**, Philo L, Nguyen P, Hofflich H, Hernandez C, Bettencourt R, Richards L, Salotti J, Bhatt A, Hooker J, Haufe W, Hooker C, Brenner DA, Sirlin CB, Loomba R. Sitagliptin vs. placebo for non-alcoholic fatty liver disease: A randomized controlled trial. *J Hepatol* 2016; **65**: 369-376 [PMID: 27151177 DOI: 10.1016/j.jhep.2016.04.021]
27 **Pan JJ**, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* 2014; **6**: 274-283 [PMID: 24868321 DOI: 10.4254/wjh.v6.i5.274]
28 **Moser M**, Lunn J. Responses to captopril and hydrochlorothiazide in black patients with hypertension. *Clin Pharmacol Ther* 1982; **32**: 307-312 [PMID: 7049502 DOI: 10.1038/clpt.1982.165]
29 **Yancy CW**, Fowler MB, Colucci WS, Gilbert EM, Bristow MR, Cohn JN, Lukas MA, Young ST, Packer M; U. S. Carvedilol Heart Failure Study Group. Race and the response to adrenergic blockade with carvedilol in patients with chronic heart failure. *N Engl J Med* 2001; **344**: 1358-1365 [PMID: 11333992 DOI: 10.1056/NEJM200105033441803]
30 **Hernaez R**, McLean J, Lazo M, Brancati FL, Hirschhorn JN, Borecki IB, Harris TB; Genetics of Obesity-

Related Liver Disease (GOLD) Consortium, Nguyen T, Kamel IR, Bonekamp S, Eberhardt MS, Clark JM, Kao WH, Speliotes EK. Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* 2013; **11**: 1183-1190.e2 [PMID: 23416328 DOI: 10.1016/j.cgh.2013.02.011]

31 **Martínez LA**, Larrieta E, Kershenobich D, Torre A. The Expression of PNPLA3 Polymorphism could be the Key for Severe Liver Disease in NAFLD in Hispanic Population. *Ann Hepatol* 2017; **16**: 909-915 [PMID: 29055919 DOI: 10.5604/01.3001.0010.5282]

32 **Rotman Y**, Koh C, Zmuda JM, Kleiner DE, Liang TJ, NASH CRN. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 894-903 [PMID: 20684021 DOI: 10.1002/hep.23759]

33 **Speliotes EK**, Butler JL, Palmer CD, Voight BF; GIANT Consortium; MIGen Consortium; NASH CRN, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010; **52**: 904-912 [PMID: 20648472 DOI: 10.1002/hep.23768]

34 **Valenti L**, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, Nobili V, Mozzi E, Roviaro G, Vanni E, Bugianesi E, Maggioni M, Fracanzani AL, Fargion S, Day CP. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1209-1217 [PMID: 20373368 DOI: 10.1002/hep.23622]

35 **Ford JG**, Howerton MW, Lai GY, Gary TL, Bolen S, Gibbons MC, Tilburt J, Baffi C, Tanpitukpongse TP, Wilson RF, Powe NR, Bass EB. Barriers to recruiting underrepresented populations to cancer clinical trials: a systematic review. *Cancer* 2008; **112**: 228-242 [PMID: 18008363 DOI: 10.1002/cncr.23157]

36 **Murthy VH**, Krumholz HM, Gross CP. Participation in cancer clinical trials: race-, sex-, and age-based disparities. *JAMA* 2004; **291**: 2720-2726 [PMID: 15187053 DOI: 10.1001/jama.291.22.2720]

37 **Garza MA**, Quinn SC, Li Y, Assini-Meytin L, Casper ET, Fryer CS, Butler J 3rd, Brown NA, Kim KH, Thomas SB. The Influence of Race and Ethnicity on Becoming a Human Subject: Factors Associated with Participation in Research. *Contemp Clin Trials Commun* 2017; **7**: 57-63 [PMID: 29226266 DOI: 10.1016/j.conctc.2017.05.009]

38 **Langford A**, Resnicow K, An L. Clinical trial awareness among racial/ethnic minorities in HINTS 2007: sociodemographic, attitudinal, and knowledge correlates. *J Health Commun* 2010; **15** Suppl 3: 92-101 [PMID: 21154086 DOI: 10.1080/10810730.2010.525296]

39 **Leiter A**, Diefenbach MA, Doucette J, Oh WK, Galsky MD. Clinical trial awareness: Changes over time and sociodemographic disparities. *Clin Trials* 2015; **12**: 215-223 [PMID: 25673636 DOI: 10.1177/1740774515571917]

40 **Fischer SM**, Kline DM, Min SJ, Okuyama S, Fink RM. Apoyo con Cariño: Strategies to Promote Recruiting, Enrolling, and Retaining Latinos in a Cancer Clinical Trial. *J Natl Compr Canc Netw* 2017; **15**: 1392-1399 [PMID: 29118231 DOI: 10.6004/jnccn.2017.7005]

41 **Sanossian N**, Rosenberg L, Liebeskind DS, Starkman S, Eckstein M, Stratton S, Pratt FD, Hamilton S, Kim-Tenser M, Sharma LK, Restrepo L, Valdes-Sueras M, Conwit R, Saver JL; FAST-MAG Investigators and Coordinators. A Dedicated Spanish Language Line Increases Enrollment of Hispanics Into Prehospital Clinical Research. *Stroke* 2017; **48**: 1389-1391 [PMID: 28389617 DOI: 10.1161/STROKEAHA.117.014745]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 519-524

DOI: 10.4254/wjh.v12.i8.519

ISSN 1948-5182 (online)

CASE REPORT

Non-islet cell tumor hypoglycemia as an initial presentation of hepatocellular carcinoma coupled with end-stage liver cirrhosis: A case report and review of literature

Bo Yu, Rana Douli, Jose Amaya Suarez, Victor Perez Gutierrez, Mohammad Aldiabat, Maria Khan

ORCID number: Bo Yu 0000-0002-7520-453X; Rana Douli 0000-0002-3200-214X; Jose Amaya Suarez 0000-0001-3330-412X; Victor Perez Gutierrez 0000-0002-5028-623X; Mohammad Aldiabat 0000-0002-7023-964X; Maria Khan 0000-0002-5100-747X.

Author contributions: Yu B wrote the manuscript; Douli R, Suarez JA reviewed the paper; Gutierrez VP edited the references; Aldiabat M and Khan M designed the figures.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

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

Bo Yu, Rana Douli, Jose Amaya Suarez, Victor Perez Gutierrez, Mohammad Aldiabat, Maria Khan, Department of Medicine, Lincoln Medical Center, Bronx, NY 10451-5504, United States

Corresponding author: Bo Yu, MD, MSc, Doctor, Department of Medicine, Lincoln Medical Center, 234 E.149th Street, Bronx, NY 10451-5504, United States. yub3@nychhc.org

Abstract

BACKGROUND

Non-islet cell tumor hypoglycemia (NICTH) is a rare cause of persistent hypoglycemia seen in patients with hepatocellular carcinoma (HCC). It is likely to be underdiagnosed especially in the patients with poor hepatic function and malnutrition. Herein, we report a rare case of NICTH as the initial presentation of HCC in a patient with chronic hypoglycemia due to end-stage liver cirrhosis.

CASE SUMMARY

A 62-year-old male with chronic fasting hypoglycemia secondary to end-stage hepatitis C-related cirrhosis, presented with altered mental status and dizziness. He was found to have severe hypoglycemia refractory to glucose supplements. Imaging studies and biopsy discovered well differentiated HCC without metastasis. Further evaluation showed low insulin, C-peptide and beta-hydroxybutyrate along with a high insulin-like growth factor-2/insulin-like growth factor ratio, consistent with the diagnosis of NICTH. As patient was not a candidate for surgical resection or chemotherapy, he was started on prednisolone with some improvements in the glucose homeostasis, but soon decompensated after a superimposed hospital acquired pneumonia.

CONCLUSION

NICTH can occur as the sole initial presentation of HCC and is often difficult to correct without tumor removal. Clinicians should maintain high clinical suspicion for early recognition of paraneoplastic NICTH in patients at risk for HCC, even those with chronic fasting hypoglycemia in the setting of severe hepatic failure and malnutrition.

Key words: Non-islet cell tumor hypoglycemia; Hepatocellular carcinoma; Liver cirrhosis; Insulin-like growth factor-2; Paraneoplastic syndrome; Case report

Manuscript source: Unsolicited manuscript

Received: March 31, 2020
Peer-review started: March 31, 2020
First decision: July 5, 2020
Revised: July 8, 2020
Accepted: July 26, 2020
Article in press: July 26, 2020
Published online: August 27, 2020

P-Reviewer: Tanaka Y
S-Editor: Zhang L
L-Editor: A
P-Editor: Li JH

Core tip: Paraneoplastic Non-islet cell tumor hypoglycemia can occur as an initial presentation in patients with hepatocellular carcinoma and is often difficult to correct. It is tend to be underdiagnosed because patients often developed tolerance to chronic fasting hypoglycemia secondary to advanced liver cirrhosis. A ratio of insulin-like growth factor-2/insulin-like growth factor-1 above 10 is often found if non-islet cell tumor hypoglycemia is induced by overproduction of incompletely processed insulin-like growth factor-2. Oral corticosteroids and frequent high carbohydrate meals are often recommended but the outcome is unfavorable in general if tumor removal is not possible.

Citation: Yu B, Douli R, Suarez JA, Gutierrez VP, Aldiabat M, Khan M. Non-islet cell tumor hypoglycemia as an initial presentation of hepatocellular carcinoma coupled with end-stage liver cirrhosis: A case report and review of literature. *World J Hepatol* 2020; 12(8): 519-524
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/519.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.519

INTRODUCTION

Non-islet cell tumor hypoglycemia (NICTH) is a rare paraneoplastic complication associated with malignancies of both epithelial and mesenchymal origin. One of the most common epithelial tumors is hepatocellular carcinoma (HCC)[1,2]. Hypoglycemia is induced either by tumor consumption of glucose (type A) or overproduction of incompletely processed insulin-like growth factor-2 (IGF-2) (type B), while levels of insulin, C-peptide, pro-insulin, and beta-hydroxybutyrate are suppressed[3]. NICTH occurs in 4% to 27% of patients with HCC[4]. However the actual prevalence might be underestimated due to limited availability of testing for IGF-2. In addition, the etiologies of hypoglycemia in HCC patients are often multifactorial. Many patients might have developed tolerance to chronic fasting hypoglycemia due to long term poor hepatic function and nutritional status at the time of discovery of HCC. Here we report a case of persistent NICTH as the initial presentation in a patient with newly diagnosed HCC overlapped with end-stage liver cirrhosis.

CASE PRESENTATION

Chief complaints

A 62-year-old Hispanic male with long-standing hepatitis C-related cirrhosis was brought to the emergency room on December 7, 2019 due to 2 episodes of altered mental status and non-vertiginous dizziness witnessed by his family. He also reported an unintentional 1-kg weight loss over the past 1 mo.

History of present illness

There was no history of loss of consciousness, falls, or head trauma. He was first found to have hepatitis C infection with concurrent liver cirrhosis and portal hypertension in 2015. Viral load became undetectable after the completion of antiviral therapy but the patient lost follow-up ever since July 2018. Child-Pugh score during the last outpatient visit was 8 (class B). AFP was within the normal limit. No signs of malignancy were found on liver ultrasound.

Physical examination upon admission

On physical exam, he was all the time conscious and had full ability to communicate. Vital signs were within normal limits. Rest of the physical exam was significant for cachectic appearance, jaundice, and bilateral lower extremity edema up to the knee.

Laboratory examination, imaging studies and diagnostic reasoning

In the emergency room, his blood glucose was detected to be 26 mg/dL. He denied poor oral intake or history of diabetes, alcohol abuse or illicit drug use. Of note, his blood glucose level tended to be on the lower side (75-85 mg/dL) seen in the records

of several outpatient visits before he lost follow-up. The blood glucose level was corrected by two immediate intravenous 50% dextrose pushes, but dropped again down to 10 mg/dL in 2 h for which continuous 10% dextrose infusion was started and the patient was instructed to consume frequent carbohydrate-rich snacks. However, recurrent hypoglycemic attacks still occurred since admission that required multiple IV 50% dextrose and glucagon pushes.

Laboratory evaluation of hypoglycemia showed undetectable insulin [< 0.4 μU/mL (2.6-24.9 μU/mL)], low C-peptide [0.2 ng/mL (1.1-4.4 ng/mL)], lower normal pro-insulin [1.3 pmol/L (0-10.0 pmol/L)], and undetectable beta-hydroxybutyrate [< 0.1 mg/dL (0.2-2.8 mg/dL)], excluding the possibility of insulinoma. Sulfonylurea screen test was negative. Adrenal insufficiency was also unlikely due to a high serum cortisol concentration. His hepatic function deteriorated [INR 2.8; albumin 2.9 g/dL (3.5-5.2 g/dL); total bilirubin 3.76 mg/dL (0.2-1.2 mg/dL); aspartate transaminase 145 U/L (< 40 U/L); alanine transaminase 93 U/L (< 41 U/L); alkaline phosphatase 263 U/L (40-130 U/L)]. Hepatic encephalopathy was also suspected due to high ammonia level [101 μmol/L (16-60 μmol/L)]. Child-Pugh score was calculated to be 11 (class C). AFP level was found to be elevated [108 ng/mL (< 8.3 ng/mL)]. Computed tomography of the abdomen with contrast showed cirrhosis and there was a centrally necrotic mass in the left hepatic lobe, measuring 6.7 cm × 6.5 cm (Figure 1). Three-phase liver computed tomography scan demonstrated suboptimal arterial phase enhancement due to the timing of the contrast with washout on delayed phase of the study. A subsequent biopsy confirmed the diagnosis of well differentiated HCC. No metastasis was found on bone scan. Therefore, NICTH was suspected. To establish the diagnosis, serum insulin-like growth factor-1 (IGF-1), IGF-2, and insulin-like growth factor-binding protein 3, the major binding protein for IGF-2 were measured. IGF-1 was suppressed [14 ng/mL (49-214 ng/mL)], IGF-2 was lower normal [303 ng/mL (300-960 ng/mL)], insulin-like growth factor-binding protein 3 was slightly decreased [2.2 μg/mL (2.6-4.8 μg/mL)], and the IGF-2/IGF-1 ratio was 21.6 (> 10), consistent with the diagnosis of NICTH.

FINAL DIAGNOSIS

NICTH (type B); well differentiated HCC, Barcelona Clinic Liver Cancer Stage D; decompensated liver cirrhosis, Child-Pugh Class C.

TREATMENT

The patient was not a candidate of transplant, surgical resection, or palliative chemotherapy due to the baseline poor hepatic function. He was started on oral prednisolone with a dose titrated up to 60 mg daily. There were less hypoglycemic episodes and the patient showed improvements in the severity of hypoglycemia. However, he still required on and off glucose supplements to maintain glucose homeostasis. While waiting for the trial of trans-arterial chemoembolization and radiotherapy, he developed hospital-acquired pneumonia. And concurrently his plasma glucose dropped and became difficult to correct again (Figure 2).

OUTCOME AND FOLLOW-UP

The patient opted for inpatient hospice care and died of septic shock on day 19 of hospitalization.

DISCUSSION

NICTH is a rare complication seen in patients with HCC. In the present case, the patient had advanced cirrhosis without regular follow up for a year, so it's unclear when the HCC first developed. Interestingly, acute hypoglycemic encephalopathy occurred as the sole initial clinical symptom prior to the diagnosis of HCC. Different from a few previously reported cases[5-9], our patient had a very poor hepatic function and nutritional status which could both contribute to his hypoglycemia to some extent.

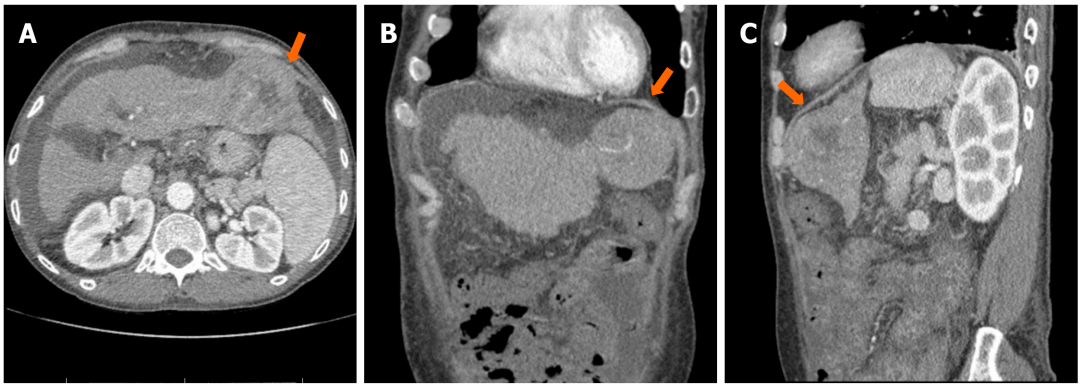

Figure 1 Arterial phase of abdominal computed tomography showed a centrally necrotic mass in the left hepatic lobe, measuring 6.7 cm × 6.5 cm (orange arrow). A: Axial view; B: Coronal view; C: Sagittal view.

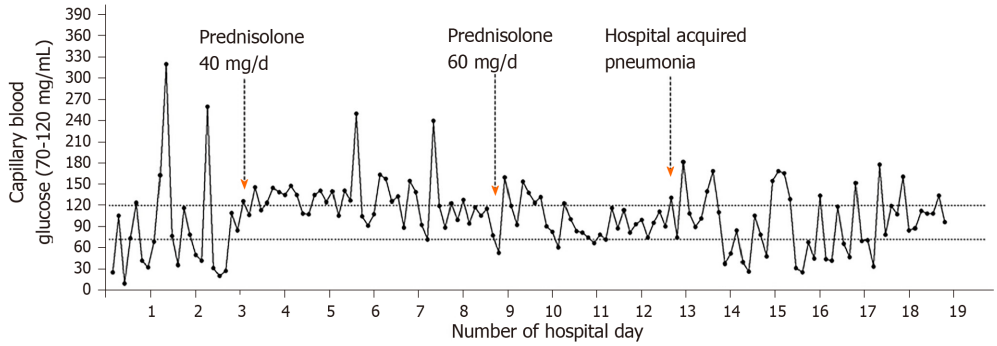

Figure 2 Capillary blood glucose trend.

He might have developed chronic hypoglycemia with diminished awareness during the past year since there were no signs of sympathetic activation. This might obscure other underlying causes of hypoglycemia if patient was not assessed thoroughly. However, his glucose level fluctuated drastically and was very difficult to correct. Further investigation for NICTH is merited given high risk of malignancy.

Two types of NICTH (type A and B) are seen in HCC patients[10]. Type A often occurs at the terminal stage of disease when there is an increased glucose consumption by the tumor on top of a progressive reduction in glucose supply due to hepatic failure on the residual liver tissue and in part due to malnutrition. The tumor mass is usually rapid growing and poorly differentiated, associated with severe anorexia, muscle wasting and weight loss. But hypoglycemia is often mild and relatively easier to correct[11,12]. Type B, less common than type A, is related to an overproduction of IGF-2 and its precursors by the tumor. It often occurs at the earlier course of the disease and is thought to be a paraneoplastic syndrome. The severity of hypoglycemia is predominant and is often difficult to control. Glucose utilization by the tumor might also contribute to the hypoglycemia but is not a significant pathway[9]. The excess of IGF-2 Messenger RNA overwhelms the enzyme transforming pro-IGF-2 to mature IGF-2, thus producing various sizes of incompletely processed and unprocessed pro-IGF-2, the so called "big IGF-2"[3,13]. Normally most of serum IGF-2 is transported in the form of a 150 kDa ternary complex together with insulin-like growth factor-binding protein 3 and acid-labile sub-unit. But the "big IGF-2" mainly forms a 50 kDa binary complex with only insulin-like growth factor-binding protein 3. These binary complexes have a higher biological activity and can readily cross the capillary membrane to interact with insulin receptors in the liver, adipose tissue, and skeletal muscle due to their smaller size, leading to more glucose uptake and inhibition of gluconeogenesis[14,15]. By interacting with the IGF-1 receptors in the hypothalamus, the excess of pro-IGF-2 and IGF-2 inhibits the secretion of growth hormone, which in turn suppresses the production of IGF-1, insulin-like growth factor-binding protein 3, and acid-labile sub-unit. Therefore, more amount of free IGF-2 might gain access to the target tissue[16-19].

As to our patient, IGF-2 was inappropriately normal for the extremely low IGF-1 level. An IGF-2/IGF-1 ratio greater than 10 has been proposed to be enough to confirm the diagnosis of NICTH[16,20,21]. IGF-2 might be falsely normal in our patient because the sample was collected after the first dose of prednisolone was administered, which was able to inhibit the production of IGF-2[22]. In addition, serum IGF-2 levels in NICTH are often not elevated partially because most "big IGF-2" are not measured by common commercially available assay[16,23,24]. It has also been found by a few case reports that the levels of serum IGF-2 were decreased or normal in contrast to an increased pro-IGF-2 in NICTH[25,26]. Pro-IGF-2 was not measured in this patient because the test was not available in our setting. Although we are not able to entirely exclude the possibility of excessive glucose consumption by the tumor, the tumor mass was not extensive, only occupying part of the left lobe, and the level of AFP was not significantly elevated, indicating mild biological activities. The hepatic failure was more likely due to his advanced cirrhosis rather than the tumor. Therefore, we believe that our case fits more into type B rather than type A NICTH.

Priority of management of NICTH is still tumor resection. In inoperable patients, several treatment options of local tumor cytoreduction are recommended, including percutaneous ethanol injection and trans-arterial chemoembolization[27,28]. Systemic chemotherapy, such as Sorafenib or FOLFOX (oxaliplatin and 5-fluorouracil/leucovorin), has also been showed to be effective[29]. In addition, emerging drugs that directly inhibit the IGF signals (PI3K-AKT-TOR or RAF-MEK-ERK) are under investigation[30]. In case that the primary malignancy cannot be treated, palliative medical management can be chosen. Glucocorticoid together with frequent high carbohydrate meals and IV glucose infusions is an ideal option to achieve long-term prevention of hypoglycemia. Glucocorticoid, on one hand, stimulates hepatic gluconeogenesis and inhibits peripheral glucose uptake; on the other hand, can reduce the level of "big IGF-2" either by decreasing tumor production or by promoting the maturation of pro-IGF-2 and the formation of normal ternary complexes[22,31-33]. Other than glucocorticoid, glucagon, growth hormone, and octreotide infusion are also recommended, but their effects are transient and limited[2,8,34,35]. Our patient initially showed responses to high-dose prednisolone, but it failed to last for a long time mainly because of a poor hepatic reserve from cirrhosis. And the concurrent sepsis and pneumonia further destroyed patient's ability to maintain the euglycemic status.

CONCLUSION

In conclusion, paraneoplastic NICTH should be considered in the evaluation of refractory hypoinsulinemic hypoglycemia in patients with risk factors of HCC, even in the setting of chronic fasting hypoglycemia induced by severe hepatic failure and malnutrition. NICTH can occur as the only initial presentation of HCC. Oral corticosteroids and frequent high carbohydrate meals are often recommended but the outcome is unfavorable in general if tumor removal is not possible.

REFERENCES

1 **Marks V**, Teale JD. Tumours producing hypoglycaemia. *Diabetes Metab Rev* 1991; **7**: 79-91 [PMID: 1665409 DOI: 10.1002/dmr.5610070202]
2 **Bodnar TW**, Acevedo MJ, Pietropaolo M. Management of non-islet-cell tumor hypoglycemia: a clinical review. *J Clin Endocrinol Metab* 2014; **99**: 713-722 [PMID: 24423303 DOI: 10.1210/jc.2013-3382]
3 **Ishida S**, Noda M, Kuzuya N, Kubo F, Yamada S, Yamanaka T, Isozaki O, Hizuka N, Kanazawa Y. Big insulin-like growth factor II-producing hepatocellular carcinoma associated with hypoglycemia. *Intern Med* 1995; **34**: 1201-1206 [PMID: 8929651 DOI: 10.2169/internalmedicine.34.1201]
4 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]
5 **Lau CI**, Wang HC, Hsu WC. Hypoglycemic encephalopathy as the initial presentation of hepatic tumor: a case report. *Neurologist* 2010; **16**: 206-207 [PMID: 20445433 DOI: 10.1097/NRL.0b013e3181a6ec56]
6 **Jha V**, Borpujari P. Hypoglycaemia presenting as sole manifestation of hepatocellular carcinoma. *Med J Armed Forces India* 2012; **68**: 75-77 [PMID: 24669040 DOI: 10.1016/S0377-1237(11)60113-5]
7 **Tsai CY**, Chou SC, Liu HT, Lin JD, Lin YC. Persistent hypoglycemia as an early, atypical presentation of hepatocellular carcinoma: A case report and systematic review of the literature. *Oncol Lett* 2014; **8**: 1810-1814 [PMID: 25202415 DOI: 10.3892/ol.2014.2365]
8 **Sharma M**, Reddy DN, Kiat TC. Refractory Hypoglycemia Presenting as First Manifestation of Advanced Hepatocellular Carcinoma. *ACG Case Rep J* 2014; **2**: 50-52 [PMID: 26157905 DOI: 10.14309/crj.2014.82]
9 **Zhou S**, Jiang L, Sun M. Recurrent hypoglycemic coma as the initial and single clinical manifestation of advanced hepatocellular carcinoma. *J Gastrointest Cancer* 2015; **46**: 64-67 [PMID: 25407746 DOI:

10.1007/s12029-014-9670-3]
10 **McFadzean AJ**, Yeung RT. Further observations on hypoglycaemia in hepatocellular carcinoma. *Am J Med* 1969; **47**: 220-235 [PMID: 4309111 DOI: 10.1016/0002-9343(69)90148-x]
11 **Yeung RT**. Hypoglycaemia in hepatocellular carcinoma: a review. *Hong Kong Med J* 1997; **3**: 297-301 [PMID: 11847375]
12 **Sorlini M**, Benini F, Cravarezza P, Romanelli G. Hypoglycemia, an atypical early sign of hepatocellular carcinoma. *J Gastrointest Cancer* 2010; **41**: 209-211 [PMID: 20204540 DOI: 10.1007/s12029-010-9137-0]
13 **Daughaday WH**, Emanuele MA, Brooks MH, Barbato AL, Kapadia M, Rotwein P. Synthesis and secretion of insulin-like growth factor II by a leiomyosarcoma with associated hypoglycemia. *N Engl J Med* 1988; **319**: 1434-1440 [PMID: 3185662 DOI: 10.1056/NEJM198812013192202]
14 **Eastman RC**, Carson RE, Orloff DG, Cochran CS, Perdue JF, Rechler MM, Lanau F, Roberts CT Jr, Shapiro J, Roth J. Glucose utilization in a patient with hepatoma and hypoglycemia. Assessment by a positron emission tomography. *J Clin Invest* 1992; **89**: 1958-1963 [PMID: 1318326 DOI: 10.1172/JCI115803]
15 **Daughaday WH**, Kapadia M. Significance of abnormal serum binding of insulin-like growth factor II in the development of hypoglycemia in patients with non-islet-cell tumors. *Proc Natl Acad Sci USA* 1989; **86**: 6778-6782 [PMID: 2771956 DOI: 10.1073/pnas.86.17.6778]
16 **Teale JD**, Marks V. Inappropriately elevated plasma insulin-like growth factor II in relation to suppressed insulin-like growth factor I in the diagnosis of non-islet cell tumour hypoglycaemia. *Clin Endocrinol (Oxf)* 1990; **33**: 87-98 [PMID: 2205424 DOI: 10.1111/j.1365-2265.1990.tb00469.x]
17 **Frohman LA**, Downs TR, Chomczynski P. Regulation of growth hormone secretion. *Front Neuroendocrinol* 1992; **13**: 344-405 [PMID: 1360911]
18 **Dynkevich Y**, Rother KI, Whitford I, Qureshi S, Galiveeti S, Szulc AL, Danoff A, Breen TL, Kaviani N, Shanik MH, Leroith D, Vigneri R, Koch CA, Roth J. Tumors, IGF-2, and hypoglycemia: insights from the clinic, the laboratory, and the historical archive. *Endocr Rev* 2013; **34**: 798-826 [PMID: 23671155 DOI: 10.1210/er.2012-1033]
19 **Rana P**, Kim B. A Unique Case of IGF-2 Induced Hypoglycemia Associated with Hepatocellular Carcinoma. *Case Rep Endocrinol* 2019; **2019**: 4601484 [PMID: 31737377 DOI: 10.1155/2019/4601484]
20 **Cryer PE**, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, Service FJ; Endocrine Society. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2009; **94**: 709-728 [PMID: 19088155 DOI: 10.1210/jc.2008-1410]
21 **Garla V**, Sonani H, Palabindala V, Gomez-Sanchez C, Subauste J, Lien LF. Non-islet Cell Hypoglycemia: Case Series and Review of the Literature. *Front Endocrinol (Lausanne)* 2019; **10**: 316 [PMID: 31156561 DOI: 10.3389/fendo.2019.00316]
22 **Teale JD**, Marks V. Glucocorticoid therapy suppresses abnormal secretion of big IGF-II by non-islet cell tumours inducing hypoglycaemia (NICTH). *Clin Endocrinol (Oxf)* 1998; **49**: 491-498 [PMID: 9876347 DOI: 10.1046/j.1365-2265.1998.00564.x]
23 **Yonei Y**, Tanaka M, Ozawa Y, Miyazaki K, Tsukada N, Inada S, Inagaki Y, Miyamoto K, Suzuki O, Okawa H. Primary hepatocellular carcinoma with severe hypoglycemia: involvement of insulin-like growth factors. *Liver* 1992; **12**: 90-93 [PMID: 1320177 DOI: 10.1111/j.1600-0676.1992.tb00563.x]
24 **Forde JJ**, Ewelukwa O, Brar T, Cabrera R. Intractable Fasting Hypoglycemia as a Manifestation of Hepatocellular Carcinoma. *Case Reports Hepatol* 2017; **2017**: 7465025 [PMID: 28785493 DOI: 10.1155/2017/7465025]
25 **Zapf J**, Futo E, Peter M, Froesch ER. Can "big" insulin-like growth factor II in serum of tumor patients account for the development of extrapancreatic tumor hypoglycemia? *J Clin Invest* 1992; **90**: 2574-2584 [PMID: 1281841 DOI: 10.1172/JCI116152]
26 **van den Berg SAA**, Krol CG. Pro-IGF2-induced hypoglycaemia associated with hepatocellular carcinoma. *Endocrinol Diabetes Metab Case Rep* 2017; 2017 [PMID: 28567293 DOI: 10.1530/EDM-17-0004]
27 **Saigal S**, Nandeesh HP, Malhotra V, Sarin SK. A case of hepatocellular carcinoma associated with troublesome hypoglycemia: management by cytoreduction using percutaneous ethanol injection. *Am J Gastroenterol* 1998; **93**: 1380-1381 [PMID: 9707076 DOI: 10.1111/j.1572-0241.1998.427_h.x]
28 **Whitsett M**, Lindenmeyer CC, Shaw CM, Civan JM, Fenkel JM. Transarterial chemoembolization for palliation of paraneoplastic hypoglycemia in a patient with advanced hepatocellular carcinoma. *J Vasc Interv Radiol* 2013; **24**: 1918-1920 [PMID: 24267531 DOI: 10.1016/j.jvir.2013.07.002]
29 **Huang JS**, Chang PH. Refractory hypoglycemia controlled by systemic chemotherapy with advanced hepatocellular carcinoma: A case report. *Oncol Lett* 2016; **11**: 898-900 [PMID: 26870302 DOI: 10.3892/ol.2015.3915]
30 **Gualberto A**, Pollak M. Emerging role of insulin-like growth factor receptor inhibitors in oncology: early clinical trial results and future directions. *Oncogene* 2009; **28**: 3009-3021 [PMID: 19581933 DOI: 10.1038/onc.2009.172]
31 **Baxter RC**, Holman SR, Corbould A, Stranks S, Ho PJ, Braund W. Regulation of the insulin-like growth factors and their binding proteins by glucocorticoid and growth hormone in nonislet cell tumor hypoglycemia. *J Clin Endocrinol Metab* 1995; **80**: 2700-2708 [PMID: 7545698 DOI: 10.1210/jcem.80.9.7545698]
32 **Thipaporn T**, Bubpha P, Varaphon V. Hepatocellular carcinoma with persistent hypoglycemia: successful treatment with corticosteroid and frequent high carbohydrate intake. *J Med Assoc Thai* 2005; **88**: 1941-1946 [PMID: 16518997]
33 **de Groot JW**, Rikhof B, van Doorn J, Bilo HJ, Alleman MA, Honkoop AH, van der Graaf WT. Non-islet cell tumour-induced hypoglycaemia: a review of the literature including two new cases. *Endocr Relat Cancer* 2007; **14**: 979-993 [PMID: 18045950 DOI: 10.1677/ERC-07-0161]
34 **Wing JR**, Panz VR, Joffe BI, Kalk WJ, Seftel HC, Zapf J, Kew MC. Hypoglycemia in hepatocellular carcinoma: failure of short-term growth hormone administration to reduce enhanced glucose requirements. *Metabolism* 1991; **40**: 508-512 [PMID: 1850816 DOI: 10.1016/0026-0495(91)90232-l]
35 **Hoff AO**, Vassilopoulou-Sellin R. The role of glucagon administration in the diagnosis and treatment of patients with tumor hypoglycemia. *Cancer* 1998; **82**: 1585-1592 [PMID: 9554538]

World Journal of Hepatology

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

World J Hepatol 2020 August 27; 12(8): 525-532

DOI: 10.4254/wjh.v12.i8.525

ISSN 1948-5182 (online)

LETTERS TO THE EDITOR

"Six-and-twelve" score for outcome prediction of hepatocellular carcinoma following transarterial chemoembolization. In-depth analysis from a multicenter French cohort

Xavier Adhoute, Guillaume Pénaranda, Jean-Luc Raoul, Jean-Pierre Bronowicki, Rodolphe Anty, Marc Bourlière

ORCID number: Xavier Adhoute 0000-0001-5977-800X; Guillaume Pénaranda 0000-0002-7461-4254; Jean-Luc Raoul 0000-0001-6305-8953; Jean-Pierre Bronowicki 0000-0003-1631-500X; Rodolphe Anty 0000-0002-8053-1957; Marc Bourlière 0000-0001-8976-9200.

Author contributions: Adhoute X, Raoul J, Bronowicki J and Bourlière M are physicians in charge of the patients; Adhoute X and Bronowicki J collected the data and Pénaranda G proceeded to statistical analysis; Adhoute X, Bronowicki J and Anty R wrote the manuscript.

Conflict-of-interest statement: The authors have no potential conflict of interest relevant to this article.

Xavier Adhoute, Marc Bourlière, Department of Gastroenterology and Hepatology, Hôpital Saint-Joseph, Marseille 13008, France

Guillaume Pénaranda, AlphaBio Laboratory, Marseille 13003, France

Jean-Luc Raoul, Department of Medical Oncology, Institut de Cancérologie de l'Ouest, Nantes 44805, France

Jean-Pierre Bronowicki, Department of Gastroenterology and Hepatology, Centre Hospitalo-Universitaire de, Nancy 54511, France

Rodolphe Anty, Department of Gastroenterology and Hepatology, Hôpital Universitaire de l'Archet, Nice 06200, France

Corresponding author: Adhoute Xavier, MD, Doctor, Department of Gastroenterology and Hepatology, Hôpital Saint-Joseph, 26 Bd de Louvain, Marseille 13008 France. adhoute.xavier@neuf.fr

Abstract

The "six-and-twelve" (6&12) score is a new hepatocellular carcinoma (HCC) prognostic index designed for recommended transarterial chemoembolization (TACE) candidates. Quick and easy to use by the sum of tumor size (cm) and number, this model identifies three groups with different survival time (the sum is ≤ 6; or > 6 but ≤ 12; or > 12); a survival benefit with TACE can be expected for HCC patients with a score not exceeding twelve. Recently, Wang ZW *et al* showed that the "6&12" model was the best system correlated with radiological response after the first TACE. Thus, we wanted to assess its survival prediction ability as well as its prognostic value and compared it to other systems (Barcelona Clinic Liver Cancer, Hong Kong Liver Cancer (HKLC) staging, Albumin-Bilirubin grade, tumor nodularity, infiltrative nature of the tumor, alpha-fetoprotein, Child-Pugh class, and Performance Status score, Cancer of the Liver Italian Program, Model to Estimate Survival for HCC scores, up-to-seven criteria) different from Wang ZW *et al* study in a multicenter French cohort of HCC including only recommended TACE candidates retrospectively enrolled. As previously demonstrated, we show that the "6&12" score can classify survival within this French cohort, with a

Manuscript source: Invited manuscript

Received: April 13, 2020
Peer-review started: April 13, 2020
First decision: April 22, 2020
Revised: July 5, 2020
Accepted: July 19, 2020
Article in press: July 19, 2020
Published online: August 27, 2020

P-Reviewer: Montasser IF
S-Editor: Liu M
L-Editor: A
P-Editor: Li JH

prognostic value comparable to that of other systems, except HKLC staging. More importantly, the "6&12" score simplicity and ability in patients' stratification outperform other systems for a routine clinical practice.

Key words: Hepatocellular carcinoma; Transarterial chemoembolization; "Six-and-twelve" score; Prognosis; Albumin-Bilirubin grade; Tumor nodularity, infiltrative nature of the tumor, alpha-fetoprotein, Child-Pugh class, and performance status score

Core tip: Not all-intermediate stage hepatocellular carcinoma (HCC) benefit from transarterial chemoembolization (TACE). The recent "six-and-twelve" (6&12) score is an easy to use prognostic model that ensure a quick and appropriate patient' selection before the first TACE in Chinese cohorts. In this multicenter French cohort of HCC, the "6&12" score can also classify survival among recommended TACE candidates with a good prognostic performance. It may help clinicians in routine clinical practice.

Citation: Adhoute X, Pénaranda G, Raoul JL, Bronowicki JP, Anty R, Bourlière M. "Six-and-twelve" score for outcome prediction of hepatocellular carcinoma following transarterial chemoembolization. In-depth analysis from a multicenter French cohort. *World J Hepatol* 2020; 12(8): 525-532
URL: https://www.wjgnet.com/1948-5182/full/v12/i8/525.htm
DOI: https://dx.doi.org/10.4254/wjh.v12.i8.525

TO THE EDITOR

We have read with great interest the study by Wang *et al*[1] who assessed and compared different prognostic models for hepatocellular carcinoma (HCC) patients undergoing transarterial chemoembolization (TACE) treatment, especially the latest "six-and-twelve" (6&12) score[2] within a nationwide Chinese HCC cohort (n = 1107). Increased survival after TACE is correlated with radiological response[3,4] and this study shows that the "6&12" index is the best system correlated with radiological response after the first TACE. The study population was more heterogeneous than the population used to develop the score, including patients with slightly altered performance status (PS) and logically a model like the 3rd version of the hepatoma arterial-embolization prognostic score[5] (which include liver function parameters) had a higher predictive value for survival. However, simplicity (using two cut-off values for risk stratification) and presumed reliability of the "6&12" score have convinced us to assess once again[6] the reproducibility and the predictive value of this new model in a multicenter French cohort of HCC patients including only recommended TACE candidates (n = 324) ie intermediate and early unresectable stages according to the treatment stage migration concept. We compared it to other systems different from Wang *et al*[1]'s study (Barcelona Clinic Liver cancer[7] (BCLC) staging, Child-Pugh (CP) class, Albumin-Bilirubin[8] (ALBI) grade, NIACE[9] [tumor nodularity, infiltrative nature of the tumor, alpha-fetoprotein (AFP), CP class, and PS] score (Table 1)) using time-dependent area under receiver operating characteristic curve (AUROC) values and C-indices.

Patients were retrospectively enrolled over a six years period in two centers (Marseille, Nancy). Demographic and clinical characteristics of HCC patients are shown in Table 2. HCC patients were mostly male (85%), with a median of age of 68 years. Cirrhosis was present in 96% of cases, CP class A (77%), CP class B7 (23%). Underlying liver disease was mostly related to alcohol abuse (38%) or viral C hepatitis (40%). Patients were BCLC stage B (n = 179), BCLC stage A (n = 145). HCC were multinodular in 71% of cases and the median tumor diameter was 35 mm (25-50). The mean session number of conventional TACE was 2.7 ± 1.8.

After a median follow-up duration of 24.4 (15.0-36.8) mo, eighty one percent of patients died. Kaplan-Meier analyses showed significant differences in overall survival (OS) distributions across subgroups of BCLC staging, "6&12" (Figure 1) and NIACE scores within this cohort (P < 0.05) (Table 3). Liver function at baseline also had an impact on survival; median OS was significantly different according to the CP class

Table 1 Summary of points-based scores

CLIP (0 to 7 points)			MESH (0 to 6 points)		NIACE (0 to 7 points)	
Portal vein thrombosis		1 point	Tumor extent: Beyond Milan criteria	1 point	Tumor nodules ≥ 3	1 point
AFP ≥ 400 ng/mL		1 point	Vascular invasion and/or Extrahepatic spread	1 point	Infiltrative HCC	1.5 points
					Nodular HCC	0 point
Child-Pugh grade	A	0 point	PS ≥ 2	1 point	AFP ≥ 200 ng/mL	1.5 points
	B	1 point	Child-Pugh grade ≥ A6	1 point		
	C	2 points				
Tumor extent	Unidolar and extension ≤ 50%	0 point	AFP ≥ 20 ng/mL	1 point	Child-Pugh grade A	0 point
	Multinodular and extension ≤ 50%	1 point	Alkaline phosphatase ≥ 200 IU/l	1 point	Child-Pugh grade B	1.5 points
	Massive or extension > 50%	2 points			PS ≥ 1	1.5 points

CLIP: Cancer of the Liver Italian Program; MESH: Model to Estimate Survival for Hepatocellular carcinoma; NIACE: Tumor nodularity, infiltrative nature of the tumor, alpha-fetoprotein, Child-Pugh class, and performance status.

[CP-A, 27 (25-31) mo; CP-B7, 21 (15-24) mo (P = 0.0003)], or ALBI grade [grade 1, 35 (25-43) mo; grade 2, 26 (22-28) mo; grade 3, 16 (12-24) mo (P = 0.0029)].

Performances of the "6&12" score and other systems for survival prediction are indicated in Table 4. Time-dependent AUROC values and C-indices of the "6&12" score was not significantly different from those of other systems. We checked our results within the main cohort from Marseille (n = 252) (Table 2) by comparing the "6&12" score to other staging scoring systems (Hong Kong Liver Cancer[10] (HKLC), Cancer of the Liver Italian Program[11] (CLIP), Model to Estimate Survival for HCC[12] (MESH), up-to-seven criteria[13]). Significant differences in survival distributions were also found across subgroups of the "6&12" score and other systems within this single center cohort (P < 0.05) (Table 5). Its predictive value remained comparable to that of other systems [C-index "6&12" 0.63 (0.56-0.70) *vs* CLIP 0.70 (0.62-0.78) *vs* "up-to-seven" 0.61 (0.56-0.66) *vs* MESH 0.71 (0.63-0.78), not significant] except for HKLC staging, which provides a better prognostication ability [3-year AUROC ("6&12") 0.56 (0.44-0.68) *vs* (HKLC) 0.69 (0.65-0.74), P = 0.0325] using a more complex stratification into five subgroups.

Firstly, our findings confirm previously published results[1,2], the "6&12" score can classify survival among recommended TACE candidates. Its prognostic performance was similar within our cohort compared to Wang *et al*[2] original study [3-year AUROC values: 0.64 (0.58-0.71) *vs* 0.65 (0.61, 0.70); C-indices: 0.66 (0.58-0.74) *vs* 0.66 (0.63, 0.69) (Table 4)], and higher than that observed in this nationwide Chinese cohort[1] [c-index: 0.58 (0.56, 0.60)]. Moreover, HCC patients with the highest tumor burden [sum of largest tumor size (cm) and number exceeding 12] have a median survival of 15 mo similar to Wang *et al*[1]'s manuscript. Thus, this model can also identify within our population a subgroup of patients with poor prognosis who may not achieve benefit from TACE. The "6&12" risk stratification into three subgroups is relevant. Indeed, the first one (sum of tumor size and number not exceeding six) identifies TACE candidates with long-term survival especially those who may achieve a complete necrosis after this treatment[14,15]. Moreover, TACE is also an effective therapy for the second subgroup (sum of tumor size and number above six and not exceeding twelve), which has clear boundaries unlike intermediate stage subclassifications[16,17] that divide tumor burden according to the up-to-seven criteria (within/out).

Secondly, in our study the "6&12" score prognostic value is comparable to that of other systems, but most of these models cannot be used to guide treatment decision directly. "6&12" simplicity outweighs other systems for a current clinical practice including models with online calculator[5]. Indeed, therapeutic management is determined using a multidisciplinary approach and control of different published prognostic scores for TACE by clinicians (surgeons, oncologists, hepatologists and radiologists) is very unusual. By adding "the sum of largest tumor size and number", it is true that consensus is easy to achieve among all clinicians. Moreover, other scores[9] encompass other baseline features that are likely to impact OS such as morphology of the tumor[18], but those parameters are not routinely recorded, which

Table 2 Baseline characteristics of hepatocellular carcinoma patients undergoing transarterial chemoembolization treatment, *n* (%)

Demographic variables	Marseille/Nancy cohort, *n* = 324	Marseille cohort[1],*n* = 252
Age - Median [Q1-Q3], year	68 [62-74]	68 [60-73]
Gender Male/female	276 (85)/48 (15)	214 (85)/38 (15)
Liver disease HCV/HBV/Alcoholism/MS/other	129 (40)/14 (4)/122 (38)/42 (13)/17 (5)	109 (43)/12 (5)/84 (33)/37 (15)/10 (4)
ECOG (PS-0)	324 (100)	252 (100)
Cirrhosis	311 (96)	243 (96)
Tumor variables:		
Tumor Size – mm - median [q1-q3]	35 [25-50]	32 [25-44]
Nodule (s): 1/2/3/4/≥ 5	95 (29)/72 (22)/80 (25)/38 (12)/39 (12)	83 (33)/67 (27)/34 (13)/31 (12)/37 (15)
Laboratory variables		
AFP – ng/mL, median [q1-q3]	16.3 [6.0-120.3]	11.2 [5.0-77.7]
PT (%), median [q1-q3]	76 [64-88]	78 [68-88]
Albumin (g/L), median [q1-q3]	35 [28-38]	36.6 [32.7-41.0]
Total bilirubin (mcmol/L), median [q1-q3]	19.0 [13.7-28.7]	17 [11-27]
Child - Pugh grade A/B7	249 (77)/75 (23)	180 (71)/72 (29)
ALBI[1] class	64 (20)/230 (71)/30 (9)	37 (15)/175 (73)/29 (12)
BCLC[1] stage A/B	145 (45%)/179 (55%)	134 (56)/107 (44)
"6&12"[1] score allocation *n* ≤ 6/> 6 - ≤ 12/> 12	154 (48)/163 (50)/7 (2)	130 (54)/106 (44)/5 (2)
NIACE score allocation ≤ 1/1.5 - 3/> 3	168 (52)/134 (41)/22 (7)	
CLIP[1] score allocation 0/1/2/≥ 3	-	55 (23)/135 (56)/45 (19)/ 6 (2)
MESH[1] score allocation 0/1/2/3/4	-	41 (17)/77 (32)/78 (32)/37 (15)/8 (4)
Up-to-Seven model[1] (In/Out)	-	176 (73)/65 (27)
HKLC[1] stage 1/2a/2b/3a/3b	-	89 (37)/43 (17)/65 (27)/24 (10)/21 (9)

[1]Available data for 241 patients for staging and scores calculation. The Albumin-Bilirubin (ALBI) score was calculated according to the . ALBI grades were defined as ALBI grade 1 (score ≤ -2.60), ALBI grade 2 (score > - 2.60 and ≤ - 1.39) and ALBI grade 3 (score > - 1.39). Bilirubin level in mcmol/L and albumin level in g/L; Up-to-seven criteria: With seven as the sum of the largest tumor size (in cm) + number of tumor(s). Barcelona Clinic Liver cancer (BCLC) classification: Current (BCLC) staging considers solitary tumor > 2 cm or no more than 3 tumors not exceeding 3 cm in diameter (Performance Status-0, Child-Pugh (CP) class A or B7 grade) as stage A. No tumor was classified at the very early stage of hepatocellular carcinoma (HCC) (BCLC 0) in this multicenter French cohort. BCLC stage B HCC encompassed patients with multiple tumors beyond 3 cm, PS-0, CP A or B7 grade. Hong Kong Liver Cancer classification: Early tumor: ≤ 5 cm, ≤ 3 tumor nodules; CP grade A (stage 1), CP grade B (stage 2a), -Intermediate tumor: ≤ 5 cm and > 3 tumor nodules or > 5 cm and ≤ 3 tumor nodules, CP grade A (stage 2b), CP grade B (stage 3a), - Locally-advanced tumor: > 5 cm, > 3 tumor nodules, CP grade A or B (stage 3b). HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; HCV: Hepatitis C virus; HBV: Hepatitis B virus; MS: Metabolic syndrome; ECOG (PS): Eastern Cooperative Oncology Group (Performance Status); AFP: Alpha-fetoprotein; PT: Prothrombin Time; ALBI: Albumin-Bilirubin; BCLC: Barcelona Clinic Liver Cancer; "6&12": "Six-and-twelve"; NIACE: Tumor nodularity, infiltrative nature of the tumor, AFP, CP, PS; CLIP: Cancer of the Liver Italian Program; MESH: Model to Estimate Survival for Hepatocellular carcinoma; HKLC: Hong Kong Liver Cancer.

limits their use.

Thirdly, TACE should be limited to HCC patients with preserved liver function, and our results also highlight the importance of liver function in our population that included only recommended TACE candidates. Our patients are older, with more cirrhotic patients, and more alcohol-related diseases. This probably explains the differences in survival observed between this multicenter French cohort and Wang *et al*[2] original study, with OS ranging from 31.0 to 15.0 mo compared to 43.3 to 16.8 mo (according to "6&12" score), respectively. However, OS observed in our cohort was comparable to that of this nationwide Chinese cohort[1] including a more heterogeneous population with OS ranging from 31.3 to 18.5 mo.

Fourthly, Wang *et al*[19] findings on ABCR score are not surprising. This model designed for further TACE combines four parameters (AFP serum level, BCLC stage, change in Child-Pugh grade, and radiological tumor Response), but unlike ART[20,21] (assessment for re-treatment with TACE) model the highest coefficient is assigned to

Table 3 Kaplan-Meier survival analysis according to "Six-and-twelve" score and other systems in the multicenter French cohort (*n* = 324)

Scoring/stage systems	OS [95%CI], mo	*P* value (log-rank)	Sidak[1]	Hazard ratio [95%CI]	*P* value
"6&12" score		< 0.0001			
sum ≤ 6 (*n* = 154)	31 [27-35]		Ref	Ref	
sum > 6 ≤ 12 (*n* = 163)	20 [17-24]		0.0009	1.55 [1.21-1.99]	0.0005
sum > 12 (*n* = 7)	15 [5-19]		< 0.0001	3.80 [1.76-8.21]	0.0007
BCLC staging		< 0.0001			
A (*n* = 145)	35 [29-38]		NR	Ref	
B (*n* = 179)	19 [17-23]		NR	1.88 [1.47-2.41]	< 0.0001
NIACE score		< 0.0001			
≤ 1 (*n* = 168)	35 [28-36]		Ref	Ref	
1.5 - 3 (*n*= 134)	20 [16-23]		< 0.0001	1.92 [1.49-2.48]	< 0.0001
> 3 (*n* = 22)	11 [5-16]		< 0.0001	6.23 [3.87-10.02]	< 0.0001
Child-Pugh class		0.0003			
A (*n* = 249)	27 [25-31]		NR	Ref	
B (*n* = 75)	21 [15-24]		NR	1.66 [1.26-2.19]	0.0003
ALBI grade		0.0029			
Grade 1 (*n* = 64)	35 [25-43]		Ref	Ref	
Grade 2 (*n* = 230)	26 [22-28]		0.1228	1.50 [1.06-2.11]	0.0216
Grade 3 (*n* = 30)	16 [12-24]		0.0016	2.30 [1.41-3.75]	0.0009

[1]Sidak test for multiple comparisons. OS: Overall Survival; CI: Confidence Interval; "6&12": "Six-and-twelve"; Ref: Reference; BCLC: Barcelona Clinic Liver Cancer; NIACE: Tumor nodularity, infiltrative nature of the tumor, alpha-fetoprotein, child-pugh class, performance status; ALBI: Albumin-Bilirubin.

Table 4 Comparison of predictive accuracy for overall survival between "Six-and-Twelve" score and staging/scoring systems (multicenter French cohort *n* = 324)

Scoring/stage systems	1-yr AUROC	*P* (*vs* ref)	2-yr AUROC	*P* (*vs* ref)	3-yr AUROC	*P* (*vs* ref)	C-index	*P* (*vs* ref)
"6&12" score	0.65 [0.57-0.74]	Ref	0.65 [0.59-0.71]	Ref	0.64 [0.58-0.71]	Ref	0.66 [0.58-0.74]	
BCLC staging	0.61 [0.54-0.67]	0.1827	0.64 [0.59-0.70]	0.7079	0.61 [0.55-0.68]	0.2317	0.61 [0.54-0.68]	NS
NIACE score	0.75 [0.68-0.83]	0.0134	0.69 [0.64-0.75]	0.2368	0.69 [0.63-0.74]	0.2827	0.70 [0.64-0.77]	NS
Child-Pugh class	0.56 [0.49-0.63]	0.1057	0.56 [0.51-0.60]	0.0217	0.55 [0.50-0.59]	0.0304	0.59 [0.55-0.64]	NS
ALBI grade	0.63 [0.57-0.69]	0.6835	0.56 [0.51-0.61]	0.0479	0.55 [0.49-0.61]	0.1033	0.62 [0.55-0.68]	NS

"6&12": "Six-and-twelve"; AUROC: Area under receiver operating characteristic curve; C-index: Concordance index; Ref: Reference; BCLC: Barcelona Clinic Liver Cancer; NS: Not significant; NIACE: Tumor nodularity, infiltrative nature of the tumor, alpha-fetoprotein, child-pugh class, performance status; ALBI: Albumin-Bilirubin.

radiological tumor response.

In summary, in this multicenter French HCC cohort different staging/scoring systems classify survival among recommended TACE candidates with a similar predictive power. However, "6&12" score simplicity and ability in patients' stratification outperform other systems for a routine clinical practice.

Table 5 Kaplan-Meier survival analysis according to "Six-and-twelve" score and other systems in the main cohort from Marseille (available data for 241 hepatocellular carcinoma patients)

Scoring/stage systems	OS [95%CI], mo	*P* value (log-rank)	Sidak[1]	Hazard ratio [95%CI]	*P* value
"6&12" score		0.0004			
sum ≤ 6 (*n* = 130)	32 [28-36]		Ref	Ref	
sum > 6 ≤ 12 (*n* = 106)	20 [17-25]		0.0017	1.61 [1.21-2.14]	0.0010
sum > 12 (*n* = 5)	16 [5-34]		0.0003	3.34 [1.35-8.25]	0.0092
CLIP		< 0.0001			
0 (*n* = 55)	35 [30-68]		Ref	Ref	
1 (*n* = 135)	28 [25-32]		0.0724	1.81 [1.23-2.67]	0.0028
2 (*n* = 45)	18 [15-23]		< 0.0001	2.86 [1.81-4.54]	< 0.0001
3 (*n* = 6)	10 [1-27]		< 0.0001	8.12 [3.35-19.67]	< 0.0001
HKLC		< 0.0001			
1 (*n* = 89)	36 [30-40]		Ref	Ref	
2a (*n* = 42)	25 [19-35]		0.0024	1.79 [1.18-2.72]	0.0060
2b (*n* = 65)	26 [19-34]		0.0749	1.45 [1.01-2.10]	0.0450
3a (*n* = 24)	17 [11-23]		< 0.0001	3.30 [2.03-5.36]	< 0.0001
3b (*n* = 21)	14 [11-16]		< 0.0001	4.55 [2.73-7.58]	< 0.0001
Up-to-Seven		0.0001			
In (*n* = 176)	30 [27-35]		NA	Ref	
Out (*n* = 65)	18 [15-24]		NA	1.81 [1.34-2.46]	0.0001
MESH		< 0.0001			
0 (*n* = 41)	43 [35-70]		Ref	Ref	
1 (*n* = 77)	30 [25-35]		0.1291	2.16 [1.33-3.48]	0.0017
2 (*n* = 78)	26 [19-34]		0.0490	2.30 [1.41-3.74]	0.0008
3 (*n* = 37)	15 [10-21]		< 0.0001	6.02 [3.51-10.33]	< 0.0001
4 (*n* = 8)	13 [4-24]		< 0.0001	9.69 [3.86-24.36]	< 0.0001

[1]Sidak test for multiple comparisons. "6&12": "Six-and-twelve"; OS: Overall Survival; CI: Confidence Interval; Ref: Reference; CLIP: Cancer of the Liver Italian Program; HKLC: Hong Kong Liver Cancer; MESH: Model to Estimate Survival for Hepatocellular carcinoma.

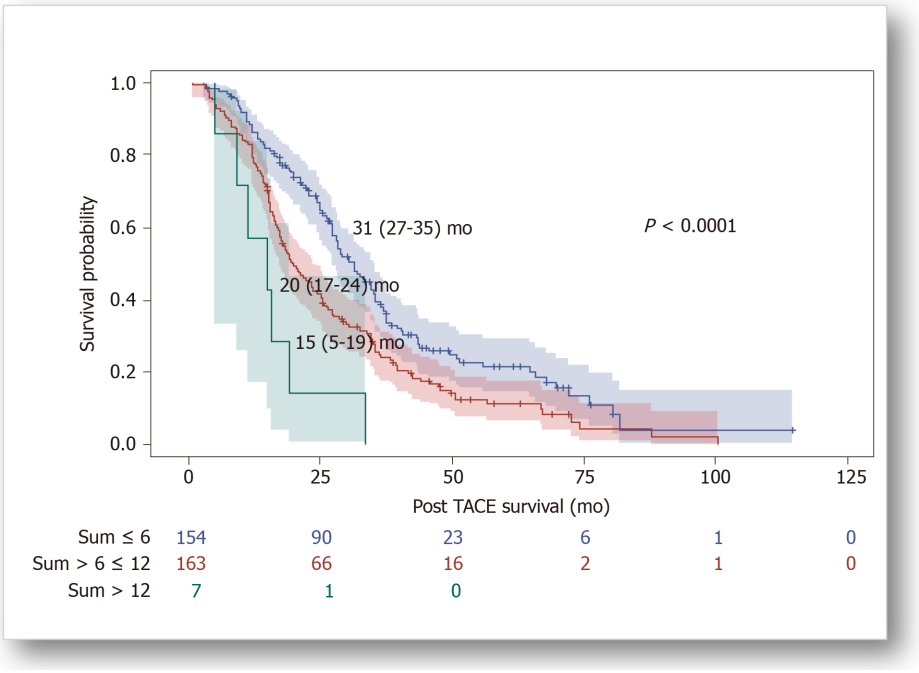

Figure 1 Kaplan-Meier analysis of overall survival according to "Six-and-twelve" criteria in the multicenter French HCC cohort (*n* = 324).
TACE: Transarterial chemoembolization.

REFERENCES

1 **Wang ZX**, Wang EX, Bai W, Xia DD, Mu W, Li J, Yang QY, Huang M, Xu GH, Sun JH, Li HL, Zhao H, Wu JB, Yang SF, Li JP, Li ZX, Zhang CQ, Zhu XL, Zheng YB, Wang QH, Li J, Yuan J, Li XM, Niu J, Yin ZX, Xia JL, Fan DM, Han GH, On Behalf Of China Hcc-Tace Study Group. Validation and evaluation of clinical prediction systems for first and repeated transarterial chemoembolization in unresectable hepatocellular carcinoma: A Chinese multicenter retrospective study. *World J Gastroenterol* 2020; **26**: 657-669 [PMID: 32103874 DOI: 10.3748/wjg.v26.i6.657]

2 **Wang Q**, Xia D, Bai W, Wang E, Sun J, Huang M, Mu W, Yin G, Li H, Zhao H, Li J, Zhang C, Zhu X, Wu J, Li J, Gong W, Li Z, Lin Z, Pan X, Shi H, Shao G, Liu J, Yang S, Zheng Y, Xu J, Song J, Wang W, Wang Z, Zhang Y, Ding R, Zhang H, Yu H, Zheng L, Gu W, You N, Wang G, Zhang S, Feng L, Liu L, Zhang P, Li X, Chen J, Xu T, Zhou W, Zeng H, Zhang Y, Huang W, Jiang W, Zhang W, Shao W, Li L, Niu J, Yuan J, Li X, Lv Y, Li K, Yin Z, Xia J, Fan D, Han G; China HCC-TACE Study Group. Development of a prognostic score for recommended TACE candidates with hepatocellular carcinoma: A multicentre observational study. *J Hepatol* 2019; **70**: 893-903 [PMID: 30660709 DOI: 10.1016/j.jhep.2019.01.013]

3 **Memon K**, Kulik L, Lewandowski RJ, Wang E, Riaz A, Ryu RK, Sato KT, Marshall K, Gupta R, Nikolaidis P, Miller FH, Yaghmai V, Senthilnathan S, Baker T, Gates VL, Abecassis M, Benson AB 3rd, Mulcahy MF, Omary RA, Salem R. Radiographic response to locoregional therapy in hepatocellular carcinoma predicts patient survival times. *Gastroenterology* 2011; **141**: 526-535, 535.e1-535.e2 [PMID: 21664356 DOI: 10.1053/j.gastro.2011.04.054]

4 **Kim BK**, Kim KA, Park JY, Ahn SH, Chon CY, Han KH, Kim SU, Kim MJ. Prospective comparison of prognostic values of modified Response Evaluation Criteria in Solid Tumours with European Association for the Study of the Liver criteria in hepatocellular carcinoma following chemoembolisation. *Eur J Cancer* 2013; **49**: 826-834 [PMID: 22995582 DOI: 10.1016/j.ejca.2012.08.022]

5 **Cappelli A**, Cucchetti A, Cabibbo G, Mosconi C, Maida M, Attardo S, Pettinari I, Pinna AD, Golfieri R. Refining prognosis after trans-arterial chemo-embolization for hepatocellular carcinoma. *Liver Int* 2016; **36**: 729-736 [PMID: 26604044 DOI: 10.1111/liv.13029]

6 **Bourlière M**, Pénaranda G, Adhoute X, Bronowicki JP. The "six-and-twelve score" for TACE treatment: Does it really help us? *J Hepatol* 2019; **71**: 1051-1052 [PMID: 31515044 DOI: 10.1016/j.jhep.2019.06.014]

7 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]

8 **Johnson PJ**, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, O'Beirne J, Fox R, Skowronska A, Palmer D, Yeo W, Mo F, Lai P, Iñarrairaegui M, Chan SL, Sangro B, Miksad R, Tada T, Kumada T, Toyoda H. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015; **33**: 550-558 [PMID: 25512453 DOI: 10.1200/JCO.2014.57.9151]

9 **Adhoute X**, Pénaranda G, Raoul JL, Bollon E, Pol B, Letreut YP, Perrier H, Bayle O, Monnet O, Beaurain P, Muller C, Hardwigsen J, Lefolgoc G, Castellani P, Bronowicki JP, Bourlière M. NIACE score for hepatocellular carcinoma patients treated by surgery or transarterial chemoembolization. *Eur J Gastroenterol Hepatol* 2017; **29**: 706-715 [PMID: 28195873 DOI: 10.1097/MEG.0000000000000852]

10 **Yau T**, Tang VY, Yao TJ, Fan ST, Lo CM, Poon RT. Development of Hong Kong Liver Cancer staging system with treatment stratification for patients with hepatocellular carcinoma. *Gastroenterology* 2014; **146**:

1691-700.e3 [PMID: 24583061 DOI: 10.1053/j.gastro.2014.02.032]
11 A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998; **28**: 751-755 [PMID: 9731568 DOI: 10.1002/hep.510280322]
12 **Liu PH**, Hsu CY, Hsia CY, Lee YH, Huang YH, Su CW, Lee FY, Lin HC, Huo TI. Proposal and validation of a new model to estimate survival for hepatocellular carcinoma patients. *Eur J Cancer* 2016; **63**: 25-33 [PMID: 27259100 DOI: 10.1016/j.ejca.2016.04.023]
13 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P; Metroticket Investigator Study Group. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]
14 **Golfieri R**, Cappelli A, Cucchetti A, Piscaglia F, Carpenzano M, Peri E, Ravaioli M, D'Errico-Grigioni A, Pinna AD, Bolondi L. Efficacy of selective transarterial chemoembolization in inducing tumor necrosis in small (<5 cm) hepatocellular carcinomas. *Hepatology* 2011; **53**: 1580-1589 [PMID: 21351114 DOI: 10.1002/hep.24246]
15 **Allard MA**, Sebagh M, Ruiz A, Guettier C, Paule B, Vibert E, Cunha AS, Cherqui D, Samuel D, Bismuth H, Castaing D, Adam R. Does pathological response after transarterial chemoembolization for hepatocellular carcinoma in cirrhotic patients with cirrhosis predict outcome after liver resection or transplantation? *J Hepatol* 2015; **63**: 83-92 [PMID: 25646884 DOI: 10.1016/j.jhep.2015.01.023]
16 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]
17 **Kudo M**, Arizumi T, Ueshima K, Sakurai T, Kitano M, Nishida N. Subclassification of BCLC B Stage Hepatocellular Carcinoma and Treatment Strategies: Proposal of Modified Bolondi's Subclassification (Kinki Criteria). *Dig Dis* 2015; **33**: 751-758 [PMID: 26488473 DOI: 10.1159/000439290]
18 **Kim HY**, Park JW, Joo J, Jung SJ, An S, Woo SM, Kim HB, Koh YH, Lee WJ, Kim CM. Severity and timing of progression predict refractoriness to transarterial chemoembolization in hepatocellular carcinoma. *J Gastroenterol Hepatol* 2012; **27**: 1051-1056 [PMID: 22098152 DOI: 10.1111/j.1440-1746.2011.06963.x]
19 **Adhoute X**, Penaranda G, Naude S, Raoul JL, Perrier H, Bayle O, Monnet O, Beaurain P, Bazin C, Pol B, Folgoc GL, Castellani P, Bronowicki JP, Bourlière M. Retreatment with TACE: the ABCR SCORE, an aid to the decision-making process. *J Hepatol* 2015; **62**: 855-862 [PMID: 25463541 DOI: 10.1016/j.jhep.2014.11.014]
20 **Sieghart W**, Hucke F, Pinter M, Graziadei I, Vogel W, Müller C, Heinzl H, Trauner M, Peck-Radosavljevic M. The ART of decision making: retreatment with transarterial chemoembolization in patients with hepatocellular carcinoma. *Hepatology* 2013; **57**: 2261-2273 [PMID: 23316013 DOI: 10.1002/hep.26256]
21 **Adhoute X**, Penaranda G, Castellani P, Perrier H, Bourliere M. Recommendations for the use of chemoembolization in patients with hepatocellular carcinoma: Usefulness of scoring system? *World J Hepatol* 2015; **7**: 521-531 [PMID: 25848475 DOI: 10.4254/wjh.v7.i3.521]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com