ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2021 July 14; 27(26): 3951-4251

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 27 Number 26 July 14, 2021

FRONTIER

3951 Liver dysfunction and SARS-CoV-2 infection

Gracia-Ramos AE, Jaquez-Quintana JO, Contreras-Omaña R, Auron M

OPINION REVIEW

3971 Chronic hepatitis B infection with concomitant hepatic steatosis: Current evidence and opinion

Shi YW, Yang RX, Fan JG

REVIEW

3984 Acute kidney injury and hepatorenal syndrome in cirrhosis

Gupta K, Bhurwal A, Law C, Ventre S, Minacapelli CD, Kabaria S, Li Y, Tait C, Catalano C, Rustgi VK

4004 Progress and challenges in the comprehensive management of chronic viral hepatitis: Key ways to achieve the elimination

Higuera-de la Tijera F, Servín-Caamaño A, Servín-Abad L

4018 Viral hepatitis update: Progress and perspectives

Pisano MB, Giadans CG, Flichman DM, Ré VE, Preciado MV, Valva P

4045 Biomarkers in the diagnosis of pancreatic cancer: Are we closer to finding the golden ticket?

O'Neill RS, Stoita A

4088 Non-occlusive mesenteric ischemia: Diagnostic challenges and perspectives in the era of artificial intelligence

Bourcier S, Klug J, Nguyen LS

4104 Hepatocellular carcinoma in patients with renal dysfunction: Pathophysiology, prognosis, and treatment challenges

Yeh H, Chiang CC, Yen TH

MINIREVIEWS

4143 Abdominal and gastrointestinal manifestations in COVID-19 patients: Is imaging useful?

Boraschi P, Giugliano L, Mercogliano G, Donati F, Romano S, Neri E

4160 Inflammatory effect on the gastrointestinal system associated with COVID-19

Delgado-Gonzalez P, Gonzalez-Villarreal CA, Roacho-Perez JA, Quiroz-Reyes AG, Islas JF, Delgado-Gallegos JL, Arellanos-Soto D, Galan-Huerta KA, Garza-Treviño EN

4172 Adult pancreatoblastoma: Current concepts in pathology

Omiyale AO

World Journal of Gastroenterology

Weekly Volume 27 Number 26 July 14, 2021

Contents

4182 Prevention of vertical transmission of hepatitis B virus infection

Veronese P, Dodi I, Esposito S, Indolfi G

4194 Endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for pancreatic masses, subepithelial lesions, and lymph nodes

Levine I, Trindade AJ

ORIGINAL ARTICLE

Basic Study

4208 Metal-organic framework IRMOFs coated with a temperature-sensitive gel delivering norcantharidin to treat liver cancer

Li XY, Guan QX, Shang YZ, Wang YH, Lv SW, Yang ZX, Wang R, Feng YF, Li WN, Li YJ

4221 Ubiquitin-specific protease 15 contributes to gastric cancer progression by regulating the Wnt/β-catenin signaling pathway

Zhong M, Zhou L, Fang Z, Yao YY, Zou JP, Xiong JP, Xiang XJ, Deng J

Retrospective Study

4236 Feasibility of totally laparoscopic gastrectomy without prophylactic drains in gastric cancer patients

Liu H, Jin P, Quan X, Xie YB, Ma FH, Ma S, Li Y, Kang WZ, Tian YT

CORRECTION

4246 Correction to "Downregulation of FoxM1 inhibits the viability and invasion of gallbladder carcinoma cells, partially dependent on the induction of cellular senescence"

Tao J, Xu XS, Song YZ, Liu C

LETTER TO THE EDITOR

4248 Impact of COVID-19 on the clinical status of patients with Wilson disease

Zhuang YP, Zhong HJ

ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, Mortada HF El-Shabrawi, MD, FAASLD, Professor of Pediatrics and Pediatric Hepatology, Faculty of Medicine, Cairo University, 3 Nablos Street, Off Shehab Street, Mohandessen, Giza 12411, Egypt. melshabrawi@medicine.cu.edu.eg

AIMS AND SCOPE

The primary aim of *World Journal of Gastroenterology* (*WJG*, *World J Gastroenterol*) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJG* mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

INDEXING/ABSTRACTING

The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2021 edition of Journal Citation Report® cites the 2020 impact factor (IF) for *WJG* as 5.742; Journal Citation Indicator: 0.79; IF without journal self cites: 5.590; 5-year IF: 5.044; Ranking: 28 among 92 journals in gastroenterology and hepatology; and Quartile category: Q2. The *WJG*'s CiteScore for 2020 is 6.9 and Scopus CiteScore rank 2020: Gastroenterology is 19/136.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Ji-Hong Liu*; **Production Department Director:** *Yu-Jie Ma*; **Editorial Office Director:** *Ze-Mao Gong*.

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Andrzej S Tarnawski, Subrata Ghosh

EDITORIAL BOARD MEMBERS
http://www.wjgnet.com/1007-9327/editorialboard.htm

PUBLICATION DATE
July 14, 2021

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 Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 3951-3970

DOI: 10.3748/wjg.v27.i26.3951

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

FRONTIER

Liver dysfunction and SARS-CoV-2 infection

Abraham Edgar Gracia-Ramos, Joel Omar Jaquez-Quintana, Raúl Contreras-Omaña, Moises Auron

ORCID number: Abraham Edgar Gracia-Ramos 0000-0003-1842-2554; Joel Omar Jaquez-Quintana 0000-0002-0990-8571; Raúl Contreras-Omaña 0000-0003-0062-5919; Moises Auron 0000-0001-6398-6047.

Author contributions: The authors contributed equally to all aspects of this manuscript preparation and have read and approved the final manuscript; each author meets the criteria for authorship established by the International Committee of Medical Journal Editors.

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

Manuscript source: Invited manuscript

Specialty type: Gastroenterology

Abraham Edgar Gracia-Ramos, Department of Internal Medicine, General Hospital, National Medical Center "La Raza", Instituto Mexicano del Seguro Social, Mexico City 02990, Mexico

Joel Omar Jaquez-Quintana, Gastroenterology Service and Department of Internal Medicine, Faculty of Medicine, University Hospital "Dr. José E. González", Universidad Autónoma de Nuevo León, Monterrey 64460, Mexico

Raúl Contreras-Omaña, Centro de Estudio e Investigación en Enfermedades Hepáticas y Toxicol ógicas (CEIHET), Pachuca 42184, Mexico

Moises Auron, Departments of Hospital Medicine and Pediatric Hospital Medicine, Cleveland Clinic, Cleveland, OH 44195, United States

Corresponding author: Abraham Edgar Gracia-Ramos, MD, MSc, Department of Internal Medicine, General Hospital, National Medical Center "La Raza", Instituto Mexicano del Seguro Social, Av. Jacaranda S/N, Col. La Raza, Del. Azcapotzalco, C.P., Mexico City 02990, Mexico. dr.gracia.dmm@gmail.com

Abstract

Severe acute respiratory syndrome coronavirus 2 infection is the cause of coronavirus disease 2019 (COVID-19), which predominantly affects the respiratory system; it also causes systemic and multi-organic disease. Liver damage is among the main extrapulmonary manifestations. COVID-19-associated liver injury is defined as any liver damage occurring during the disease course and treatment of COVID-19 in patients with or without pre-existing liver disease, and occurs in approximately one in five patients. Abnormal liver test results have been associated with a more severe course of COVID-19 and other complications, including death. Mechanisms linking COVID-19 to liver injury are diverse. Particular consideration should be made for patients with pre-existing liver disease, such as metabolic dysfunction-associated fatty liver disease, chronic liver disease due to viral or autoimmune disease, liver transplant carriers, or cirrhosis, given the risk for more severe outcomes. This manuscript summarizes the current lines of evidence on COVID-19-associated liver injury regarding pathophysiology, clinical significance, and management in both patients with or without pre-existing liver disease, to facilitate clinicians' access to updated information and patient care. Finally, we mention the ideas and recommendations to be considered for future research.

Key Words: SARS-CoV-2; Coronavirus; COVID-19; Liver; Liver diseases; Liver failure; Liver injury; Cirrhosis

and hepatology

Country/Territory of origin: Mexico

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

Received: March 16, 2021
Peer-review started: March 16, 2021
First decision: May 1, 2021
Revised: May 9, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 14, 2021

P-Reviewer: Chen S, Tamori A, Zhong HJ
S-Editor: Gao CC
L-Editor: Wang TQ
P-Editor: Xing YX

Core Tip: Coronavirus disease 2019 (COVID-19)-associated liver injury is defined as any liver damage occurring during the disease course and treatment of COVID-19 in patients with or without pre-existing liver disease, with an observed ratio of 1:5. The presence of abnormal liver biochemical parameters has been associated with a severe course of severe acute respiratory syndrome coronavirus 2 infection and other complications, including death. Pathophysiology of COVID-19-induced liver injury is complex. Also, special consideration should be made in patients with pre-existing liver disease, such as metabolic dysfunction-associated fatty liver disease, chronic liver disease due to viral or autoimmune disease, liver transplant carriers, or cirrhosis.

Citation: Gracia-Ramos AE, Jaquez-Quintana JO, Contreras-Omaña R, Auron M. Liver dysfunction and SARS-CoV-2 infection. *World J Gastroenterol* 2021; 27(26): 3951-3970
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/3951.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.3951

INTRODUCTION

In December 2019, multiple cases of unexplained pneumonia were reported in Wuhan, China[1]. The etiology of the outbreak was attributed to a newly identified coronavirus, initially named '2019-nCoV' (human), and subsequently renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease was denominated coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO)[1,2]. Due to the constantly increasing number of cases worldwide, on March 11, 2020, the WHO formally declared the COVID-19 outbreak as a pandemic[3]. More than a year after its appearance, SARS-CoV-2 has infected almost 10 million people worldwide and caused more than 2 million deaths[4].

Coronaviruses are members of the subfamily Coronavirinae in the family Coronaviridae and the order Nidovirales (International Committee on Taxonomy of Viruses). This subfamily consists of four genera (*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*)[5]. The *Betacoronavirus* include the Middle East respiratory syndrome (MERS) coronavirus, SARS-CoV, and SARS-CoV-2. These viruses have a positive-sense single-stranded RNA genome[6]. The angiotensin-converting enzyme 2 (ACE2) has been identified as the main viral receptor for SARS-CoV and SARS-CoV-2[5,7]. ACE2 is ubiquitously and widely expressed in many organs and systems, including the lungs, cardiovascular system, kidneys, pancreas, intestines, liver, adipose tissue, and muscular and nervous systems[8]. Another cellular protein, the transmembrane protease serine 2 (*i.e.*, TMPRSS2), facilitates viral entry into the host cells through plasma membrane surface interaction[9].

SARS-CoV-2 could be transmitted from person to person through close contact, respiratory droplets, and aerosol[10]. The manifestations of COVID-19 represent a wide clinical spectrum, which ranges from asymptomatic individuals or mild respiratory symptoms to severe-critical illness; overall, it is categorized as a mild, severe, or critical illness[11]. Although SARS-CoV-2 predominantly causes respiratory symptoms, it can also result in extrapulmonary disease, including thrombotic complications, myocardial damage, acute kidney failure, gastrointestinal symptoms, hepatocellular injury, hyperglycemia and ketosis, neurologic illnesses, ocular symptoms, and dermatologic manifestations[12,13]. These manifestations can occur in subjects without identified pre-existing organic disease, as well as in individuals with comorbidities, such as patients with hypertension, obesity, and chronic liver disease, among others.

The objective of this review is to discuss and show current data regarding liver dysfunction caused by SARS-CoV-2 infection in patients with or without pre-existing liver disease, its pathophysiology and management, as well as the prospects for future research.

SARS-COV-2 INFECTION AND LIVER DYSFUNCTION IN PATIENTS WITH NO PREVIOUS LIVER DISEASE

Epidemiology

COVID-19-associated liver injury is defined as any liver damage occurring during disease course and treatment of COVID-19 in patients with or without pre-existing liver disease[14]. A summary of the principal studies about liver damage in COVID-19 patients is showed in Table 1. Studies have shown that one in five patients with COVID-19 develop abnormalities in liver function tests[15]. A large systematic review that included 64 studies with 11245 patients with SARS-CoV-2 infection showed the following prevalence of abnormal liver function parameters: Elevated aspartate aminotransferase (AST) in 23.2%; alanine aminotransferase (ALT) in 21.2%; elevated total bilirubin in 9.7%; increased gamma-glutamyltransferase (GGT) in 15.0%; and increased alkaline phosphatase in 4.0%[16]. The presentation of liver injury during COVID-19 infection occurs mostly during the acute hospitalization period and it is associated with increased length of hospital stay, worse pulmonary score on computed tomography (commonly referred to as CT), overall severity of disease, and increased mortality.

In a single-center retrospective study that described temporal variations of liver injury during hospitalization due to SARS-CoV-2 infection, the percent of subjects with elevated aminotransferases (transaminitis) in mild cases was 12.6% *vs* 46.2% in severe cases. Most of the patients presented ALT elevations between days 4 and 17 of their hospitalization, with a mean of 10.7 d and 7.3 d in mild and severe cases, respectively. During treatment, increases in liver function test parameters were predominantly mild and elevations in ALT and AST were largely isolated, occurring in 19% of patients. The majority of patients were discharged with normal liver function parameters[17]. A large retrospective multicenter cohort study that included 5771 patients with COVID-19 pneumonia determined the distribution and temporal patterns of liver injury indicators in these patients; an initial elevation of AST, followed by ALT in severe patients, and mild fluctuation in total bilirubin levels in both non-severe and severe disease were found[18]. Another study of 79 in-patients with COVID-19 found that the extent of pulmonary lesions observed on CT was predictive of liver function damage [19]. In a systematic review that included 45 studies, abnormal liver biochemical indicators were detected at admission in 27.2% of cases, which increased to 36% during hospitalization, and there was a higher incidence of severe and/or critical cases [20]. Another meta-analysis revealed that, among 15407 patients with SARS-CoV-2 infection, the incidence of elevated liver chemistries was 23.1% at early presentation and 24.4% throughout the course of illness[21]. A prospective cohort study in 1611 hospitalized patients from 11 Latin American countries found abnormal liver tests on admission in 45.2% and that such was independently associated with death [odds ratio (OR): 1.5, 95% confidence interval (CI): 1.1-2.0] and severe COVID-19 (OR: 2.6, 95%CI: 2.0-3.3)[22]. A systematic review of 24 studies (5961 subjects) found that, among COVID-19 patients who were critically ill, the OR of hypoalbuminemia was 7.1, of AST elevation was 3.4, of ALT elevation was 2.5, and of hyperbilirubinemia was 1.7[23]. Systematic reviews with meta-analyses showed that patients with prolonged prothrombin time had a higher odds for progression to severe disease (OR: 1.82) and intensive care unit (ICU) admission (OR: 2.18)[24,25]. A synthesis of the literature that compared survivors and non-survivors with severe COVID-19 patients showed an OR of 1.98 (95%CI: 1.39-2.82) for liver dysfunction and mortality[26]. Similarly, previous investigations have shown that liver injury was common among patients infected by SARS-CoV and MERS coronavirus, and associated with the severity of diseases[27].

In patients with SARS-CoV-2 infection, the degree of transaminitis is generally mild [22,23], defined as less than 5 times the upper reference limit, and severe liver failure occurs infrequently[28]. In a cohort of 5700 patients from New York, United States, AST and ALT were both commonly increased (58.4% and 39.0% of subjects, respectively). In this same study, 56 (2.1%) patients had developed severe acute liver injury (defined as an increase in ALT or AST of > 15 times the upper limit of normal) and an association with mortality was found in 95%[29]. Finally, abnormal liver function test has been observed in patients with subclinical disease (elevated AST in 8.7% and elevated ALT in 8.9%)[30].

Pathophysiology

The mechanisms of liver injury in patients with SARS-CoV-2 infection are diverse. It has been postulated that SARS-CoV-2 may cause cytopathic effects due to viral replication after entrance into the liver and bile duct cells *via* interaction with ACE2

Table 1 Principal studies about liver damage in coronavirus disease 2019 patients

Ref.	Study	Findings
Mao *et al*[15]	SR (35 studies, *n* = 6686)	The prevalence of abnormal liver functions was 19% (CI: 9-32). Patients with severe COVID-19 had higher rates of abnormal liver function including increased ALT (OR: 1.89, CI: 1·30-2·76) and increased AST (OR: 3.08, CI: 2.14-4.42) compared with those with non-severe disease
Wijarnpreecha *et al*[16]	SR (64 studies, *n* = 11245)	The prevalence of elevated AST, ALT, total bilirubin, GGT, and alkaline phosphatase was 23.2%, 21.2%, 9.7%, 15.0%, and 4.0%, respectively. The prevalence of elevated AST was higher among those with severe cases (45.5%) compared to non-severe cases (15.0%). Co-existing CLD presented in up to 37.6% of patients with COVID-19
Wang *et al*[17]	Single-center retrospective study (*n* = 105)	Fifty-six percent of the patients had abnormal ALT, AST, or total bilirubin during the illness (91.4% cases were ≤ 3 fold of the ULN). The percentage of patients with elevated both ALT and AST was 12.7% in mild cases *vs* 46.2% in severe cases. One third of patients with severe disease started to have abnormal ALT after admission, and 73.3% of all patients had normal ALT before discharge
Lei *et al*[18]	Multicenter retrospective cohort study (*n* = 5771)	The distributional and temporal patterns of liver injury indicators were following: AST elevated first, followed by ALT, in severe patients. Alkaline phosphatase modestly increased during hospitalization and largely remained in the normal range. The fluctuation in total bilirubin levels was mild in the non-severe and severe groups
Xie *et al*[19]	Retrospective study (*n* = 79)	Logistic regression analyses suggested that the extent of pulmonary lesions on CT was a predictor of liver function damage
Wu *et al*[20]	SR (45 studies, *n* = 7228)	The incidence of any abnormal liver biochemical indicator at admission and during hospitalization was 27.2% and 36%, respectively
Kulkarni *et al* [21]	SR (107 studies, *n* = 20874)	The prevalence of CLD was 3.6% (CI: 2.5-5.1). The incidence of elevated liver chemistries was 23.1% (CI: 19.3-27.3) at initial presentation and 24.4% (CI: 13.5-40) during the illness. The incidence of DILI was 25.4% (CI: 14.2-41.4). The prevalence of CLD among 1587 severely infected patients was 3.9% (3%-5.2%). CLD was not associated with the developing severe COVID-19 (OR: 0.81, CI: 0.31-2.09) compared to non-CLD patients. COVID-19 patients with elevated liver chemistries had an increased risk of mortality (OR: 3.46 CI: 2.42-4.95) and severe disease (OR: 2.87, CI: 2.29-3.6) compared to patients without
Mendizabal *et al*[22]	Multicenter prospective cohort study (*n* = 1611)	Abnormal liver tests on admission were present on 45.2% and were independently associated with death (OR: 1.5, CI: 1.1-2.0), and severe COVID-19 (OR: 2.6, CI: 2.0-3.3). The prevalence of CLD was 8.5%
Wong *et al*[23]	SR (24 studies, *n* = 5961)	In subjects with critical COVID-19, the OR of hypoalbuminemia was 7.1 (CI: 2.1-24.1), of AST elevation was 3.4 (CI: 2.3-5.0), of ALT elevation was 2.5 (CI: 1.6-3.7), and of hyperbilirubinemia was 1.7 (CI: 1.2-2.5)
Zhu *et al*[24]	SR (34 studies, *n* = 6492)	Patients with severe COVID-19 showed significantly longer PT, and a longer PT was associated with a higher risk to die
Elshazli *et al* [25]	SR (52 studies, *n* = 6320)	Prolonged PT was associated with a higher risk of progression to severe COVID-19 (OR: 1.82) and ICU admission (OR: 2.18)
Wu and Yang [26]	SR (13 studies, *n* = 3722)	The comparison between survivors and non-survivors with severe COVID-19 patients showed an OR of 1.98 (CI: 1.39-2.82) for liver dysfunction and mortality
Richardson *et al* [29]	Multicenter prospective cohort study (*n* = 5700)	In hospitalized COVID-19 patients, AST and ALT were both commonly increased (58.4% and 39.0% of patients, respectively). Fifty-six (2.1%) subjects developed a severe acute liver injury with a mortality of 95%
Shi *et al*[30]	Two-center retrospective study (*n* = 81)	Abnormal liver function test was found in patients with subclinical disease (elevated AST in 8.7% and elevated ALT in 8.9%
Sultan *et al*[58]	SR (47 studies, *n* = 10980)	The prevalence estimates of elevated liver abnormalities were as follows: AST 15.0% (CI: 13.6-16.5), ALT 15.0% (CI: 13.6-16.4), and abnormal bilirubin 16.7% (CI: 15.0-18.5)

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; CLD: Chronic liver disease; COVID-19: Coronavirus disease 2019; CT: Computed tomography; DILI: Drug-induced liver injury; GGT: Gamma-glutamyltransferase; ICU: Intensive care unit; PT: Prothrombin time; OR: Odds ratio; SR: Systematic review; ULN: Upper limit of normal.

and TMPRSS2[31]. ACE2 expression is considerably higher in cholangiocytes (59.7%) than in hepatocytes (2.6%)[32]. Cholangiocytes have an important role in immune response, inflammation, and liver regeneration[33]. Furthermore, the expression of ACE2 in hepatocytes increases in cases of liver injury[34]. In postmortem liver biopsies from two patients who died from COVID-19, typical coronavirus particles were identified in the cytoplasm of hepatocytes, with cytopathic damage characterized by mitochondrial swelling, endoplasmic reticulum dilatation, and glycogen granule decrease[35]. These findings support the hypothesis of virus-related hepatic damage. However, other liver biopsy specimens of a patient who died from COVID-19 showed moderate microvesicular steatosis and mild lobular and portal activity, which are not specific and could have been caused by the viral infection, drug-induced liver injury (DILI), or nonalcoholic fatty liver disease (NAFLD)[36,37]. In addition, viral inclusion

bodies were not detected in liver tissue[37]. Another postmortem liver histopathologic study also reported microvesicular steatosis, accompanied by overactivation of T cells, suggesting a component of immune-mediated liver injury[38]. SARS-CoV-2 could also cause liver damage through the generation of endothelitis[39]. Endothelial cells are involved in ischemia-reperfusion liver damage and promote oxidative stress through reactive oxygen species and derivatives of nitric oxide[40]. Post-mortem wedge liver biopsies from 48 patients who died from severe COVID-19 disease showed vascular alterations characterized by an increased number of portal vein branches associated with massive lumen dilatation, partial or complete luminal thrombosis of portal and sinusoidal vessels, and marked focal enlargement and fibrosis of the portal tract[41]. In addition, transaminitis has been reported in some cases of portal thrombosis due to SARS-CoV-2 infection[42,43].

The immune overactivation associated with SARS-CoV-2 infection may also be involved in liver injury. Prominent elevations in serum inflammatory cytokine levels, such as interferon-γ, interleukin (IL)-1β, IL-6, IL-10, soluble IL-2 receptor α, and tumor necrosis factor, are present in patients with COVID-19, especially those with severe pneumonia[44,45]. This can lead to immune-mediated liver injury *via* activation of intrahepatic CD4+ and CD8+ cells, T cells, Kupffer cells, and a dysregulated innate immune response[46,47]. This phenomenon has also been described in infections caused by herpes viruses (Epstein-Barr virus, cytomegalovirus, and herpes simplex virus), parvovirus, adenovirus, and SARS-CoV[47]. Moreover, COVID-19 patients with increased AST also have elevated IL-6, ferritin, lactate dehydrogenase, and C-reactive protein compared to patients with normal AST[48].

In the course of infection by SARS-CoV-2, hepatic ischemia and hypoxia with impaired tissue perfusion can develop as a consequence of pneumonia-associated hypoxemia, circulatory failure, respiratory distress syndrome, and multiple organ failure[49]. Hepatic congestion secondary to high positive end-respiratory pressure in mechanically-ventilated patients may also enhance the degree of hypoxic damage in hepatocytes[32,46].

Liver injury associated with COVID-19 may also occur secondary to the potentially hepatotoxic effects of many drugs used for its treatment, such as acetaminophen, antivirals, antibiotics, corticosteroids, and immune modulators, among others. The presence of microvesicular steatosis and liver inflammation in liver biopsies of patients with SARS-CoV-2 infection could also be drug-related[37]. The drug-cytochrome P-450 interaction could explain some of the liver toxicity secondary to such drugs as azithromycin, lopinavir/ritonavir, hydroxychloroquine, and acetaminophen[50]. Additionally, patients with underlying NAFLD might be more susceptible to DILI because the cytokine monocyte chemoattractant protein-1 (*i.e.*, MCP-1) is often elevated in COVID-19 patients and could exacerbate steatohepatitis[51]. In a systematic review which included 107 articles (n = 20874 patients), the pooled incidence of DILI in COVID-19 patients was 25.4%[21]. A more detailed description of the drugs to treat SARS-CoV-2 infection and their potential risk of liver damage is discussed later.

SARS-CoV-2 RNA has been detected in feces, and it appears plausible that virus and inflammatory mediators present within the gut lumen could reach the liver through the portal circulation. Kupffer cells could attempt to clear the viral particles, consequently increasing the inflammatory response[39,50].

Other causes that are not necessarily associated with direct hepatocyte injury may explain the abnormal liver biochemical indicators in patients with SARS-CoV-2 infection. Transaminitis could originate from myositis rather than liver damage[52]. Muscular injury [defined as the presence of myalgias and creatinine kinase (CK) > 200 U/L] has been documented in 10% of hospitalized patients by COVID-19 and some studies have reported increased levels of myoglobin of CK in association with COVID-19 severity[46,53,54]. Hypoalbuminemia could be explained by decreased hepatic synthesis, malnutrition, increased catabolism, and albumin extravasation because of increased capillary permeability[55,56]; we must recall that hypoalbuminemia is also an acute phase reactant. Alkaline phosphatase and GGT are considered as cholangiocyte-related enzymes, but the higher prevalence of abnormal GGT may be attributed to acute inflammatory stress because the GGT is recognized as a surrogate marker for increased oxidative stress and inflammation[57].

Management

The recommendations by the American Gastroenterology Association and the World Gastroenterology Organization regarding the general approach to patients with SARS-CoV-2 infection and liver injury are as follows[58,59]: (1) In patients with abnormal liver function test results in the context of suspected or known COVID-19, evaluate for

alternative etiologies, including proof of viral hepatitis, particularly in developing countries; (2) Routine outpatient testing of liver biochemistries is not recommended; (3) In in-patients with COVID-19, obtain baseline liver indicators at the time of admission and consider its monitoring throughout the hospitalization; and (4) Avoid routine liver imaging, unless it will alter management.

FATTY LIVER DISEASE

General implications and epidemiology

The presence of metabolic dysfunction-associated fatty liver disease (MAFLD; previously known as NAFLD)[60] in the patients with infection by SARS-CoV-2 (*i.e.*, COVID-19) is important given that specific metabolic and cardiovascular comorbidities intrinsically related to MAFLD, like hypertension, diabetes, obesity, coronary artery disease, and cerebrovascular disease, were identified as independent risk factors associated with increased risk of infection by SARS-CoV-2[61,62], especially hypertension[52], diabetes[63,64] , and obesity [body mass index (BMI) > 30 kg/m^2][65]; furthermore, morbid obesity (BMI > 40 kg/m^2) is a strong risk predictor of hospitalization in patients with COVID-19[66].

MAFLD has been associated with an increased risk for mortality in patients with community-acquired pneumonia, which is further enhanced in patients with advanced liver fibrosis[67]. Also, MAFLD has been associated with an increased risk for bacterial infections, independent of the presence of metabolic syndrome and especially among patients with vitamin D deficiency[68]. The relevance of this is the recognition of MAFLD as a risk factor for severe infections.

MAFLD is an independent risk factor for progression of COVID-19 respiratory disease (OR: 6.4, 95%CI: 1.5-31.2), and this risk is heightened in patients with associated liver fibrosis[38,69,70]. In addition, MAFLD is associated with a higher likelihood of abnormal levels of aminotransferases at time of discharge as well as increased duration of virus shedding, which renders the individual infectious for 5 d longer[38,59]. The increased risk for viral infection in patients with MAFLD may be related to the pre-existent intrinsic up-regulation of ACE2 receptors that occurs in this disease, as well as in liver injury; in addition, the ACE2 receptors have been identified as the cellular point of entry of SARS-CoV-2[59].

A multicenter study of COVID-19 patients in the United States found a significant association between MAFLD and ICU admissions (OR: 2.30, 95%CI: 1.27-4.17, P = 0.03) as well as need for mechanical ventilation (OR: 2.15, 95%CI: 1.18-3.91, P = 0.02) but did not find a correlation with increased mortality[71]. A cohort study in the United Kingdom (Forlano *et al*[72]) showed that patients with MAFLD were younger than their counterparts without MAFLD. MAFLD *per se* had no direct correlation with increased mortality; however, among those who died in hospital, the risk was associated with male sex (71% *vs* survivors: 50%, P = 0.01), elevated ferritin (2076 μg/L *vs* survivors: 688 μg/L, P = 0.003), and early weaning score (n = 7 *vs* survivors: 3, P = 0.047). A recent systematic review of eight studies, including 8142 patients with COVID-19 and 833 of those with MAFLD, found that MAFLD by itself conferred an increased risk for severe COVID-19 of 2-fold (OR: 2.358, 95%CI: 1.902-2.923, P < 0.001) [73]. Finally, a meta-analysis of six studies (n = 1293) found an increase in the risk of COVID-19 disease severity of almost 3-fold (OR: 2.93, 95%CI: 1.87-4.60, I^2 = 34.3%, P = 0.166) among patients with MAFLD[74].

The comorbidities and increased inflammatory state in patients with MAFLD confer a hypothetical increased risk for DILI and, hence, careful monitoring of liver function is warranted in these patients, as are efforts to minimize exposure to polypharmacy [75].

Age and MAFLD

A cohort study in the United Kingdom found that most patients with COVID-19 and MAFLD were younger than 60 years old, as compared with patients with no MAFLD [72]. Among younger patients (age < 60 years old), the risk of severe COVID-19 is increased by 4-fold among those with concomitant NAFLD (OR: 4.07, 95%CI: 1.20-13.79, P = 0.02)[76,77].

Histopathologic changes in COVID and MAFLD

Although the severity of hepatic visceral fat correlates with the risk of COVID-19 infection[78], in general, the histopathologic findings in the liver in patients with SARS-CoV-2 infection have been presumed to be related mostly to the underlying liver

disease (*e.g.*, MAFLD) or other comorbidities (*e.g.*, drug toxicity and ICU care) rather than to a direct effect of the viral infection[79]. However, Nardo *et al*[80] described several mechanisms in which there is increased liver steatosis as a consequence of the viral infection; these include impaired mitochondrial dysfunction, endoplasmic reticulum stress-induced lipogenesis, and inflammation (including cytokine storm) with increased IL-6 and hyperstimulation of the mammalian target of rapamycin (*i.e.*, mTOR). The mTOR is also activated by glucose and insulin, and insulin resistance is also intrinsically associated with MAFLD; therefore, not only is there already an underlying inflammatory state but it can also be enhanced further by direct viral cytopathic effect[80].

Obesity and MAFLD

When considering the correlation of obesity and metabolic disease with the increased risk of COVID-19 as well as of severity of clinical presentation, one of the most accepted hypotheses is the presence of underlying chronic inflammatory state in these patients enhancing oxidative stress and increasing atherosclerosis and cardiovascular disease[81,82]. In addition, it is well evidenced that obesity confers an impaired immune response to viruses, with associated prolonged viral shedding as well as emergence of virulent minor variants[83]. If the readers would like to explore more intricate descriptions of the pathophysiology of inflammation in MAFLD and obesity, they are referred to the excellent manuscript by Portincasa *et al*[84].

In a study conducted in a Chinese population by Gao *et al*[65], the presence of obesity was found to increase the risk of severe COVID-19 by almost 3-fold (OR: 2.91, 95%CI: 1.31-6.47); furthermore, this risk was incrementally raised by 12% per unit of increase in BMI (OR: 1.12, 95%CI: 1.01-1.23). A prospective study of 5279 patients admitted to a hospital in New York, United States found that BMI > 40 kg/m^2 increased the risk of hospitalization by more than 2-fold (OR: 2.5, 95%CI: 1.8-3.4) and the risk of critical illness by 50% (OR: 1.5, 95%CI: 1.0-2.2)[66]. A very important epidemiological risk factor was reported by Kass *et al*[85], who identified a negative correlation of increased BMI and age among patients with severe COVID-19 infection, which showcases its impact in young patients. The co-existence of obesity and MAFLD has also been associated with an almost 6-fold increase in the risk of severe COVID-19 infection[38,86]. Furthermore, the severity of steatosis also correlates with the risk of infection as demonstrated by Roca-Fernández *et al*[78], who reported that among obese patients (BMI > 30 kg/m^2) with liver fat > 10%, the risk of symptomatic COVID-19 infection was increased almost 3-fold (OR: 2.96, 95%CI: 1.12-7.78, P = 0.02).

Management of patients with MAFLD in the era of COVID-19

The World Gastroenterology Organization recently published its recommendations for management of patients with MAFLD in the COVID-19 era, which essentially recommends to[59]: (1) Recognize the presence of MAFLD in patients with underlying metabolic disease, formally identifying its stage and grade; (2) Recognize that obesity and diabetes mellitus increase the risk of mortality from respiratory illnesses, including COVID-19; (3) Recognize that the risk of respiratory disease progression is higher in patients with MAFLD; and (4) Encourage patients with MAFLD to make lifestyle changes that will mitigate risk factors (*e.g.*, obesity) that can worsen the prognosis of COVID-19.

SARS-COV-2 INFECTION IN LIVER TRANSPLANT PATIENTS

In this section, we will focus on the assessment and management of patients with a transplanted liver who present with infection by SARS-CoV-2 (COVID-19).

Liver transplant patients are frail and have many risk factors for COVID-19 infection, including immunosuppression, in addition to other underlying comorbidities[87]. The symptomatology among patients with solid organ transplant who are infected with COVID-19 is similar to that among the general population; however, the severity and outcomes are worse, especially as both are impacted by their comorbidities[88,89].

Epidemiology

Imam *et al*[87] reported a review of ten studies from all over the world that included 22 patients with orthotopic liver transplant, among which 72% experienced clinical recovery from COVID-19, with a median duration of illness of 17 d. ICU admission was required in 28.6% of patients and the mortality rate in the cohort was 13.6%. On

the other hand, a European liver transplant cohort study of 57 patients with COVID-19 (70% male; median age of 65 years) found no significant impact of decreasing immunosuppression (37% of patients). The rate of hospitalization was 72%, and acute respiratory distress syndrome was present in 19% of cases. The overall mortality in the cohort was 12%, which increased to 17% among hospitalized patients. Among those who died, a history of cancer was common (5 out of 7 patients)[90]. An international multicenter cohort study of 151 adult liver transplant recipients from 18 countries (68% male; median age of 60 years) performed a comparison with 627 patients without a history of liver transplant (52% male; median age of 73 years). The liver transplant cohort had more frequent rates of ICU admission (28% *vs* 8%, $P < 0.0001$) and invasive ventilation (20% *vs* 5%, $P < 0.0001$). The mortality rate was 19% in the liver transplant cohort *vs* 27% in the comparison cohort ($P = 0.046$). After adjusting for comorbidities (age, sex, creatinine concentration, obesity, hypertension, diabetes, and ethnicity), liver transplantation was not associated with a significant increase in the risk of mortality in patients with COVID-19; however, multivariable logistic regression analysis demonstrated that the mortality increase in liver transplant patients was associated with age [(OR: 1.06, 95%CI: 1.01-1.11) per 1 year increase], serum creatinine [(OR: 1.57, 95%CI: 1.05-2.36) per 1 mg/dL increase], and cancer (OR: 18.30, 95%CI: 1.96-170.75) [91].

Recommendations for management of liver transplant patients with COVID-19

Multiple guidelines and reviews have been published with the aim of outlining the management of patients with COVID-19 who are either liver transplant candidates or have post-liver transplant status[92-98]. Most have very similar recommendations to the ones by the American Association for the Study of Liver Diseases (AASLD)[99] and Asian-Pacific Association for the Study of the Liver (APASL)[100] summarized below.

The AASLD published an Expert Panel Consensus Statement for Management of Liver Transplant During the COVID-19 Pandemic[99].

Recommendations that apply to the patient post-transplant status: (1) Given the associated high risk for severe COVID-19, these patients must be prioritized for testing; (2) In patients with COVID-19 and elevated aminotransferases, other etiologies unrelated to COVID-19 should be considered, such as viral hepatitis, myositis (especially if AST > ALT), cytokine release syndrome, and ischemia; (3) Ancillary studies should be minimized (*e.g.*, ultrasound and magnetic resonance imaging) to avoid the risk of healthcare personnel exposure, unless it will change management (*e.g.*, venous thrombosis and biliary obstruction); and (4) In the post-transplant time, which includes concerns for acute cellular rejection, a formal histopathologic confirmation with biopsy is necessary.

In patients who are candidates for transplantation: (1) The pandemic may affect the waiting time to transplant. Care teams must consider the evaluation of patients with a high model for end-stage liver disease score or hepatocellular carcinoma with severe disease (upper levels of Milan criteria), who would have a higher priority; (2) Screening for COVID-19 must be done on both the donor and the recipient. At this time, donors who are positive for SARS-CoV-2 are not considered eligible for organ donation. In the same tenure, transplantation is not recommended for COVID-19-positive patients; (3) Care teams should aim to select donor livers with a low risk of delayed graft function, in order to avoid complications and duration of postoperative hospitalization; and (4) Care teams may consider postponing a liver donor program during the pandemic.

In post-transplant patients with COVID-19 infection: (1) It is adequate to consider decreasing the dosage of high-dose prednisone. Although, a dosage that is sufficient to avoid adrenal insufficiency must be maintained; and (2) Reduction of azathioprine, mycophenolate, or daily calcineurin inhibitor dosages can be considered, especially in the setting of lymphopenia, fever, or worsening pneumonia attributed to COVID-19.

Very similar recommendations have been published by the APASL[100]. In addition, they recommend immunization of all patients with liver transplant against pneumococcus and influenza. Other recommendations include avoiding drugs that would have a significant impact on the tacrolimus levels, such as would occur in any other clinical setting[98].

One of the considerations to keep in mind for patients with liver transplant who become infected with COVID-19 is their public health impact, given their risk to be long-term carriers not only due to the slower clearance of the virus but also as they can be asymptomatic carriers[96]. This increases their risk for viral spread in the community, as well as nosocomially as they may have prolonged hospitalizations due

to their medical complexity[96].

Conclusions

Patients with liver transplant must be managed with similar protocols as non-transplanted patients; yet, clinicians must be mindful of the impact of immunosuppression on these patients' viral shedding and carrier status, as well as of medication interaction.

COVID-19 AND LIVER CIRRHOSIS

General considerations and epidemiology

The current evidence that describes the overall impact of COVID-19 in patients with liver cirrhosis, either compensated or decompensated, is scant. However, extrapolating from the current knowledge of the physiopathology of both diseases, the expected morbidity and mortality are more severe when compared to other groups. Many factors must be considered in the interaction of COVID-19 and the liver; for instance, most of the drugs used in the treatment of COVID-19, including biologic agents, can have either a direct hepatotoxic effect or reactivate chronic viral diseases, such as hepatitis B virus[14]. Other studies have detected the presence of SARS-CoV-2 in the liver tissues of patients who had died from COVID-19[101], suggesting viral replication at this level. In patients with liver cirrhosis, both effects have a critical impact as they may worsen the course of the disease by damaging the remaining liver parenchyma[96,102]. Otherwise, there are studies with findings suggesting that if the liver damage induced by COVID-19 is immunologically driven, then the immunocompromised status of cirrhotic patients might be more protective than harmful[103]. However, due to the limited number of patients with chronic liver disease within individual studies on COVID-19 to date, the true impact of underlying liver disease on viral progression and outcomes is unknown.

Existing evidence about outcomes of COVI-19 infection in patients with chronic liver disease is contradictory. A pooled analysis of six studies estimating the impact of chronic liver disease in COVID-19 patients suggested that chronic liver disease and cirrhosis seem to play a minor role in determining patient progression towards the severe forms of the disease; in that study, there was no correlation found between chronic liver disease and increased odds of the severe form of COVID-19 (OR: 0.96, 95%CI: 0.36-2.52) nor with increased odds of mortality (OR: 2.33, 95%CI: 0.77-7.04) [104]. Similar data were reported by Bangash *et al*[46]; specifically, a mortality rate of 0 to 2% was shown by COVID-19 patients with liver cirrhosis. A study of 22 patients with chronic liver disease, among which only three had liver cirrhosis, found that the only significant difference between patients with chronic liver diseases *vs* those without was the risk of progression to severe forms of COVID-19 ($P < 0.001$); however, there were no statistical differences in other variables, such as in-hospital days, death/discharge, or significant changes in liver enzyme values[69]. Finally, a meta-analysis found that the pooled prevalence of chronic liver disease among studies reporting on severity of COVID-19 was 2.64% (95%CI: 1.73-4.00), with 3.03% (95%CI: 1.97-4.64) among severe and 2.20% (95%CI: 1.16 - -4-15) among non-severe COVID-19. The relative risk of chronic liver disease in severe *vs* non-severe patients was 1.69 (95%CI: 1.05-2.73)[105].

The controversy in the data involves evidence generated by another meta-analysis which demonstrated that patients with a pre-existing chronic liver disease have an increased risk for severe COVID-19 (53.33%) and higher mortality (17.65%)[106]. This outcome is likely related to coexistent thrombocytopenia and lymphopenia[32,107] as well as cirrhosis-associated immune dysfunction[108]; therefore, precautions against SARS-CoV-2 infection are warranted among patients with cirrhosis. In addition, stress and sepsis related to over-imposed bacterial infections in COVID-19 are particularly risky and problematic in patients with decompensated liver cirrhosis, given the associated risk of developing acute-on-chronic liver failure, increasing the underlying risk of death from 26.2% to 63.2%; however, most of the studies have shown the cause of death in most liver cirrhosis patients with COVID-19 not to be due to progressive liver disease but rather to pulmonary disease[107,109]. Nonetheless, recent studies have found a higher 30-d mortality rate among patients with cirrhosis and COVID-19 [110], and the presence of cirrhosis has even been proposed as an independent predictor of mortality[71].

Treatment recommendations

The current available evidence suggests that COVID-19 patients with liver cirrhosis have worse outcomes and disease progression than those without. Thus, the treatment recommendations by most international associations are as follows: (1) Minimal exposure to medical staff, ideally leveraging telemedicine as the preferred method; (2) Listing for liver transplantation being restricted to patients with acute liver failure or poor short-term prognosis; (3) Prophylaxis regimens for spontaneous bacterial peritonitis and hepatic encephalopathy being strictly followed at home, to prevent decompensation and the need for hospital admissions; (4) Testing for SARS-CoV-2 for every patient with cirrhosis and acute decompensation or acute-on-chronic liver failure[95]; (5) In-person new patient visits being restricted to only those with significant liver diseases, such as jaundice, elevated transaminases > 500 U/L, or recent decompensation; (6) Rescheduling elective procedures, such as screening for varices and hepatocellular carcinoma; and (7) Urgent procedures, such as paracentesis, being performed using a COVID-19-free path in either the hospital or home care[111-113].

The data regarding vaccination against SARS-CoV-2 in patients with liver cirrhosis is scarce. Despite the inclusion of nearly 100000 participants in all the vaccination trials, data for patients with liver disease are extremely limited. For example, in the Pfizer vaccination study, 217 (0.6%) of 37706 participants had liver disease and only three (< 0.1%) had moderate to severe liver disease. Similar numbers can be seen in the Moderna trial. Importantly, criteria used to classify liver disease and its severity in each study were not specified. Therefore, the real SARS-CoV-2 vaccine safety profile and its immunological response in patients with liver cirrhosis will almost completely come from post-licensing, real-world data[114].

We must not forget the underlying deficiencies in innate and humoral immunity, termed cirrhosis-associated immune dysfunction, that are present in patients with advanced liver disease. It can be hypothesized that this may confer an attenuated immune response to vaccination, but this remains to be verified[115]. Nonetheless, taking into account the risk of COVID-19 progression in these patients (as described above) and considering that there are no absolute contraindications to SARS-CoV-2 vaccination in cirrhosis, it is fundamental to prioritize immunization in this subgroup. AASLD recommendations establish that, when the supply of COVID-19 vaccine is limited, it is reasonable to prioritize patients with higher model for end-stage liver disease and Child-Turcotte-Pugh scores for vaccination together with those who are anticipated to undergo imminent liver transplantation; ideally, however, all chronic liver disease patients should be vaccinated whenever possible[114,116,117].

MISCELLANEOUS

Autoimmune hepatitis

Treatment of autoimmune hepatitis (AIH) has posed a challenge during this COVID-19 pandemic. One of the main challenges is the management with immunosuppressive drugs, since these medications are associated with an increased risk of severe viral infections[118]. COVID-19 has been hypothesized to decompensate or increase the risk of an unfavorable course of liver disease[99]. In a small cohort in northern Italy of ten AIH patients on immunosuppressive treatment who became infected with COVID-19, five developed COVID-19 pneumonia, with only one patient dying (who had decompensated cirrhosis previously), while the rest of the patients fully recovered. Regarding the impact of the COVID-19 on AIH, only one patient presented relapse associated with the interruption of immunosuppressive treatment; it was concluded that patients with AIH under immunosuppressive and COVID-19 treatment have no increased risk of severity or complications of COVID-19 disease when compared to the general population[119]. A multicenter study that included 70 AIH patients with COVID-19, where 58 patients were on immunosuppressant therapy, and of whom 52% received combined immunosuppressant therapy, found that 65 (93%) patients reported clinical symptoms, mainly respiratory (74%) and gastrointestinal (26%), and 15% were asymptomatic. Mortality occurred in 16 (22.8%) patients; among those who died, the causes were attributed to a pulmonary etiology in nine (56%), liver etiology in five (31%), and cardiac etiology in two (13%). The factors associated with death in AIH patients were age (OR: 2.01 per 10 years, 95%CI: 1.07-3.81, P = 0.031), Child-Pugh B score (OR: 42.48, 95%CI: 4.41–409.53, P = 0.001), and Child Pugh C score (OR: 69.30, 95%CI: 2.83-1694.50, P = 0.009) unrelated to immunosuppressant use and death[120]. When comparing this group of patients with a cohort of patients with liver disease

without AIH, the authors did not find a statistical difference among groups, concluding that AIH patients on immunosuppressive therapy are not associated with an increased risk or severity of SARS-COV-2 infection; therefore, the recommendation is not to decrease or discontinue immunosuppressive treatment in patients with AIH and COVID-19, due to the risk of decompensation of liver disease.

Viral hepatitis

Hepatitis B (HB) and hepatitis C (HC) represent major global public health problems [121,122]. The coinfection of SARS-CoV-2 and HB and/or HC depends on local prevalence. For example, a Chinese study of a cohort of 1099 cases of COVID-19 patients demonstrated that 23 (2.1%) had pre-existing HB; in contrast, in the northeastern United States, a series of 5700 patients hospitalized with COVID-19 showed a prevalence of 0.1% HB and < 0.1% HC[29,52].

The impact on the evolution of COVID-19 and HB superinfection is uncertain. The first reports of the cohort in Wuhan, China found that 2.1% (23/1099) of patients with HB accounted for 0.6% of severe cases[52]. Another report from different hospitals in China involving a cohort of 571 patients showed that 15 (2.63%) patients had underlying HB; the incidence of admission to ICU and death in the HB group was 0% and 6.47% (36/556), respectively, in the non-HB group[123]. Contradictory data stem from other studies. A retrospective study of 70 patients with COVID-19 and HB documented a higher susceptibility of acquiring COVID-19, as well as higher rates of hepatic damage and coagulation disorders and severity of the disease, without having an impact on hospital stay or mortality[124]. A retrospective study of 123 patients with COVID-19, found that HB was present in 15 (12.2%) patients, among who 11 (73.3%) evolved favorably and were discharged from the hospital uneventfully; out of the four who remained in the hospital, two (13.3%) died from digestive bleeding. In comparison, the mortality rate was lower in the group of 108 patients with COVID-19 without HB, among which only eight (5.6%) remained in the hospital and three (2.8%) died due to respiratory failure[125]. Theoretically, this association of poor clinical forecast is due to the common lymphopenia caused in patients with COVID-19, which generates a loss of immune tolerance over HB, which itself can cause viral reactivation [126]. However, there is one study showing that COVID-19 was not associated with reactivation or seroconversion in chronic HB patients, despite using immunomodulatory treatment in a short course for severe COVID-19[127].

The current data are controversial and contradictory; therefore, it is necessary to take into account the number of patients and the heterogeneity of the population studied based on HB activity, the presence of cirrhosis, and stage of the liver disease. A recent systematic review and meta-analysis concluded that the association of co-infection of HB and SARS-CoV-2 does not have a serious adverse impact in patients hospitalized with COVID-19[128].

In regards to the treatment of COVID-19 in patients with chronic HB, we must be cautious as the use of corticosteroids or tocilizumab may reactivate HB[129]; although, as previously mentioned, this has not been shown to happen[127]. Finally, the evolution of patients with COVID-19 and HB superinfection is not clear, as the studies have yielded contradictory results and prospective studies with large numbers of patients and control of variables such as presence of other comorbidities, viral replication, cirrhosis, and stage of the liver disease are required. In the case of patients with COVID-19 and recently diagnosed HC, the HC treatment should be postponed until the remission of COVID-19; however, if treatment has already been ongoing, it is necessary to monitor the interactions of HC and COVID-19 treatments[99].

Drug-induced damage

Since the onset of the COVID-19 pandemic, multiple medications have been used as potential treatments, including antimalarials, antiparasitics, antivirals, monoclonal antibodies, *etc*. Some of these medications have hepatotoxic effects, which can be reviewed on the website http://www.livertox.nih.gov, where updated data of all drugs are available (Table 2)[58,99,130]. Of similar relevance is the consideration of drug interactions, as some treatments are experimental. Interactions can be reviewed at: https://www.covid19-druginteractions.org of Liverpool University.

Most of the data collected have come from case reports, particularly of serious cases and cohorts, for which there may be uncontrolled variables, with patients having pre-existing liver disease, interaction with unreported medicines, and use of traditional medicine, herbal products, or substances. Heightened awareness of both hepatotoxicity and drug interactions in patients with COVID-19 must continue, as should further research efforts regarding these interactions.

Table 2 Therapeutic management of patients with coronavirus disease 2019 and hepatotoxicity

Medication	Hepatotoxicity	Action mechanism	Currently recommended use for COVID-19
Hydroxychloroquine	Likelihood score: D (possible). Rare cause of clinically-apparent liver injury	Altered metabolism of other medications	Not recommended
Azithromycin	Likelihood score: A (well-known). Transient and asymptomatic elevation in serum aminotransferases; Typical cholestatic hepatitis	Unknown	Not recommended
Ivermectin	Likelihood score: D (possible). Mild elevation of serum aminotransferases; Reports of acute liver failure	Unknown	Not recommended
Dexamethasone	Likelihood score: A (well-known). Long-term use effects; Symptoms usually represent the worsening or triggering of an underlying liver disease	Drug-associated fatty liver disease	Recommended as emergency use
Remdesivir	Likelihood score: D (possible). Mild to moderate transient elevation of serum aminotransferases	Inhibition of mitochondrial RNA polymerase or idiosyncratic injury	Recommended as emergency use
Lopinavir/ritonavir	Likelihood score: D (possible). Moderate to severe elevation of serum aminotransferases (pattern hepatocellular to cholestatic or mixed); Duration 1-2 mo; Reports of acute liver failure; Caution in patients with co-infection by hepatitis B virus-hepatitis C virus-human immunodeficiency virus	Inhibits both of the isoforms of CYP3A del P450, which may result in production of a toxic intermediate	Not recommended
Baricitinib	Likelihood score: E (unlikely). Moderate transient elevation of serum aminotransferases (17% of patients); Hepatitis B reactivation	Unknown	Recommended as emergency use
Tocilizumab	Likelihood score: C (probably). Mild to moderate transient elevation of serum aminotransferases; Duration 8 wk	Unknown	Recommended as emergency use

COVID-19: Coronavirus disease 2019.

Vaccination controversies

Chronic hepatic disease (CHD) is considered a state of immunosuppression due to a multifactorial state of systemic immunological diffusion[131], which predisposes to infections and a cause of decompensation and mortality in cirrhotic patients[132]. Immunization is, therefore, recommended in patients with cirrhosis and pre-transplantation and post-transplanted patients, with specifications for the different types of vaccines[133]. Inactivated vaccines (*e.g.*, influenza, pneumococcal, viral hepatitis A, viral HB, diphtheria, tetanus, poliomyelitis, and acellular pertussis) are preferred over live attenuated vaccines (*e.g.*, tuberculosis vaccine, measles, mumps, rubella, varicella zoster virus, and Herpes zoster)[132,133].

The development of the SARS-CoV-2 vaccine has evolved favorably with phase 3 trials, offering effectiveness and safety. Currently, 53 vaccines have been authorized by the United States' Federal Drug Administration, including those from Pfizer/BioNTech, Moderna, Oxford/AstraZeneca/Sputnik V, and Janssen. Due to the haste of the trials, very strict inclusion and exclusion criteria have been applied to avoid adverse effects. Patients with CHD are preferably not included. In the Pfizer vaccination study, 217 (0.6%) of 37706 participants had liver disease, and only three (< 0.1%) had moderate to severe liver disease. In the Moderna trial, 196 (0.6%) of 30351 participants had liver disease; the Oxford/AstraZeneca, Sputnik V, and Janssen trials completely excluded patients with pre-existent CHD. On the other hand, trials of the 53 vaccines excluded patients with systemic immunosuppression which involves post-transplant liver patients and AIH patients[114,134,135].

As mentioned, CHD patients are a susceptible and high-risk population for COVID-19 complications, and should be classified as a vulnerable population. It is paramount to define the effectiveness and safety of immunization against SARS-CoV-2. As the development and trial of new vaccines occur and vaccination programs are started, information will be generated in different subgroups of populations, including patients with hepatic disease.

FUTURE RESEARCH DIRECTIONS

After a year of pandemic, the information that has emerged regarding SARS-CoV-2

infection and liver injury in patients without or with pre-existing liver disease has opened the course of new lines of research that should be addressed in future studies. The pathophysiology of COVID-19-induced liver injury is complex and more research is necessary to determine the degree of relevance of each of the described mechanisms. Abnormal liver biochemical parameters have been associated with a more serious course and a worse prognosis in patients with SARS-CoV-2 infection, so the usefulness of such measurements in the identification and staging of those patients with related alterations should be evaluated in depth in prospective studies. It is necessary to investigate the impact of SARS-CoV-2 infection in the clinical course of pre-existing liver disease (*e.g.*, fatty liver disease, viral or AIH, and cirrhosis). Long-term follow-up in liver transplant patients suffering from COVID-19 should be investigated to determine if the infection alters graft viability. It is necessary to include patients with liver diseases in the vaccination protocols, to determine the related effectiveness and safety.

CONCLUSION

Liver injury in patients with infection due to SARS-CoV-2 is a frequent extrapulmonary manifestation, particularly in hospitalized patients, and its presence has been associated with an increased risk of complications, including death. The pathophysiology of liver damage in COVID-19 patients is multifactorial and various mechanisms interact. On the other hand, SARS-CoV-2 infection in patients with pre-existing liver disease (*i.e.*, fatty liver disease, cirrhosis, autoimmune or viral hepatitis, and liver transplant patients) presents an increased risk of an ominous course of the disease. Therefore, the presence of liver damage (both acute onset or as a pre-existing condition) requires close monitoring and individualized management according to the individual conditions of the patients. Further research is required to have a better understanding of the SARS-CoV-2 and liver interaction that can improve the therapeutic approach for patients.

REFERENCES

1 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

2 **Hu B**, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* 2021; **19**: 141-154 [PMID: 33024307 DOI: 10.1038/s41579-020-00459-7]

3 **World Health Organization**. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. [cited 12 March 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

4 **World Health Organization**. Weekly epidemiological update - 19 January 2021. [cited 12 March 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update---19-january-2021

5 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270-273 [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7]

6 **Ortiz-Prado E**, Simbaña-Rivera K, Gómez-Barreno L, Rubio-Neira M, Guaman LP, Kyriakidis NC, Muslin C, Jaramillo AMG, Barba-Ostria C, Cevallos-Robalino D, Sanches-SanMiguel H, Unigarro L, Zalakeviciute R, Gadian N, López-Cortés A. Clinical, molecular, and epidemiological characterization of the SARS-CoV-2 virus and the Coronavirus Disease 2019 (COVID-19), a comprehensive literature review. *Diagn Microbiol Infect Dis* 2020; **98**: 115094 [PMID: 32623267 DOI: 10.1016/j.diagmicrobio.2020.115094]

7 **Gracia-Ramos AE**. Is the ACE2 Overexpression a Risk Factor for COVID-19 Infection? *Arch Med Res* 2020; **51**: 345-346 [PMID: 32279908 DOI: 10.1016/j.arcmed.2020.03.011]

8 **Gheblawi M**, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, Raizada MK, Grant MB, Oudit GY. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. *Circ Res* 2020; **126**: 1456-1474 [PMID: 32264791 DOI: 10.1161/CIRCRESAHA.120.317015]

9 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry

Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280. e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]

10 **Harrison AG**, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends Immunol* 2020; **41**: 1100-1115 [PMID: 33132005 DOI: 10.1016/j.it.2020.10.004]

11 **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; **323**: 1239-1242 [PMID: 32091533 DOI: 10.1001/jama.2020.2648]

12 **Gupta A**, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, Bikdeli B, Ahluwalia N, Ausiello JC, Wan EY, Freedberg DE, Kirtane AJ, Parikh SA, Maurer MS, Nordvig AS, Accili D, Bathon JM, Mohan S, Bauer KA, Leon MB, Krumholz HM, Uriel N, Mehra MR, Elkind MSV, Stone GW, Schwartz A, Ho DD, Bilezikian JP, Landry DW. Extrapulmonary manifestations of COVID-19. *Nat Med* 2020; **26**: 1017-1032 [PMID: 32651579 DOI: 10.1038/s41591-020-0968-3]

13 **Baral R**, Ali O, Brett I, Reinhold J, Vassiliou VS. COVID-19: a pan-organ pandemic. *Oxf Med Case Reports* 2020; **2020**: omaa107 [PMID: 33304597 DOI: 10.1093/omcr/omaa107]

14 **Sun J**, Aghemo A, Forner A, Valenti L. COVID-19 and liver disease. *Liver Int* 2020; **40**: 1278-1281 [PMID: 32251539 DOI: 10.1111/liv.14470]

15 **Mao R**, Qiu Y, He JS, Tan JY, Li XH, Liang J, Shen J, Zhu LR, Chen Y, Iacucci M, Ng SC, Ghosh S, Chen MH. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 667-678 [PMID: 32405603 DOI: 10.1016/S2468-1253(20)30126-6]

16 **Wijarnpreecha K**, Ungprasert P, Panjawatanan P, Harnois DM, Zaver HB, Ahmed A, Kim D. COVID-19 and liver injury: a meta-analysis. *Eur J Gastroenterol Hepatol* 2021; **33**: 990-995 [PMID: 32639420 DOI: 10.1097/MEG.0000000000001817]

17 **Wang Q**, Zhao H, Liu LG, Wang YB, Zhang T, Li MH, Xu YL, Gao GJ, Xiong HF, Fan Y, Cao Y, Ding R, Wang JJ, Cheng C, Xie W. Pattern of liver injury in adult patients with COVID-19: a retrospective analysis of 105 patients. *Mil Med Res* 2020; **7**: 28 [PMID: 32507110 DOI: 10.1186/s40779-020-00256-6]

18 **Lei F**, Liu YM, Zhou F, Qin JJ, Zhang P, Zhu L, Zhang XJ, Cai J, Lin L, Ouyang S, Wang X, Yang C, Cheng X, Liu W, Li H, Xie J, Wu B, Luo H, Xiao F, Chen J, Tao L, Cheng G, She ZG, Zhou J, Wang H, Lin J, Luo P, Fu S, Ye P, Xiao B, Mao W, Liu L, Yan Y, Chen G, Huang X, Zhang BH, Yuan Y. Longitudinal Association Between Markers of Liver Injury and Mortality in COVID-19 in China. *Hepatology* 2020; **72**: 389-398 [PMID: 32359177 DOI: 10.1002/hep.31301]

19 **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]

20 **Wu Y**, Li H, Guo X, Yoshida EM, Mendez-Sanchez N, Levi Sandri GB, Teschke R, Romeiro FG, Shukla A, Qi X. Incidence, risk factors, and prognosis of abnormal liver biochemical tests in COVID-19 patients: a systematic review and meta-analysis. *Hepatol Int* 2020; **14**: 621-637 [PMID: 32710250 DOI: 10.1007/s12072-020-10074-6]

21 **Kulkarni AV**, Kumar P, Tevethia HV, Premkumar M, Arab JP, Candia R, Talukdar R, Sharma M, Qi X, Rao PN, Reddy DN. Systematic review with meta-analysis: liver manifestations and outcomes in COVID-19. *Aliment Pharmacol Ther* 2020; **52**: 584-599 [PMID: 32638436 DOI: 10.1111/apt.15916]

22 **Mendizabal M**, Piñero F, Ridruejo E, Anders M, Silveyra MD, Torre A, Montes P, Urzúa A, Pages J, Toro LG, Díaz J, Gonzalez Ballerga E, Miranda-Zazueta G, Peralta M, Gutiérrez I, Michelato D, Venturelli MG, Varón A, Vera-Pozo E, Tagle M, García M, Tassara A, Brutti J, Ruiz García S, Bustios C, Escajadillo N, Macias Y, Higuera-de la Tijera F, Gómez AJ, Dominguez A, Castillo-Barradas M, Contreras F, Scarpin A, Schinoni MI, Toledo C, Girala M, Mainardi V, Sanchez A, Bessone F, Rubinstein F, Silva MO. Prospective Latin American cohort evaluating outcomes of patients with COVID-19 and abnormal liver tests on admission. *Ann Hepatol* 2021; **21**: 100298 [PMID: 33359234 DOI: 10.1016/j.aohep.2020.100298]

23 **Wong YJ**, Tan M, Zheng Q, Li JW, Kumar R, Fock KM, Teo EK, Ang TL. A systematic review and meta-analysis of the COVID-19 associated liver injury. *Ann Hepatol* 2020; **19**: 627-634 [PMID: 32882393 DOI: 10.1016/j.aohep.2020.08.064]

24 **Zhu J**, Pang J, Ji P, Zhong Z, Li H, Li B, Zhang J, Lu J. Coagulation dysfunction is associated with severity of COVID-19: A meta-analysis. *J Med Virol* 2021; **93**: 962-972 [PMID: 32706396 DOI: 10.1002/jmv.26336]

25 **Elshazli RM**, Toraih EA, Elgaml A, El-Mowafy M, El-Mesery M, Amin MN, Hussein MH, Killackey MT, Fawzy MS, Kandil E. Diagnostic and prognostic value of hematological and immunological markers in COVID-19 infection: A meta-analysis of 6320 patients. *PLoS One* 2020; **15**: e0238160 [PMID: 32822430 DOI: 10.1371/journal.pone.0238160]

26 **Wu ZH**, Yang DL. A meta-analysis of the impact of COVID-19 on liver dysfunction. *Eur J Med Res* 2020; **25**: 54 [PMID: 33148326 DOI: 10.1186/s40001-020-00454-x]

27 **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]

28 **Weber S**, Mayerle J, Irlbeck M, Gerbes AL. Severe liver failure during SARS-CoV-2 infection. *Gut* 2020; **69**: 1365-1367 [PMID: 32327526 DOI: 10.1136/gutjnl-2020-321350]

29 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; 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; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]

30 **Shi H**, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng C. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *Lancet Infect Dis* 2020; **20**: 425-434 [PMID: 32105637 DOI: 10.1016/S1473-3099(20)30086-4]

31 **Lozano-Sepulveda SA**, Galan-Huerta K, Martínez-Acuña N, Arellanos-Soto D, Rivas-Estilla AM. SARS-CoV-2 another kind of liver aggressor, how does it do that? *Ann Hepatol* 2020; **19**: 592-596 [PMID: 32858226 DOI: 10.1016/j.aohep.2020.08.062]

32 **Metawea MI**, Yousif WI, Moheb I. COVID 19 and liver: An A-Z literature review. *Dig Liver Dis* 2021; **53**: 146-152 [PMID: 32988758 DOI: 10.1016/j.dld.2020.09.010]

33 **Banales JM**, Huebert RC, Karlsen T, Strazzabosco M, LaRusso NF, Gores GJ. Cholangiocyte pathobiology. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 269-281 [PMID: 30850822 DOI: 10.1038/s41575-019-0125-y]

34 **Herath CB**, Warner FJ, Lubel JS, Dean RG, Jia Z, Lew RA, Smith AI, Burrell LM, Angus PW. Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1-7) levels in experimental biliary fibrosis. *J Hepatol* 2007; **47**: 387-395 [PMID: 17532087 DOI: 10.1016/j.jhep.2007.03.008]

35 **Wang Y**, Liu S, Liu H, Li W, Lin F, Jiang L, Li X, Xu P, Zhang L, Zhao L, Cao Y, Kang J, Yang J, Li L, Liu X, Li Y, Nie R, Mu J, Lu F, Zhao S, Lu J, Zhao J. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol* 2020; **73**: 807-816 [PMID: 32437830 DOI: 10.1016/j.jhep.2020.05.002]

36 **Working Group***. Association of Pathologists** for Guidebook of NASH and NAFLD, 2015: The Japan Society of Hepatology. Pathological Findings of NASH and NAFLD: for Guidebook of NASH and NAFLD, 2015: The Japan Society of Hepatology. *Hepatol Res* 2017; **47**: 3-10 [PMID: 27889947 DOI: 10.1111/hepr.12847]

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 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: 32278005 DOI: 10.1016/j.jhep.2020.03.044]

39 **Bertolini A**, van de Peppel IP, Bodewes FAJA, Moshage H, Fantin A, Farinati F, Fiorotto R, Jonker JW, Strazzabosco M, Verkade HJ, Peserico G. Abnormal Liver Function Tests in Patients With COVID-19: Relevance and Potential Pathogenesis. *Hepatology* 2020; **72**: 1864-1872 [PMID: 32702162 DOI: 10.1002/hep.31480]

40 **Dar WA**, Sullivan E, Bynon JS, Eltzschig H, Ju C. Ischaemia reperfusion injury in liver transplantation: Cellular and molecular mechanisms. *Liver Int* 2019; **39**: 788-801 [PMID: 30843314 DOI: 10.1111/liv.14091]

41 **Sonzogni A**, Previtali G, Seghezzi M, Grazia Alessio M, Gianatti A, Licini L, Morotti D, Zerbi P, Carsana L, Rossi R, Lauri E, Pellegrinelli A, Nebuloni M. Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. *Liver Int* 2020; **40**: 2110-2116 [PMID: 32654359 DOI: 10.1111/liv.14601]

42 **Singh B**, Kaur P, Maroules M. Splanchnic vein thrombosis in COVID-19: A review of literature. *Dig Liver Dis* 2020; **52**: 1407-1409 [PMID: 33067157 DOI: 10.1016/j.dld.2020.09.025]

43 **Hassan W**, Ramadan HK. COVID-19 as a novel etiology of portal vein thrombosis: change in the current management concepts. *Infect Dis (Lond)* 2021; **53**: 148-150 [PMID: 33090034 DOI: 10.1080/23744235.2020.1837943]

44 **Zhong J**, Tang J, Ye C, Dong L. The immunology of COVID-19: is immune modulation an option for treatment? *Lancet Rheumatol* 2020; **2**: e428-e436 [PMID: 32835246 DOI: 10.1016/S2665-9913(20)30120-X]

45 **Fajgenbaum DC**, June CH. Cytokine Storm. *N Engl J Med* 2020; **383**: 2255-2273 [PMID: 33264547 DOI: 10.1056/NEJMra2026131]

46 **Bangash MN**, Patel J, Parekh D. COVID-19 and the liver: little cause for concern. *Lancet Gastroenterol Hepatol* 2020; **5**: 529-530 [PMID: 32203680 DOI: 10.1016/S2468-1253(20)30084-4]

47 **Adams DH**, Hubscher SG. Systemic viral infections and collateral damage in the liver. *Am J Pathol* 2006; **168**: 1057-1059 [PMID: 16565481 DOI: 10.2353/ajpath.2006.051296]

48 **Effenberger M**, Grander C, Grabherr F, Griesmacher A, Ploner T, Hartig F, Bellmann-Weiler R, Joannidis M, Zoller H, Weiss G, Adolph TE, Tilg H. Systemic inflammation as fuel for acute liver injury in COVID-19. *Dig Liver Dis* 2021; **53**: 158-165 [PMID: 32873520 DOI: 10.1016/j.dld.2020.08.004]

49 **Ali N**, Hossain K. Liver injury in severe COVID-19 infection: current insights and challenges. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 879-884 [PMID: 32649840 DOI: 10.1080/17474124.2020.1794812]

50 **Portincasa P**, Krawczyk M, Machill A, Lammert F, Di Ciaula A. Hepatic consequences of COVID-

19 infection. Lapping or biting? *Eur J Intern Med* 2020; **77**: 18-24 [PMID: 32507608 DOI: 10.1016/j.ejim.2020.05.035]

51 **Boeckmans J**, Rodrigues RM, Demuyser T, Piérard D, Vanhaecke T, Rogiers V. COVID-19 and drug-induced liver injury: a problem of plenty or a petty point? *Arch Toxicol* 2020; **94**: 1367-1369 [PMID: 32266419 DOI: 10.1007/s00204-020-02734-1]

52 **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]

53 **Tsivgoulis G**, Palaiodimou L, Katsanos AH, Caso V, Köhrmann M, Molina C, Cordonnier C, Fischer U, Kelly P, Sharma VK, Chan AC, Zand R, Sarraj A, Schellinger PD, Voumvourakis KI, Grigoriadis N, Alexandrov AV, Tsiodras S. Neurological manifestations and implications of COVID-19 pandemic. *Ther Adv Neurol Disord* 2020; **13**: 1756286420932036 [PMID: 32565914 DOI: 10.1177/1756286420932036]

54 **Berth SH**, Lloyd TE. Secondary Causes of Myositis. *Curr Treat Options Neurol* 2020; **22**: 38 [PMID: 33041620 DOI: 10.1007/s11940-020-00646-0]

55 **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; **40**: 2095-2103 [PMID: 32239796 DOI: 10.1111/liv.14455]

56 **Soeters PB**, Wolfe RR, Shenkin A. Hypoalbuminemia: Pathogenesis and Clinical Significance. *JPEN J Parenter Enteral Nutr* 2019; **43**: 181-193 [PMID: 30288759 DOI: 10.1002/jpen.1451]

57 **Corti A**, Belcastro E, Dominici S, Maellaro E, Pompella A. The dark side of gamma-glutamyltransferase (GGT): Pathogenic effects of an 'antioxidant' enzyme. *Free Radic Biol Med* 2020; **160**: 807-819 [PMID: 32916278 DOI: 10.1016/j.freeradbiomed.2020.09.005]

58 **Sultan S**, Altayar O, Siddique SM, Davitkov P, Feuerstein JD, Lim JK, Falck-Ytter Y, El-Serag HB; AGA Institute. AGA Institute Rapid Review of the Gastrointestinal and Liver Manifestations of COVID-19, Meta-Analysis of International Data, and Recommendations for the Consultative Management of Patients with COVID-19. *Gastroenterology* 2020; **159**: 320-334. e27 [PMID: 32407808 DOI: 10.1053/j.gastro.2020.05.001]

59 **Hamid S**, Alvares da Silva MR, Burak KW, Chen T, Drenth JPH, Esmat G, Gaspar R, LaBrecque D, Lee A, Macedo G, McMahon B, Ning Q, Reau N, Sonderup M, van Leeuwen DJ, Armstrong D, Yurdaydin C. WGO Guidance for the Care of Patients With COVID-19 and Liver Disease. *J Clin Gastroenterol* 2021; **55**: 1-11 [PMID: 33230011 DOI: 10.1097/MCG.0000000000001459]

60 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

61 **Wang B**, Li R, Lu Z, Huang Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. *Aging (Albany NY)* 2020; **12**: 6049-6057 [PMID: 32267833 DOI: 10.18632/aging.103000]

62 **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]

63 **Mantovani A**, Byrne CD, Zheng MH, Targher G. Diabetes as a risk factor for greater COVID-19 severity and in-hospital death: A meta-analysis of observational studies. *Nutr Metab Cardiovasc Dis* 2020; **30**: 1236-1248 [PMID: 32571616 DOI: 10.1016/j.numecd.2020.05.014]

64 **Lim S**, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol* 2021; **17**: 11-30 [PMID: 33188364 DOI: 10.1038/s41574-020-00435-4]

65 **Gao F**, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, Chen YP, Targher G, Byrne CD, George J, Zheng MH. Obesity Is a Risk Factor for Greater COVID-19 Severity. *Diabetes Care* 2020; **43**: e72-e74 [PMID: 32409499 DOI: 10.2337/dc20-0682]

66 **Petrilli CM**, Jones SA, Yang J, Rajagopalan H, O'Donnell L, Chernyak Y, Tobin KA, Cerfolio RJ, Francois F, Horwitz LI. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ* 2020; **369**: m1966 [PMID: 32444366 DOI: 10.1136/bmj.m1966]

67 **Nseir WB**, Mograbi JM, Amara AE, Abu Elheja OH, Mahamid MN. Non-alcoholic fatty liver disease and 30-day all-cause mortality in adult patients with community-acquired pneumonia. *QJM* 2019; **112**: 95-99 [PMID: 30325458 DOI: 10.1093/qjmed/hcy227]

68 **Nseir W**, Taha H, Khateeb J, Grosovski M, Assy N. Fatty liver is associated with recurrent bacterial infections independent of metabolic syndrome. *Dig Dis Sci* 2011; **56**: 3328-3334 [PMID: 21562784 DOI: 10.1007/s10620-011-1736-5]

69 **Ji D**, Zhang D, Yang T, Mu J, Zhao P, Xu J, Li C, Cheng G, Wang Y, Chen Z, Qin E, Lau G. Effect

of COVID-19 on patients with compensated chronic liver diseases. *Hepatol Int* 2020; **14**: 701-710 [PMID: 32734407 DOI: 10.1007/s12072-020-10058-6]

70 **Targher G**, Mantovani A, Byrne CD, Wang XB, Yan HD, Sun QF, Pan KH, Zheng KI, Chen YP, Eslam M, George J, Zheng MH. Risk of severe illness from COVID-19 in patients with metabolic dysfunction-associated fatty liver disease and increased fibrosis scores. *Gut* 2020; **69**: 1545-1547 [PMID: 32414813 DOI: 10.1136/gutjnl-2020-321611]

71 **Hashemi N**, Viveiros K, Redd WD, Zhou JC, McCarty TR, Bazarbashi AN, Hathorn KE, Wong D, Njie C, Shen L, Chan WW. Impact of chronic liver disease on outcomes of hospitalized patients with COVID-19: A multicentre United States experience. *Liver Int* 2020; **40**: 2515-2521 [PMID: 32585065 DOI: 10.1111/liv.14583]

72 **Forlano R**, Mullish BH, Mukherjee SK, Nathwani R, Harlow C, Crook P, Judge R, Soubieres A, Middleton P, Daunt A, Perez-Guzman P, Selvapatt N, Lemoine M, Dhar A, Thursz MR, Nayagam S, Manousou P. In-hospital mortality is associated with inflammatory response in NAFLD patients admitted for COVID-19. *PLoS One* 2020; **15**: e0240400 [PMID: 33031439 DOI: 10.1371/journal.pone.0240400]

73 **Sachdeva S**, Khandait H, Kopel J, Aloysius MM, Desai R, Goyal H. NAFLD and COVID-19: a Pooled Analysis. *SN Compr Clin Med* 2020; 1-4 [PMID: 33173850 DOI: 10.1007/s42399-020-00631-3]

74 **Pan L**, Huang P, Xie X, Xu J, Guo D, Jiang Y. Metabolic associated fatty liver disease increases the severity of COVID-19: A meta-analysis. *Dig Liver Dis* 2021; **53**: 153-157 [PMID: 33011088 DOI: 10.1016/j.dld.2020.09.007]

75 **Ferron PJ**, Gicquel T, Mégarbane B, Clément B, Fromenty B. Treatments in Covid-19 patients with pre-existing metabolic dysfunction-associated fatty liver disease: A potential threat for drug-induced liver injury? *Biochimie* 2020; **179**: 266-274 [PMID: 32891697 DOI: 10.1016/j.biochi.2020.08.018]

76 **Zhou YJ**, Zheng KI, Wang XB, Yan HD, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, George J, Zheng MH. Younger patients with MAFLD are at increased risk of severe COVID-19 illness: A multicenter preliminary analysis. *J Hepatol* 2020; **73**: 719-721 [PMID: 32348790 DOI: 10.1016/j.jhep.2020.04.027]

77 **Zhou YJ**, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, George J, Zheng MH. Metabolic-associated fatty liver disease is associated with severity of COVID-19. *Liver Int* 2020; **40**: 2160-2163 [PMID: 32573883 DOI: 10.1111/liv.14575]

78 **Roca-Fernández A**, Dennis A, Nicolls R, McGonigle J, Kelly M, Banerjee R. High Liver Fat Associates with Higher Risk of Developing Symptomatic COVID-19 Infection - Initial UK Biobank Observations. 2020 Preprint. Available from: medRxiv:2020.06.04.20122457 [DOI: 10.1101/2020.06.04.20122457]

79 **Saviano A**, Wrensch F, Ghany MG, Baumert TF. Liver disease and COVID-19: from Pathogenesis to Clinical Care. *Hepatology* 2020 [PMID: 33332624 DOI: 10.1002/hep.31684]

80 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int* 2021; **41**: 20-32 [PMID: 33190346 DOI: 10.1111/liv.14730]

81 **Saltiel AR**, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest* 2017; **127**: 1-4 [PMID: 28045402 DOI: 10.1172/JCI92035]

82 **GBD 2015 Obesity Collaborators**. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi A, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anjana RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennett DA, Beyene AS, Biadgilign S, Biryukov S, Bjertness E, Boneya DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaeian A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang YH, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah GA, Mensink GBM, Mezgebe HB, Mirrakhimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Qorbani M, Rafay A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sanabria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin MJ, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaja KN, Uthman OA, Vaezghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass E, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; **377**: 13-27 [PMID: 28604169 DOI: 10.1056/NEJMoa1614362]

83 **Honce R**, Schultz-Cherry S. Impact of Obesity on Influenza A Virus Pathogenesis, Immune Response, and Evolution. *Front Immunol* 2019; **10**: 1071 [PMID: 31134099 DOI: 10.3389/fimmu.2019.01071]

84 **Portincasa P**, Krawczyk M, Smyk W, Lammert F, Di Ciaula A. COVID-19 and non-alcoholic fatty liver disease: Two intersecting pandemics. *Eur J Clin Invest* 2020; **50**: e13338 [PMID: 32589264

DOI: 10.1111/eci.13338]
85 **Kass DA**, Duggal P, Cingolani O. Obesity could shift severe COVID-19 disease to younger ages. *Lancet* 2020; **395**: 1544-1545 [PMID: 32380044 DOI: 10.1016/S0140-6736(20)31024-2]
86 **Zheng KI**, Gao F, Wang XB, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, Liu WY, George J, Zheng MH. Letter to the Editor: Obesity as a risk factor for greater severity of COVID-19 in patients with metabolic associated fatty liver disease. *Metabolism* 2020; **108**: 154244 [PMID: 32320741 DOI: 10.1016/j.metabol.2020.154244]
87 **Imam A**, Abukhalaf SA, Merhav H, Abu-Gazala S, Cohen-Arazi O, Pikarsky AJ, Safadi R, Khalaileh A. Prognosis and Treatment of Liver Transplant Recipients in the COVID-19 Era: A Literature Review. *Ann Transplant* 2020; **25**: e926196 [PMID: 33106469 DOI: 10.12659/AOT.926196]
88 **Zhang H**, Dai H, Xie X. Solid Organ Transplantation During the COVID-19 Pandemic. *Front Immunol* 2020; **11**: 1392 [PMID: 32612614 DOI: 10.3389/fimmu.2020.01392]
89 **Ranabothu S**, Kanduri SR, Nalleballe K, Cheungpasitporn W, Onteddu S, Kovvuru K. Outcomes of COVID-19 in Solid Organ Transplants. *Cureus* 2020; **12**: e11344 [PMID: 33173653 DOI: 10.7759/cureus.11344]
90 **Becchetti C**, Zambelli MF, Pasulo L, Donato MF, Invernizzi F, Detry O, Dahlqvist G, Ciccarelli O, Morelli MC, Fraga M, Svegliati-Baroni G, van Vlierberghe H, Coenraad MJ, Romero MC, de Gottardi A, Toniutto P, Del Prete L, Abbati C, Samuel D, Pirenne J, Nevens F, Dufour JF; COVID-LT group. COVID-19 in an international European liver transplant recipient cohort. *Gut* 2020; **69**: 1832-1840 [PMID: 32571972 DOI: 10.1136/gutjnl-2020-321923]
91 **Webb GJ**, Marjot T, Cook JA, Aloman C, Armstrong MJ, Brenner EJ, Catana MA, Cargill T, Dhanasekaran R, García-Juárez I, Hagström H, Kennedy JM, Marshall A, Masson S, Mercer CJ, Perumalswami PV, Ruiz I, Thaker S, Ufere NN, Barnes E, Barritt AS 4th, Moon AM. Outcomes following SARS-CoV-2 infection in liver transplant recipients: an international registry study. *Lancet Gastroenterol Hepatol* 2020; **5**: 1008-1016 [PMID: 32866433 DOI: 10.1016/S2468-1253(20)30271-5]
92 **Taher M**, Miroliaee A, Ebrahimi Daryani N, Alborzi Avanaki F, Aletaha N, Nasiri-Toosi M, Dashti H, Basirat V, Jafarian A. Management of Patients with Liver Transplant and Chronic Liver Diseases During COVID-19 Pandemic: A Brief Review. *Arch Iran Med* 2020; **23**: 713-717 [PMID: 33107315 DOI: 10.34172/aim.2020.92]
93 **Kabaçam G**, Dayangaç M, Üçbilek E, Erçin CN, Günsar F, Akyıldız M, Akarsu M, Demir M, Kaymakoğlu S, Karasu Z, İdilman R. The COVID-19 pandemic: Clinical practice advice for gastroenterologists, hepatologists, and liver transplant specialists. *Turk J Gastroenterol* 2020; **31**: 348-355 [PMID: 32519953 DOI: 10.5152/tjg.2020.20413]
94 **Di Maira T**, Berenguer M. COVID-19 and liver transplantation. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 526-528 [PMID: 32651555 DOI: 10.1038/s41575-020-0347-z]
95 **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]
96 **Sahin TT**, Akbulut S, Yilmaz S. COVID-19 pandemic: Its impact on liver disease and liver transplantation. *World J Gastroenterol* 2020; **26**: 2987-2999 [PMID: 32587443 DOI: 10.3748/wjg.v26.i22.2987]
97 **Saigal S**, Gupta S, Sudhindran S, Goyal N, Rastogi A, Jacob M, Raja K, Ramamurthy A, Asthana S, Dhiman RK, Singh B, Perumalla R, Malik A, Shanmugham N, Soin AS. Liver transplantation and COVID-19 (Coronavirus) infection: guidelines of the liver transplant Society of India (LTSI). *Hepatol Int* 2020; **14**: 429-431 [PMID: 32270388 DOI: 10.1007/s12072-020-10041-1]
98 **Liu H**, He X, Wang Y, Zhou S, Zhang D, Zhu J, He Q, Zhu Z, Li G, Sun L, Wang J, Cheng G, Liu Z, Lau G. Management of COVID-19 in patients after liver transplantation: Beijing working party for liver transplantation. *Hepatol Int* 2020; **14**: 432-436 [PMID: 32277387 DOI: 10.1007/s12072-020-10043-z]
99 **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]
100 **APASL Covid-19 Task Force**. Lau G, Sharma M. Clinical practice guidance for hepatology and liver transplant providers during the COVID-19 pandemic: APASL expert panel consensus recommendations. *Hepatol Int* 2020; **14**: 415-428 [PMID: 32447721 DOI: 10.1007/s12072-020-10054-w]
101 **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]
102 **Sarin SK**. "Fast, faster, and fastest: science on the run during COVID-19 drama"-"do not forget the liver". *Hepatol Int* 2020; **14**: 454-455 [PMID: 32277386 DOI: 10.1007/s12072-020-10042-0]
103 **Gambato M**, Burra P. Clinical implications of COVID-19 in patients with chronic liver disease and liver tumor. *Updates Surg* 2020; **72**: 237-239 [PMID: 32504266 DOI: 10.1007/s13304-020-00804-8]
104 **Lippi G**, de Oliveira MHS, Henry BM. Chronic liver disease is not associated with severity or mortality in Coronavirus disease 2019 (COVID-19): a pooled analysis. *Eur J Gastroenterol Hepatol*

2021; **33**: 114-115 [PMID: 32282549 DOI: 10.1097/MEG.0000000000001742]

105 **Kumar-M P**, Mishra S, Jha DK, Shukla J, Choudhury A, Mohindra R, Mandavdhare HS, Dutta U, Sharma V. Coronavirus disease (COVID-19) and the liver: a comprehensive systematic review and meta-analysis. *Hepatol Int* 2020; **14**: 711-722 [PMID: 32623633 DOI: 10.1007/s12072-020-10071-9]

106 **Oyelade T**, Alqahtani J, Canciani G. Prognosis of COVID-19 in Patients with Liver and Kidney Diseases: An Early Systematic Review and Meta-Analysis. *Trop Med Infect Dis* 2020; **5** [PMID: 32429038 DOI: 10.3390/tropicalmed5020080]

107 **Qi X**, Liu Y, Wang J, Fallowfield JA, Li X, Shi J, Pan H, Zou S, Zhang H, Chen Z, Li F, Luo Y, Mei M, Liu H, Wang Z, Li J, Yang H, Xiang H, Liu T, Zheng MH, Liu C, Huang Y, Xu D, Kang N, He Q, Gu Y, Zhang G, Shao C, Liu D, Zhang L, Kawada N, Jiang Z, Wang F, Xiong B, Takehara T, Rockey DC; COVID-Cirrhosis-CHESS Group. Clinical course and risk factors for mortality of COVID-19 patients with pre-existing cirrhosis: a multicentre cohort study. *Gut* 2021; **70**: 433-436 [PMID: 32434831 DOI: 10.1136/gutjnl-2020-321666]

108 **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]

109 **Strnad P**, Tacke F, Koch A, Trautwein C. Liver - guardian, modifier and target of sepsis. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 55-66 [PMID: 27924081 DOI: 10.1038/nrgastro.2016.168]

110 **Iavarone M**, D'Ambrosio R, Soria A, Triolo M, Pugliese N, Del Poggio P, Perricone G, Massironi S, Spinetti A, Buscarini E, Viganò M, Carriero C, Fagiuoli S, Aghemo A, Belli LS, Lucà M, Pedaci M, Rimondi A, Rumi MG, Invernizzi P, Bonfanti P, Lampertico P. High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol* 2020; **73**: 1063-1071 [PMID: 32526252 DOI: 10.1016/j.jhep.2020.06.001]

111 **Lleo A**, Invernizzi P, Lohse AW, Aghemo A, Carbone M. Management of patients with autoimmune liver disease during COVID-19 pandemic. *J Hepatol* 2020; **73**: 453-455 [PMID: 32283134 DOI: 10.1016/j.jhep.2020.04.002]

112 **Bollipo S**, Kapuria D, Rabiee A, Ben-Yakov G, Lui RN, Lee HW, Kumar G, Siau K, Turnes J, Dhanasekaran R. One world, one pandemic, many guidelines: management of liver diseases during COVID-19. *Gut* 2020; **69**: 1369-1372 [PMID: 32499304 DOI: 10.1136/gutjnl-2020-321553]

113 **Tapper EB**, Asrani SK. The COVID-19 pandemic will have a long-lasting impact on the quality of cirrhosis care. *J Hepatol* 2020; **73**: 441-445 [PMID: 32298769 DOI: 10.1016/j.jhep.2020.04.005]

114 **Marjot T**, Webb GJ, Barritt AS, Ginès P, Lohse AW, Moon AM, Pose E, Trivedi P, Barnes E. SARS-CoV-2 vaccination in patients with liver disease: responding to the next big question. *Lancet Gastroenterol Hepatol* 2021; **6**: 156-158 [PMID: 33444545 DOI: 10.1016/S2468-1253(21)00008-X]

115 **Aggeletopoulou I**, Davoulou P, Konstantakis C, Thomopoulos K, Triantos C. Response to hepatitis B vaccination in patients with liver cirrhosis. *Rev Med Virol* 2017; **27** [PMID: 28905444 DOI: 10.1002/rmv.1942]

116 **Marjot T**, Moon AM, Cook JA, Abd-Elsalam S, Aloman C, Armstrong MJ, Pose E, Brenner EJ, Cargill T, Catana MA, Dhanasekaran R, Eshraghian A, García-Juárez I, Gill US, Jones PD, Kennedy J, Marshall A, Matthews C, Mells G, Mercer C, Perumalswami PV, Avitabile E, Qi X, Su F, Ufere NN, Wong YJ, Zheng MH, Barnes E, Barritt AS 4th, Webb GJ. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: An international registry study. *J Hepatol* 2021; **74**: 567-577 [PMID: 33035628 DOI: 10.1016/j.jhep.2020.09.024]

117 **Fix OK**, Blumberg EA, Chang KM, Chu J, Chung RT, Goacher EK, Hameed B, Kaul DR, Kulik LM, Kwok RM, McGuire BM, Mulligan DC, Price JC, Reau NS, Reddy KR, Reynolds A, Rosen HR, Russo MW, Schilsky ML, Verna EC, Ward JW, Fontana RJ; AASLD COVID-19 Vaccine Working Group. AASLD Expert Panel Consensus Statement: Vaccines to Prevent COVID-19 Infection in Patients with Liver Disease. *Hepatology* 2021 [PMID: 33577086 DOI: 10.1002/hep.31751]

118 **Kaltsas A**, Sepkowitz K. Community acquired respiratory and gastrointestinal viral infections: challenges in the immunocompromised host. *Curr Opin Infect Dis* 2012; **25**: 423-430 [PMID: 22766648 DOI: 10.1097/QCO.0b013e328355660b]

119 **Gerussi A**, Rigamonti C, Elia C, Cazzagon N, Floreani A, Pozzi R, Pozzoni P, Claar E, Pasulo L, Fagiuoli S, Cristoferi L, Carbone M, Invernizzi P. Coronavirus Disease 2019 (COVID-19) in autoimmune hepatitis: a lesson from immunosuppressed patients. *Hepatol Commun* 2020 [PMID: 32838102 DOI: 10.1002/hep4.1557]

120 **Marjot T**, Buescher G, Sebode M, Barnes E, Barritt AS 4th, Armstrong MJ, Baldelli L, Kennedy J, Mercer C, Ozga AK, Casar C, Schramm C; contributing Members and Collaborators of ERN RARE-LIVER/COVID-Hep/SECURE-Cirrhosis, Moon AM, Webb GJ, Lohse AW. SARS-CoV-2 infection in patients with autoimmune hepatitis. *J Hepatol* 2021; **74**: 1335-1343 [PMID: 33508378 DOI: 10.1016/j.jhep.2021.01.021]

121 **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]

122 **World Health Organization**. Global hepatitis report 2017. 2017. [cited 13 March 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/

123 **He Q**, Zhang G, Gu Y, Wang J, Tang Q, Jiang Z, Shao C, Zhang H, Chen Z, Ma B, Liu D, Xie G, Xu D, Huang Y, Liang M, Huang H, Wang Y, Liu H, Yang J, Pan H, Zou S, Li F, Wang F, Liu C, Wang W, Xiong B, Li X, Liu L, Qi X. Clinical Characteristics of COVID-19 Patients With Pre-existing Hepatitis B Virus Infection: A Multicenter Report. *Am J Gastroenterol* 2021; **116**: 420-421 [PMID: 32925195 DOI: 10.14309/ajg.0000000000000924]

124 **Wu J**, Yu J, Shi X, Li W, Song S, Zhao L, Zhao X, Liu J, Wang D, Liu C, Huang B, Meng Y, Jiang B, Deng Y, Cao H, Li L. Epidemiological and clinical characteristics of 70 cases of coronavirus disease and concomitant hepatitis B virus infection: A multicentre descriptive study. *J Viral Hepat* 2021; **28**: 80-88 [PMID: 32929826 DOI: 10.1111/jvh.13404]

125 **Chen X**, Jiang Q, Ma Z, Ling J, Hu W, Cao Q, Mo P, Yao L, Yang R, Gao S, Gui X, Hou W, Xiong Y, Li J, Zhang Y. Clinical Characteristics of Hospitalized Patients with SARS-CoV-2 and Hepatitis B Virus Co-infection. *Virol Sin* 2020; **35**: 842-845 [PMID: 32839868 DOI: 10.1007/s12250-020-00276-5]

126 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: 32105632 DOI: 10.1016/S2213-2600(20)30079-5]

127 **Rodríguez-Tajes S**, Miralpeix A, Costa J, López-Suñé E, Laguno M, Pocurull A, Lens S, Mariño Z, Forns X. Low risk of hepatitis B reactivation in patients with severe COVID-19 who receive immunosuppressive therapy. *J Viral Hepat* 2021; **28**: 89-94 [PMID: 32969557 DOI: 10.1111/jvh.13410]

128 **Zhu JH**, Peltekian KM. HBV coinfection and in-hospital outcomes for COVID-19: a systematic review and meta-analysis. *Can Liver J* 2021; **4**: 16-22 [DOI: 10.3138/canlivj-2020-0029]

129 **Liu J**, Wang T, Cai Q, Sun L, Huang D, Zhou G, He Q, Wang FS, Liu L, Chen J. Longitudinal changes of liver function and hepatitis B reactivation in COVID-19 patients with pre-existing chronic hepatitis B virus infection. *Hepatol Res* 2020; **50**: 1211-1221 [PMID: 32761993 DOI: 10.1111/hepr.13553]

130 **National Institute of Diabetes and Digestive and Kidney Diseases**. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012

131 **Bonnel AR**, Bunchorntavakul C, Reddy KR. Immune dysfunction and infections in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2011; **9**: 727-738 [PMID: 21397731 DOI: 10.1016/j.cgh.2011.02.031]

132 **Ekpanyapong S**, Reddy KR. Infections in Cirrhosis. *Curr Treat Options Gastroenterol* 2019; **17**: 254-270 [PMID: 30980335 DOI: 10.1007/s11938-019-00229-2]

133 **Valour F**, Conrad A, Ader F, Launay O. Vaccination in adult liver transplantation candidates and recipients. *Clin Res Hepatol Gastroenterol* 2020; **44**: 126-134 [PMID: 31607643 DOI: 10.1016/j.clinre.2019.08.007]

134 **Sadoff J**, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, Stoop J, Tete S, Van Damme W, Leroux-Roels I, Berghmans PJ, Kimmel M, Van Damme P, de Hoon J, Smith W, Stephenson KE, De Rosa SC, Cohen KW, McElrath MJ, Cormier E, Scheper G, Barouch DH, Hendriks J, Struyf F, Douoguih M, Van Hoof J, Schuitemaker H. Interim Results of a Phase 1-2a Trial of Ad26.COV2.S Covid-19 Vaccine. *N Engl J Med* 2021; **384**: 1824-1835 [PMID: 33440088 DOI: 10.1056/NEJMoa2034201]

135 **Logunov DY**, Dolzhikova IV, Shcheblyakov DV, Tukhvatulin AI, Zubkova OV, Dzharullaeva AS, Kovyrshina AV, Lubenets NL, Grousova DM, Erokhova AS, Botikov AG, Izhaeva FM, Popova O, Ozharovskaya TA, Esmagambetov IB, Favorskaya IA, Zrelkin DI, Voronina DV, Shcherbinin DN, Semikhin AS, Simakova YV, Tokarskaya EA, Egorova DA, Shmarov MM, Nikitenko NA, Gushchin VA, Smolyarchuk EA, Zyryanov SK, Borisevich SV, Naroditsky BS, Gintsburg AL; Gam-COVID-Vac Vaccine Trial Group. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet* 2021; **397**: 671-681 [PMID: 33545094 DOI: 10.1016/S0140-6736(21)00234-8]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 3971-3983

DOI: 10.3748/wjg.v27.i26.3971

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

OPINION REVIEW

Chronic hepatitis B infection with concomitant hepatic steatosis: Current evidence and opinion

Yi-Wen Shi, Rui-Xu Yang, Jian-Gao Fan

ORCID number: Yi-Wen Shi 0000-0002-7049-9551; Rui-Xu Yang 0000-0001-9384-6408; Jian-Gao Fan 0000-0001-7443-5056.

Author contributions: Shi YW, Yang RX and Fan JG contributed to the study concept and design; Shi YW and Fan JG contributed to drafting the manuscript; Fan JG contributed to critical revision of the manuscript for important intellectual content; All authors confirmed critical revision of the manuscript for important intellectual content.

Supported by National Key Research and Development Program of China, No. 2017YFC0908903; and National Natural Science Foundation of China, No. 81873565 and No. 81900507.

Conflict-of-interest statement: The authors declare that theyhaveno competinginterests.

Yi-Wen Shi, Rui-Xu Yang, Jian-Gao Fan, Center for Fatty Liver, Department of Gastroenterology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Key Lab of Pediatric Gastroenterology and Nutrition, Shanghai 200092, China

Corresponding author: Jian-Gao Fan, MD, PhD, Chief Physician, Professor, Center for Fatty Liver, Department of Gastroenterology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai Key Lab of Pediatric Gastroenterology and Nutrition, No. 1665 Kongjiang Road, Shanghai 200092, China. fanjiangao@xinhuamed.com.cn

Abstract

With the increasing incidence of obesity and metabolic syndrome worldwide, concomitant nonalcoholic fatty liver disease (NAFLD) in patients with chronic hepatitis B (CHB) has become highly prevalent. The risk of dual etiologies, outcome, and mechanism of CHB with concomitant NAFLD have not been fully characterized. In this review, we assessed the overlapping prevalence of metabolic disorders and CHB, assessed the risk of advanced fibrosis/hepatocellular carcinoma in CHB patients concomitant with NAFLD, and discussed the remaining clinical issues to be addressed in the outcome of such patients. We also explored the possible roles of hepatitis B virus in the development of steatosis and discussed difficultiesof histological evaluation. For CHB patients, it is important to address concomitant NAFLD through lifestyle management and disease screening to achieve better prognoses. The assessment of progressive changes and novel therapies for CHB patients concomitant with NAFLD deserve further research.

Key Words: Nonalcoholic fatty liver disease; Hepatitis B; Metabolic disorders; Steatosis; Mechanism; Disease burden

Core Tip: The pathophysiology of concomitant hepatitis B and hepatic steatosis remains unclear. This review comprehensively discusses the epidemiology, risk factors, long-term outcomes, histological assessment, potential mechanisms, and therapeutic options in this field. We believe further studies can clarify the interactions of hepatitis B virus and steatosis, and provide novel strategies for the management of hepatitis B patients

Manuscript source: Unsolicited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: China

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

Received: February 6, 2021
Peer-review started: February 6, 2021
First decision: April 19, 2021
Revised: April 28, 2021
Accepted: May 27, 2021
Article in press: May 27, 2021
Published online: July 14, 2021

P-Reviewer: Kishida Y, Lee SW, Moriya K, Tai DI
S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Xing YX

with concomitant steatosis.

Citation: Shi YW, Yang RX, Fan JG. Chronic hepatitis B infection with concomitant hepatic steatosis: Current evidence and opinion. *World J Gastroenterol* 2021; 27(26): 3971-3983
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/3971.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.3971

INTRODUCTION

Chronic hepatitis B (CHB) has become highly prevalent worldwide in recent decades, affecting 350 million people, especially in Africa, Latin America and the Asia-Pacific region[1]. Although the incidence of hepatitis B virus (HBV) infection has recently decreased because of the widespread use of vaccines, the number of existing CHB patients remains significant[2]. CHB patients are at risk of severe liver-related adverse events, including decompensation, hepatocellular carcinoma (HCC), and even death. The persistence of covalently closed circular DNA (cccDNA) and incomplete immune tolerance lead to continuing HBV reproduction, resulting in chronic liver inflammation and fibrosis[3]. Despite the availability of potent antiviral treatments, we have not yet been able to eradicate HBV.

Nonalcoholic fatty liver disease (NAFLD) has become epidemic in those with chronic liver disease, with a worldwide annual incidence ranging from 6% to 35%[4]. The constantly increasing prevalence of NAFLD is paralleled by global increases of obesity and insulin resistance[5]. The natural course of NAFLD is asymptomatic and slowly progressive. A considerable proportion of CHB patients have concomitant hepatic steatosis or even steatohepatitis. A number of studies have investigated the relationship between CHB and NAFLD. Current evidence suggests that hepatic steatosis may have a protective effect on CHB by decreasing HBV viral markers, but CHB patients with concomitant NAFLD are faced with increased risks of advanced liver disease and HCC[6]. The management of such patients is challenging. We know little about the mechanisms of the interactions between HBV and steatosis. Therefore, this review was performed to determine the impact of HBV on hepatic steatosis and its underlying mechanisms.

EPIDEMIOLOGY OF STEATOSIS IN CHB

Prevalence and incidence of steatosis in patients with CHB

NAFLD is defined as the presence of steatosis (*i.e.* more than 5% liver fat content) without coexisting etiologies of secondary steatosissuch as alcohol abuse, metabolic dysfunction, and drug-induced liver injury[7]. Of the viral etiologies, hepatitis C virus (HCV) infection is known to influencechanges in insulin resistance and lipid metabolismthat would lead to hepatic steatosis and more severe inflammation in patients with chronic hepatitis C (CHC)[8]. The prevalence of fatty liver in CHC patients has been reported to range from 40% to 80%[9], depending on metabolic status, alcohol abuse and, virus genotypes[10]. Unlike HCV, there is currently no direct evidence that HBV increases the risk of steatosis. Even so, concomitant hepatic steatosis is not uncommon in HBV-infected patients.

NAFLD is reported to account for nearly 25% of the causes of elevated serum alanine aminotransferase (ALT) among CHB persons[11]. The prevalence of biopsy-proven NAFLD in CHB patients has been estimated to range from 14% to 30%[12-17]. Our recent study reported a prevalence of hepatic steatosis in CHB of 17.3%[18].A meta-analysis reported a higher prevalence of 29.6%[19]. Another recent study found a lower prevalence of NAFLD in CHB patients than in controls (13.5% *vs* 28.3%) using proton magnetic resonance spectroscopy, a highly reliable steatosis assay[20]. We performed a meta-analysis that found a lower prevalence of steatosis in CHB than in the general population (Supplementary material). The results of nine studies indicated a negative association with a possible risk for steatosis in CHB (pooled odds ratio (OR)= 0.81, 95%CI: 0.71-0.920, P = 0.001; Figure 1). Furthermore, the incidence of steatosis in a Korean cohort study was significantly lower in CHB patients than in the

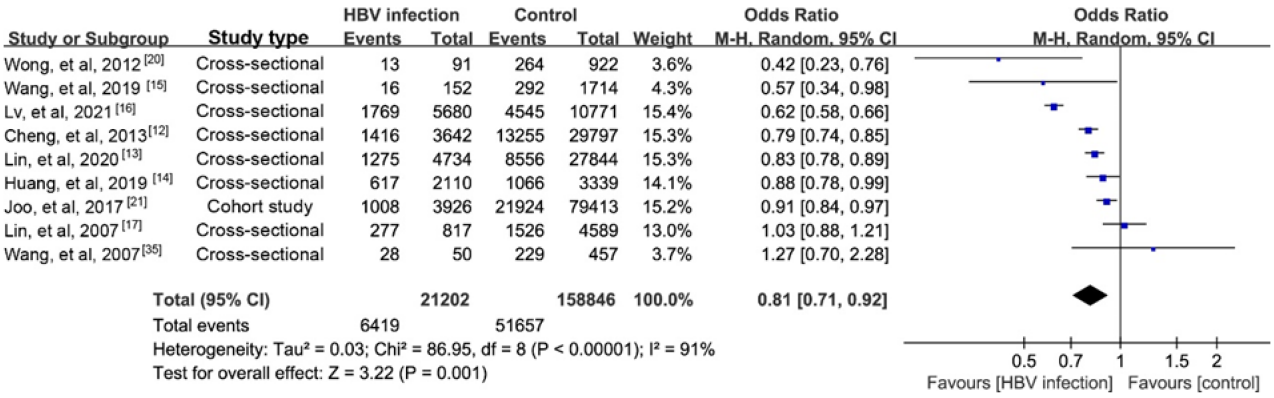

Figure 1 Meta-analysis of the prevalence of hepatic steatosis in patients with hepatitis B virus infection*vs* control.

controls (40.6 *vs* 43.5 per 1000 person-years)[21], and that was lower than an estimate of 52.34 per 1000 person-years in the general population reported by another meta-analysis[22].

Various factors may have contributed to the low prevalence of steatosis in patients with CHB. A study with propensity score analyses reported that a concurrent HBV infection was associated a lower risk of NAFLD than that in subjects who were only hepatitis B core antibody (anti-HBc) positive[23]. Other viral factors, including HBV genotypes, serum HBV DNA level, and hepatitis B e-antigen (HBeAg)positivity, were reported not to be associated with the prevalence of steatosis[20]. Our previous study reported that subclinical hypothyroidism had a role the development of steatosis in CHB patients, and that elevated thyroid stimulating hormone levels, even at normal ranges, were associated with an increased odds ratio of steatosis (OR = 1.54)[24]. Host metabolism has a role the development of steatosis. It was reported that overweight (OR = 5.99), hypertriglyceridemia (OR = 2.95), and type 2 diabetes (OR = 1.88) were risk factors for hepatic steatosis in CHB patients[25,26]. CHB patients with NAFLD presented with altered metabolic profiles and unhealthy lifestyle habits[27,28]. We speculate that differences in the estimated prevalence of NAFLD reported in these studies may be partly explainedby the modified metabolic status in CHB.

Metabolic dysfunctions in CHB

Metabolic dysfunctions have been considered as key factors for incident steatosis in CHB,with oxidative stress, insulin resistance, and hyperglycemia as contributors to hepatic steatosis. Insulin resistance increases fatty acid synthesis, delivery of free fatty acids to the liver, and accumulation of triglycerides in hepatocytes. Chronic inflammatory processes are activated in obesity, type 2 diabetes mellitus (T2DM),and other insulin-resistant states. In this context, activated macrophages release tumor necrosis factor-α and interleukin-6, which promote low-grade inflammation of adipose tissue and even the progression of hepatic damage[29,30]. Proinflammatory cytokines play a crucial role in liver inflammatory responses by promoting hepatocyte apoptosis, hepatic stellate cell proliferation, and angiogenesis[31]. In chronic liver disease, inflammation, fibrosis, and liver function decompensation disrupt liver synthesis functions. Decreased lipoprotein biosynthesis results in lower serum triglyceride and cholesterol levels[32]. Several large studies have described the associations between HBsAg positivity and disorders of lipid metabolism. HBsAg-positive patients had decreased serum cholesterol and triglyceride levels and a decreased prevalence of hyperlipemia [27,33,34]. Our study revealed a lower levels of hepatoxic lipids in serum from NAFLD-HBV patients than in those with only NAFLD[35]. The natural course of HBV infection may play a role in changes in lipid metabolism, especially in elderly patients [36]. This inverse relationship between HBV infection and serum lipid profile may also contribute to reducing the prevalence of metabolic syndrome[37,38].

Another aspect of steatosis in CHB is that impaired glucose and lipid metabolism make intrahepatic lipid content more sensitive to changes in energy intake. Liver inflammation and elevated ALT have been reported to be related to insulin resistance [39,40]. Evidence suggests that the prevalence of insulin resistance is higher in patients with CHB concomitant with NAFLD than in patients with HBV or NAFLD alone[41]. Numerous studies have reported a negative association of CHB and steatosis without aparallel risk associated with insulin resistance[40]. First, decreased liver functional reserve was found to promote insulin resistance, and because it is involved in glucose

metabolism, liver damage from hepatitis causeddisorders of glucose metabolism. The risk of developing diabetes was decreased in CHB after excluding patients with cirrhosis. Second, the association of insulin resistance and steatosis was attenuated by multiple host factors other than viruses, andage and obesity were both confounders of the risk of diabetes in CHB patients[42].

In CHB patients, steatosis resultsfrom a combination of metabolic abnormalities and the status of HBV infection. Thataccounts for the reported differences in the prevalence of steatosis in CHB patients and explains why previous HBV infection does not affect the prevalence of NAFLD[23]. The design of early studies failed to comprehensively evaluate metabolic status, calorie intake, and physical activity of CHB patients. Therefore, it was not possible to adjust for all confounding factors. Causes associated with those factors deserve investigation.

PROGRESSION AND OUTCOMES OF CHB WITH NAFLD

Disease severity of CHB with NAFLD

Chronic HBV infectionand NAFLD are the leading causes of chronic liver disease worldwide. Previous studies have considered steatosis to be an irrelevant or even a protective factor of CHB[25,43],but few focused on the effect of HBV on the severity and long-term outcome of NAFLD. The meta-analysis mentioned above revealed a strong negative association between serum viral load (*e.g.*, HBV DNA level and HBsAg positivity) and hepatic steatosis[19]. Similarly, a Korean cohort with non-CHB controls found an association between HBsAg positivity and a reduced risk of NAFLD [21]. After adjusting for metabolic factors, including insulin resistance, the association was attenuated[21], which indicated that metabolic and viral factors should both be taken into consideration.

Nonalcoholic steatohepatitis (NASH) is a severe form of NAFLD, that is prevalent in CHB patients. In a North American and European cohort, the prevalence of biopsy-proven NASH was approximately 17%[44]. NASH is characterized by necroinflammation and hepatocyte ballooning and is the major cause of advanced liver fibrosis, cirrhosis, and HCC in NAFLD[45]. Compared with bland steatosis, NASH has a more rapid progression in fibrosis[46], and it has been associated with anincreasedincidence of HCC, of up to 5.29 per 1000 person-years[47]. There is no doubt that CHB patients with NASH have a higher risk of developing advanced fibrosis, HCC,or even death than patients without steatohepatitis[44,48]. Concomitant NASH should thus be taken seriously in CHB patients. Hepatic inflammationis key fordisease progression. Although it would be difficult to differentiate the cause of inflammation from steatohepatitis in CHB patients, the risk of disease progression would be decreased if HBV replication could be suppressed before age 40. Therefore, the outcome of CHB patients with NASH would be improved in patients with early-stage NAFLD and low HBV replication phase. Comprehensive assessment and close monitoring are required in the management of CHB patients, irrespective of their viral load.

Risk of fibrosis in CHB patients with NAFLD

In patients with NAFLD, fibrosis is the characteristic that is most closely related to long-term adverse events compared with other histological features[49]. In the development of fibrosis in NASH, sustained lipotoxicity and endoplasmic reticulum stress induce cell death in steatotic hepatocytes. Developmental pathways including Notch, Hedgehog and YAP–TAZ are persistently activated to cope with the chronic insult. As a result, crosstalk of hepatocytes-macrophages-hepatic stellate cells and activation of resident Kupffer cells lead to inflammatory and fibrogenic responses[50].

Accumulating evidence suggests an increased risk of advanced fibrosis and long-term adverse prognosis in CHB patients with NAFLD. Our cross-sectional study found that CHB patients with steatosis had less severe fibrosis than those without steatosis [51]; but in prospective cohort studies, the baseline severity of steatosis was associated with more progressive fibrosis[52-54]. Furthermore, Charatcharoenwitthaya *et al*[25] reported that steatohepatitis but not simple steatosis was an independent predictor of significant, advanced fibrosis.The additive effect of steatosis has also been reported in the progression of fibrosis. Persistent severe steatosis led to a 2-fold increased risk of fibrosis progression over a 3-year follow-up[43]. A retrospective cohort study with biopsy-confirmed cirrhosis progression found that CHB patients with concomitant steatosishad a higher proportion of incident cirrhosis (36%) than those without steatosis (22%)[55]. There is little direct evidence of the effect of steatosis on fibrosis regression. It has been reported that low body mass index (BMI) and steatosis

resolution during tenofovir antiviral treatment were associated with fibrosis regression in CHB patients[43,56], suggesting that management of metabolic disorders and concomitant steatosis were key considerations of anti-fibrotic treatment.

Risk of HCC in CHB patients with NAFLD

Previously, more than 70% of HCC morbidity was attributed to chronic viral hepatitis. NAFLD has been predicted to replace viral etiologies in contributing to the HCC burden. NAFLD could account for more than 30% of HCC cases, especially in developed countries[57]. The progression of HCC in CHB patients with NAFLD is complicated, with direct evidence remaining elusive. As previously discussed, liver fibrosis and cirrhosis are recognized as key drivers of HCC[47]. Evidence suggests that metabolic factors are also responsible for disease progression. CHB patients with high BMI values were reported to have increased incidences of cirrhosis and HCC[58],and long-term follow-up has indicatedthat the incidence of HCC and the risks of liver-related mortality increase with the number of associated metabolic factors[59]. Two retrospective liver biopsy-proven cohort studies reported a 2-7-fold increase in the risks of HCC in CHB patients with NAFLD[48,55]. Recent studies reported similar results, but they found the association was reduced after adjusting for metabolic factors and age[6,60]. We speculate that metabolic factors, especially T2DM, play an important role in the development of HCC.

HCC remains the second leading cause of death related to malignancy worldwide [61].Screening and management of metabolic disorders in CHB patients are crucial for the prevention of HCC, andcoexisting factors should be taken into consideration. In the above-mentioned study, the association of hepatic steatosis and HCC development was observed only in patients receiving antiviral treatment, not in the overall population. That is because confounding factors including significant alcohol drinking were not considered[48]. In addition, the prevalence of NAFLD andthe HBsAg seroclearance rate both increase with age[62]. Therefore, patient age may be a confounding factor in the association of HBV infection with the long-term outcome of NAFLD. Noninvasive methods have often been used to identify steatosis in population-based studies, considering the injury risk of liver biopsy and the infeasibility of large numbers of patients,and using different measurements leads to bias in the definition of steatosis. Trial-based studies have carefully selected homogeneous patient samples that were matched for the presence of confounders. If patients with significant metabolic dysfunctions such asT2DM and cardiovascular disease were excluded, then the study results might not be representative of all types of real-world situations.

The overall long-term outcome of patients with CHB concomitant with steatosis is subject to avariety of risk factors. Liver conditionsincluding NASH and advanced fibrosis were found to have additive effects on event-free survival (HCC, decompensation and transplantation)[44]. Wong *et al*[52] reported that steatosis had no direct predictive effect on these events including cardiovascular events, liver-related complications, malignancy and mortality.

Important issues in clinical management

The effects of steatosis on the progression and remission of CHB have been widely investigated but few studies have focused on the outcome of NAFLD in the natural course of CHB or during antiviral treatment. Issues that should be addressed are: (1) The incidence of NAFLD in CHB and decreased risk of NAFLD in CHB[21] and diabetes[26]. Metabolic factors including weight change and lifestyle habits have not been comprehensively evaluated but a negative association may not reflect the etiology; (2) The progression of fibrosis in NAFLD needs study because the findings of cross-sectional studies are inconsistent. Concurrent HBV infection has been associated with advanced fibrosis[63], but anti-HBc-positive NAFLD patients are reported to have increased risks of cirrhosis, HCC, and liver-related complications[64]. The role of HBV infection status requires investigation; (3) The regression of fibrosis in NAFLD needs study. Steatosis resolution has been reported to be associated with fibrosis regression in CHB[43]; but whether HBV cures or antiviral treatment responses affect fibrosis regression in NAFLD remains unknown; and (4) The resolution of NASH. Given the interaction of steatosis resolution and fibrosis regression, the impact of fibrosis improvement after antiviral treatment of steatosis-related inflammation remains unknown.To address these issues, the interaction between HBV and metabolic homeostasis in the progression of liver disease requires further study.

EFFECT OF ANTIVIRAL TREATMENT ON NAFLD

Few studies have investigated the incidence of hepatic steatosis during antiviral treatment with pegylated interferon and nucleos(t)ide analogs (NAs). NA therapy reduces HBV replication, suppresses inflammation, and improves fibrosis in CHB[56]. Most studies have shown that NAFLD has no impact on viral suppression and biochemical responses during NAs antiviral treatment[65,66]. Whereas, decreased virological responses were also observed in CHB patients concomitant with steatosis in several studies[43,67,68]. In those cases, the authors speculated that the elevated ALT caused by NAFLD could lead to premature antiviral treatment and a poor response.

A recent study reported that lamivudine, entecavir, or adefovir dipivoxil increased the BMI and increased the visceral fat area in CHB patients[69]. It is worth noting that tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) were found toimprove the lipid metabolic profile of CHB patients. Compared with patients treated with entecavir, greater declines in serum lipid components were observed in patients treated with TDF[70]. An *in vitro* study reported that TDF modulated lipid metabolism by upregulating hepatic CD36 by activating PPAR-α[71]. Overexpression of hepatic CD36 improved hepatic steatosis and insulin resistance by reducing hepatic lipids, which might explain the findings above. In a study of CHB patients, switching to TAF improved metabolic dysfunction, reduced serum ALT levels, and improved ALT normalization in patients with or without diabetes despite significant increases in body weight and BMI[72].

Myrcludex B is a novel agent for CHB treatment that inhibits hepatic bile acid uptake transporter Na^+ taurocholate cotransporting polypeptide (NTCP). It has been shown to be safe and welltolerated and is currently in phase 2b clinical trials for the treatment of HBV infection. Recently, a study showed that Myrcludex B induced weight loss and decreased hepatic adiposity by inhibiting the hepatic clearance of bile acids from portal and systemic blood, stimulating glucagon-like peptide-1 (GLP-1) secretion[73].Because these agents potentially improve dyslipidemia and metabolic dysfunctions, TDF, TAF and Myrcludex B could be used to treat metabolic diseases, including NAFLD. They may be the best choice for CHB patients with concomitant NAFLD.

MECHANISMS OF INTERACTION BETWEEN HEPATITIS B AND STEATOSIS

Currently, the majority of CHB patients are on antiviral treatments that provide potent virological suppression. Viral factors are attenuated, and the relative influence of metabolic factors are increased in the course of NAFLD[74], which was verified in a study in HBsAg transgenic (HBs-Tg) mice. High-fat methionine-choline-deficient diet (MCD)-fed HBs-Tg mice had more liver fat accumulation and macrovesicular fat droplets than wild-type C57BL/6 mice. HBsAg increased susceptibility to steatohepatitis in those mice[75]. The evidence indicates that CHB patients should manage their lifestyle to prevent the incidence of NASH.

Accumulating evidence on single nucleotide polymorphisms (SNPs) and NAFLD severity and progression has helped to elucidate the genetic basis of NAFLD. SNPs of patatin-like phospholipase domain-containing protein 3 (PNPLA3) and transmembrane 6 superfamily member 2 (TM6SF2) are two common genetic determinants of NAFLD[76,77]. In cohort studies of biopsy-proven CHB patients, several SNPs of PNPLA3 were independently associated with steatosis, lobular inflammation, and steatohepatitis andwere similar to the findings of NAFLD studies[48,78,79], in which patients with SNPs of the T allele of rs1010023 in PNPLA3 were more susceptible to hepatic steatosis[78]. The T allele of rs58542926 in TM6SF2 has been associated with altered lipids and hepatic steatosis in CHB patients; this substitution was associated with increased HBV DNA[80]. As the T allele has a low prevalence of 7% worldwide, it may play a role in steatosis in a minority of the population. The evidence suggests the possibilityof genetic susceptibility to fatty liver in CHB.

Clinical studies that describe macroscopic results are often limited by the heterogenous characteristics of enrolled patients. Basic science studies would better balance confounding factors, and provide clues for elucidating the mechanism of interactions between HBV infection and fatty liver. Hepatitis B protein X (HBx), one of the four HBV proteins, has an important role in HBV infection. Previous studies in HepG2-HBx stable cells and in HBx-transgenic mice confirmed that overexpression of HBx induces

hepatic lipid accumulation, and that HBx is a risk factor for steatosis[15]. HBx is mediated by sterol regulatory element binding protein 1 (SREBP-1) and peroxisome proliferator-activated receptor gamma (PPAR-γ)[81]. HBx has been reported to upregulate fatty acid binding protein 1 (FABP1) to promote hepatic lipid accumulation in the development of steatosis in HBV-induced cells[82]. During treatment, the expression of HBx and downstream factors were downregulated by antiviral agents [83], which might be helpful for the improvement of steatosis during antiviral treatments in clinical studies.

As a regulator of adipocyte differentiation, CCAAT/enhancer-binding protein α (C/EBPα) triggers adipocyte differentiation by inducing complex cascades of transcription. In HBx-transfected hepatocytes, HBx stimulates the expression and transcriptional activation of C/EBPα and PPAR-γ[81]. Endoplasmic reticulum stress is associated with liver injury and fibrosis. C/EBPα is also the effector of endoplasmic reticulum stress, but whether HBV-induced endoplasmic reticulum stress plays a role in the development of concomitant steatosis requires further research. The involvement of adiponectin in adipogenic conversion in CHB has been extensively studied. Adiponectin improves hepatic insulin sensitivity and decreases lipid accumulation in macrophages. CHB patients have been reported to have higher serum adiponectin levels[27,84], which could account for the low prevalence of steatosis in HBV-infected subjects. The metabolic changes related to HBV infection at the cellular level could help explain the clinical,but phenotypic differences related to NAFLD at the individual level require further study.

CHALLENGES IN HISTOLOGICAL EVALUATION

NAFLD and CHB use different scoring systems for histological assessment. The fatty liver inhibition of progression algorithm and steatosis, activity, and fibrosis (FLIP-SAF) score[85] and NAFLD activity score (NAS)[86] are used to evaluate histological activity.The criteria include steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis. In the assessment of CHB, Ishaket al[87] and The METAVIR study group [88]have described two major scoring systems to evaluate necroinflammation and fibrosis. Because the pathogeneses of CHB and NAFLD are complex, the coexistence of HBV and steatosis-induced injury may affect each other. The steatosis distribution patterns in CHB patients with concomitant NAFLD and in those with NAFLD alone. In CHB, SHG/TPEF scores of the steatosis distribution and in the peripheral region and that in lobule region were similar.In NAFLD, the steatosis percentage was significantly lower in the peripheral region than in the lobule region[89]. Whether CHB concomitant with NAFLD has novel pathophysiological characteristics remains unclear.

The inflammation of CHB and NAFLD has been differentially evaluated by hepatocyte injury. The modified Knodell necroinflammatory score of the Ishak scoring system is used to assess CHB activity and is based on four variables, periportal or periseptal interface hepatitis, confluent necrosis, focal apoptosis and portal inflammation[87]. The NAS and SAF activity scoresare used to quantify inflammation in NAFLD. Ballooning is the most specific inflammatory characteristic of NAFLD, andin CHB concomitant with NAFLD, ballooning is predictive for clinical outcomes[44]. A cross-sectional studyreported that CHB with steatosis had less necroinflammation and fibrosis than CHB without steatosis[19], but CHB activity has not been associated with the degree of steatosis[90]. Although both algorithms score fibrosis on a scale of from 0 to 4, they are based on different zones and severities. In contrast to viral hepatitis, fibrosis characteristic of NASH is predominantly seen with lobular inflammation. Thus, zone-3 perisinusoidal fibrosis has been the primary focus during evaluations [86].

The dynamic assessment of inflammation and fibrosis are major problems faced in evaluating CHB concomitant with NAFLD. During antiviral treatment, viral suppression attenuates necroinflammation in CHB, inducing fibrosis improvement. Although the pathogenesis of HBV infection and NASH differ, they share a common pathway to fibrogenesis because of necroinflammation. Histological improvement in CHB is defined as a more than 2-point reduction in the Knodell necroinflammatory score with no worsening of fibrosis.Resolution of NASH is defined an inflammation score of 0 to 1 and a ballooning score of 0[91,92]. It is difficult to determine whether changes in CHB inflammation severity influence the NAFLD inflammation score orwhether fibrosis regression in CHB induces improvement of NAFLD. These problems have complicated the assessment of CHB regressionconcomitant with

NAFLD.Currently, with the new nomenclature of metabolic-associated fatty liver disease[93], it is no longer a diagnosis of exclusion. Based on the presence of steatosis and metabolic dysfunction, the diagnosis of NAFLD coexisting with CHB might be more feasible[94]. Thus, new definitions are needed to correctly classify patients during histopathological evaluation in clinical practice.

CONCLUSION

The decreased prevalence and incidence of steatosis in CHB patients are mainly due to altered metabolic profiles. However, concomitant steatosis increases the occurrence of adverse liver-related events, including cirrhosis and HCC. Lifestyle management and screening of metabolic changes associated with steatosis are recommended in CHB patients regardless of viral load. Traditional antiviral therapy has no impact on the incidence of steatosis, but tenofovir and NTCP inhibitors have strong metabolic effects, which could be promising in the treatment of CHB patients concomitant with NAFLD. Further study is necessary to determine whether these associations cause macro changes. As the mechanisms of interactions between steatosis and HBV infection become more clear, future studies will provide novel strategies for the clinical management and treatment of CHB concomitant with NAFLD.

REFERENCES

1 **Polaris Observatory Collaborators**. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* 2018; **3**: 383-403 [PMID: 29599078 DOI: 10.1016/s2468-1253(18)30056-6]

2 **Trépo C**, Chan HL, Lok A. Hepatitis B virus infection. *Lancet* 2014; **384**: 2053-2063 [PMID: 24954675 DOI: 10.1016/s0140-6736(14)60220-8]

3 **Werle-Lapostolle B**, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C, Marcellin P, Goodman Z, Delaney WE 4th, Xiong S, Brosgart CL, Chen SS, Gibbs CS, Zoulim F. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; **126**: 1750-1758 [PMID: 15188170 DOI: 10.1053/j.gastro.2004.03.018]

4 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

5 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

6 **Lee YB**, Ha Y, Chon YE, Kim MN, Lee JH, Park H, Kim KI, Kim SH, Rim KS, Hwang SG. Association between hepatic steatosis and the development of hepatocellular carcinoma in patients with chronic hepatitis B. *Clin Mol Hepatol* 2019; **25**: 52-64 [PMID: 30360031 DOI: 10.3350/cmh.2018.0040]

7 **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]

8 **Bugianesi E**, Salamone F, Negro F. The interaction of metabolic factors with HCV infection: does it matter? *J Hepatol* 2012; **56** Suppl 1: S56-S65 [PMID: 22300466 DOI: 10.1016/s0168-8278(12)60007-5]

9 **Negro F**. Abnormalities of lipid metabolism in hepatitis C virus infection. *Gut* 2010; **59**: 1279-1287 [PMID: 20660700 DOI: 10.1136/gut.2009.192732]

10 **Rubbia-Brandt L**, Fabris P, Paganin S, Leandro G, Male PJ, Giostra E, Carlotto A, Bozzola L, Smedile A, Negro F. Steatosis affects chronic hepatitis C progression in a genotype specific way. *Gut* 2004; **53**: 406-412 [PMID: 14960525 DOI: 10.1136/gut.2003.018770]

11 **Spradling PR**, Bulkow L, Teshale EH, Negus S, Homan C, Simons B, McMahon BJ. Prevalence and causes of elevated serum aminotransferase levels in a population-based cohort of persons with chronic hepatitis B virus infection. *J Hepatol* 2014; **61**: 785-791 [PMID: 24911461 DOI: 10.1016/j.jhep.2014.05.045]

12 **Cheng YL**, Wang YJ, Kao WY, Chen PH, Huo TI, Huang YH, Lan KH, Su CW, Chan WL, Lin HC, Lee FY, Wu JC. Inverse association between hepatitis B virus infection and fatty liver disease: a large-scale study in populations seeking for check-up. *PLoS One* 2013; **8**: e72049 [PMID: 23991037 DOI: 10.1371/journal.pone.0072049]

13 **Lin S**, Wang M, Liu Y, Huang J, Wu Y, Zhu Y, Wang X. Concurrence of HBV infection and non-alcoholic fatty liver disease is associated with higher prevalence of chronic kidney disease. *Clin Res Hepatol Gastroenterol* 2021; **45**: 101483 [PMID: 32646847 DOI: 10.1016/j.clinre.2020.06.009]

14 **Huang J**, Jing M, Wang C, Wang M, You S, Lin S, Zhu Y. The impact of hepatitis B virus infection status on the prevalence of nonalcoholic fatty liver disease: A population-based study. *J Med Virol* 2020; **92**: 1191-1197 [PMID: 31691993 DOI: 10.1002/jmv.25621]

15 **Wang B**, Li W, Fang H, Zhou H. Hepatitis B virus infection is not associated with fatty liver disease: Evidence from a cohort study and functional analysis. *Mol Med Rep* 2019; **19**: 320-326 [PMID: 30387826 DOI: 10.3892/mmr.2018.9619]

16 **Lv DD**, Wang YJ, Wang ML, Chen EQ, Tao YC, Zhang DM, Tang H. Effect of silibinin capsules combined with lifestyle modification on hepatic steatosis in patients with chronic hepatitis B. *Sci Rep* 2021; **11**: 655 [PMID: 33436935 DOI: 10.1038/s41598-020-80709-z]

17 **Lin YC**, Hsiao ST, Chen JD. Sonographic fatty liver and hepatitis B virus carrier status: synergistic effect on liver damage in Taiwanese adults. *World J Gastroenterol* 2007; **13**: 1805-1810 [PMID: 17465470 DOI: 10.3748/wjg.v13.i12.1805]

18 **Wang MM**, Wang GS, Shen F, Chen GY, Pan Q, Fan JG. Hepatic steatosis is highly prevalent in hepatitis B patients and negatively associated with virological factors. *Dig Dis Sci* 2014; **59**: 2571-2579 [PMID: 24838496 DOI: 10.1007/s10620-014-3180-9]

19 **Machado MV**, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. *J Gastroenterol Hepatol* 2011; **26**: 1361-1367 [PMID: 21649726 DOI: 10.1111/j.1440-1746.2011.06801.x]

20 **Wong VW**, Wong GL, Chu WC, Chim AM, Ong A, Yeung DK, Yiu KK, Chu SH, Chan HY, Woo J, Chan FK, Chan HL. Hepatitis B virus infection and fatty liver in the general population. *J Hepatol* 2012; **56**: 533-540 [PMID: 22027575 DOI: 10.1016/j.jhep.2011.09.013]

21 **Joo EJ**, Chang Y, Yeom JS, Ryu S. Hepatitis B virus infection and decreased risk of nonalcoholic fatty liver disease: A cohort study. *Hepatology* 2017; **65**: 828-835 [PMID: 28035771 DOI: 10.1002/hep.28917]

22 **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]

23 **Zhong GC**, Wu YL, Hao FB, Rao XW, Yuan XW, Zhao Y, Gong JP. Current but not past hepatitis B virus infection is associated with a decreased risk of nonalcoholic fatty liver disease in the Chinese population: A case-control study with propensity score analysis. *J Viral Hepat* 2018; **25**: 842-852 [PMID: 29406564 DOI: 10.1111/jvh.12878]

24 **Ding WJ**, Wang MM, Wang GS, Shen F, Qin JJ, Fan JG. Thyroid function is associated with non-alcoholic fatty liver disease in chronic hepatitis B-infected subjects. *J Gastroenterol Hepatol* 2015; **30**: 1753-1758 [PMID: 25974331 DOI: 10.1111/jgh.12998]

25 **Charatcharoenwitthaya P**, Pongpaibul A, Kaosombatwattana U, Bhanthumkomol P, Bandidniyamanon W, Pausawasdi N, Tanwandee T. The prevalence of steatohepatitis in chronic hepatitis B patients and its impact on disease severity and treatment response. *Liver Int* 2017; **37**: 542-551 [PMID: 27740738 DOI: 10.1111/Liv.13271]

26 **Zhu L**, Jiang J, Zhai X, Baecker A, Peng H, Qian J, Zhou M, Song C, Zhou Y, Xu J, Liu H, Hang D, Hu Z, Shen H, Zhang ZF, Zhu F. Hepatitis B virus infection and risk of non-alcoholic fatty liver disease: A population-based cohort study. *Liver Int* 2019; **39**: 70-80 [PMID: 30025200 DOI: 10.1111/Liv.13933]

27 **Hsu CS**, Liu CH, Wang CC, Tseng TC, Liu CJ, Chen CL, Chen PJ, Chen DS, Kao JH. Impact of hepatitis B virus infection on metabolic profiles and modifying factors. *J Viral Hepat* 2012; **19**: e48-e57 [PMID: 22239526 DOI: 10.1111/j.1365-2893.2011.01535.x]

28 **Kim CH**, Kallman JB, Bai C, Pawloski L, Gewa C, Arsalla A, Sabatella ME, Younossi ZM. Nutritional assessments of patients with non-alcoholic fatty liver disease. *Obes Surg* 2010; **20**: 154-160 [PMID: 18560947 DOI: 10.1007/s11695-008-9549-0]

29 **Rosso C**, Kazankov K, Younes R, Esmaili S, Marietti M, Sacco M, Carli F, Gaggini M, Salomone F, Møller HJ, Abate ML, Vilstrup H, Gastaldelli A, George J, Grønbæk H, Bugianesi E. Crosstalk between adipose tissue insulin resistance and liver macrophages in non-alcoholic fatty liver disease. *J Hepatol* 2019; **71**: 1012-1021 [PMID: 31301321 DOI: 10.1016/j.jhep.2019.06.031]

30 **De Taeye BM**, Novitskaya T, McGuinness OP, Gleaves L, Medda M, Covington JW, Vaughan DE. Macrophage TNF-alpha contributes to insulin resistance and hepatic steatosis in diet-induced obesity. *Am J Physiol Endocrinol Metab* 2007; **293**: E713-E725 [PMID: 17578885 DOI: 10.1152/ajpendo.00194.2007]

31 **Yin Z**, Ma T, Lin Y, Lu X, Zhang C, Chen S, Jian Z. IL-6/STAT3 pathway intermediates M1/M2 macrophage polarization during the development of hepatocellular carcinoma. *J Cell Biochem* 2018; **119**: 9419-9432 [PMID: 30015355 DOI: 10.1002/jcb.27259]

32 **Arain SQ**, Talpur FN, Channa NA, Ali MS, Afridi HI. Serum lipid profile as a marker of liver impairment in hepatitis B Cirrhosis patients. *Lipids Health Dis* 2017; **16**: 51 [PMID: 28249586 DOI: 10.1186/s12944-017-0437-2]

33 **Liu PT**, Hwang AC, Chen JD. Combined effects of hepatitis B virus infection and elevated alanine aminotransferase levels on dyslipidemia. *Metabolism* 2013; **62**: 220-225 [PMID: 22938729 DOI: 10.1016/j.metabol.2012.07.022]

34 **Chen JY**, Wang JH, Lin CY, Chen PF, Tseng PL, Chen CH, Chang KC, Tsai LS, Chen SC, Lu SN. Lower prevalence of hypercholesterolemia and hyperglyceridemia found in subjects with seropositivity for both hepatitis B and C strains independently. *J Gastroenterol Hepatol* 2010; **25**: 1763-1768 [PMID: 21039839 DOI: 10.1111/j.1440-1746.2010.06300.x]

35 **Li H**, Xu QY, Xie Y, Luo JJ, Cao HX, Pan Q. Effects of chronic HBV infection on lipid metabolism in non-alcoholic fatty liver disease: A lipidomic analysis. *Ann Hepatol* 2021; **24**: 100316 [PMID: 33515803 DOI: 10.1016/j.aohep.2021.100316]

36 **McMahon BJ**. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009; **49**: S45-S55 [PMID: 19399792 DOI: 10.1002/hep.22898]

37 **Huang CY**, Lu CW, Liu YL, Chiang CH, Lee LT, Huang KC. Relationship between chronic hepatitis B and metabolic syndrome: A structural equation modeling approach. *Obesity (Silver Spring)* 2016; **24**: 483-489 [PMID: 26719030 DOI: 10.1002/oby.21333]

38 **Zhao X**, Shah D, Sambamoorthi U. Association between chronic hepatitis B infection and metabolic syndrome. *J Diabetes Metab Disord* 2018; **17**: 223-232 [PMID: 30918858 DOI: 10.1007/s40200-018-0364-4]

39 **Simental-Mendía LE**, Rodríguez-Morán M, Gómez-Díaz R, Wacher NH, Rodríguez-Hernández H, Guerrero-Romero F. Insulin resistance is associated with elevated transaminases and low aspartate aminotransferase/alanine aminotransferase ratio in young adults with normal weight. *Eur J Gastroenterol Hepatol* 2017; **29**: 435-440 [PMID: 28009717 DOI: 10.1097/meg.0000000000000811]

40 **Wang CC**, Hsu CS, Liu CJ, Kao JH, Chen DS. Association of chronic hepatitis B virus infection with insulin resistance and hepatic steatosis. *J Gastroenterol Hepatol* 2008; **23**: 779-782 [PMID: 18028349 DOI: 10.1111/j.1440-1746.2007.05216.x]

41 **Ye J**, Hu X, Wu T, Wu Y, Shao C, Li F, Lin Y, Feng S, Wang W, Zhong B. Insulin resistance exhibits varied metabolic abnormalities in nonalcoholic fatty liver disease, chronic hepatitis B and the combination of the two: a cross-sectional study. *Diabetol Metab Syndr* 2019; **11**: 45 [PMID: 31223344 DOI: 10.1186/s13098-019-0440-z]

42 **Lei S**, Chen S, Zhao X, Zhang Y, Cheng K, Zhang X, Wang Z, Sun Y, Wu S, Wang L. Hepatitis B virus infection and diabetes mellitus: the Kailuan prospective cohort study in China. *Hepatol Int* 2020; **14**: 743-753 [PMID: 32885367 DOI: 10.1007/s12072-020-10086-2]

43 **Mak LY**, Hui RW, Fung J, Liu F, Wong DK, Cheung KS, Yuen MF, Seto WK. Diverse effects of hepatic steatosis on fibrosis progression and functional cure in virologically quiescent chronic hepatitis B. *J Hepatol* 2020; **73**: 800-806 [PMID: 32504663 DOI: 10.1016/j.jhep.2020.05.040]

44 **Choi HSJ**, Brouwer WP, Zanjir WMR, de Man RA, Feld JJ, Hansen BE, Janssen HLA, Patel K. Nonalcoholic Steatohepatitis Is Associated With Liver-Related Outcomes and All-Cause Mortality in Chronic Hepatitis B. *Hepatology* 2020; **71**: 539-548 [PMID: 31309589 DOI: 10.1002/hep.30857]

45 **Kleiner DE**, Brunt EM, Wilson LA, Behling C, Guy C, Contos M, Cummings O, Yeh M, Gill R, Chalasani N, Neuschwander-Tetri BA, Diehl AM, Dasarathy S, Terrault N, Kowdley K, Loomba R, Belt P, Tonascia J, Lavine JE, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Association of Histologic Disease Activity With Progression of Nonalcoholic Fatty Liver Disease. *JAMA Netw Open* 2019; **2**: e1912565 [PMID: 31584681 DOI: 10.1001/jamanetworkopen.2019.12565]

46 **Singh S**, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver *vs* nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015; **13**: 643-54. quiz e39-40 [PMID: 24768810 DOI: 10.1016/j.cgh.2014.04.014]

47 **Golabi P**, Rhea L, Henry L, Younossi ZM. Hepatocellular carcinoma and non-alcoholic fatty liver disease. *Hepatol Int* 2019; **13**: 688-694 [PMID: 31701393 DOI: 10.1007/s12072-019-09995-8]

48 **Brouwer WP**, van der Meer AJ, Boonstra A, Pas SD, de Knegt RJ, de Man RA, Hansen BE, ten Kate FJ, Janssen HL. The impact of PNPLA3 (rs738409 C>G) polymorphisms on liver histology and long-term clinical outcome in chronic hepatitis B patients. *Liver Int* 2015; **35**: 438-447 [PMID: 25284145 DOI: 10.1111/Liv.12695]

49 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-97. e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]

50 **Zhu C**, Tabas I, Schwabe RF, Pajvani UB. Maladaptive regeneration - the reawakening of developmental pathways in NASH and fibrosis. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 131-142 [PMID: 33051603 DOI: 10.1038/s41575-020-00365-6]

51 **Shi JP**, Fan JG, Wu R, Gao XQ, Zhang L, Wang H, Farrell GC. Prevalence and risk factors of hepatic steatosis and its impact on liver injury in Chinese patients with chronic hepatitis B infection. *J Gastroenterol Hepatol* 2008; **23**: 1419-1425 [PMID: 18853998 DOI: 10.1111/j.1440-1746.2008.05531.x]

52 **Wong SW**, Chan WK, Mohamed R. Fatty liver is associated with advanced fibrosis but does not predict adverse outcomes in patients with chronic hepatitis B. *J Viral Hepat* 2020; **27**: 1297-1305 [PMID: 32668489 DOI: 10.1111/jvh.13361]

53 **Seto WK**, Fung J, Cheung KS, Mak LY, Hui RW, Liu KS, Lai CL, Yuen MF. Body-mass index is associated with fibrosis regression during long-term nucleoside analogue therapy in chronic hepatitis B. *Aliment Pharmacol Ther* 2016; **44**: 1071-1079 [PMID: 27659292 DOI: 10.1111/apt.13804]

54 **Chalasani N**, Wilson L, Kleiner DE, Cummings OW, Brunt EM, Unalp A; NASH Clinical Research Network. Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. *J Hepatol* 2008; **48**: 829-834 [PMID: 18321606 DOI: 10.1016/j.jhep.2008.01.016]

55 **Chan AW**, Wong GL, Chan HY, Tong JH, Yu YH, Choi PC, Chan HL, To KF, Wong VW. Concurrent fatty liver increases risk of hepatocellular carcinoma among patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2017; **32**: 667-676 [PMID: 27547913 DOI: 10.1111/jgh.13536]

56 **Marcellin P**, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013; **381**: 468-475 [PMID: 23234725 DOI: 10.1016/s0140-6736(12)61425-1]

57 **Caines A**, Selim R, Salgia R. The Changing Global Epidemiology of Hepatocellular Carcinoma. *Clin Liver Dis* 2020; **24**: 535-547 [PMID: 33012444 DOI: 10.1016/j.cld.2020.06.001]

58 **Yu MW**, Shih WL, Lin CL, Liu CJ, Jian JW, Tsai KS, Chen CJ. Body-mass index and progression of hepatitis B: a population-based cohort study in men. *J Clin Oncol* 2008; **26**: 5576-5582 [PMID: 18955457 DOI: 10.1200/jco.2008.16.1075]

59 **Yu MW**, Lin CL, Liu CJ, Yang SH, Tseng YL, Wu CF. Influence of Metabolic Risk Factors on Risk of Hepatocellular Carcinoma and Liver-Related Death in Men With Chronic Hepatitis B: A Large Cohort Study. *Gastroenterology* 2017; **153**: 1006-1017. e5 [PMID: 28711626 DOI: 10.1053/j.gastro.2017.07.001]

60 **Lim CT**, Goh GBB, Li H, Lim TK, Leow WQ, Wan WK, Azhar R, Chow WC, Kumar R. Presence of Hepatic Steatosis Does Not Increase the Risk of Hepatocellular Carcinoma in Patients With Chronic Hepatitis B Over Long Follow-Up. *Microbiol Insights* 2020; **13**: 1178636120918878 [PMID: 32435130 DOI: 10.1177/1178636120918878]

61 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

62 **Tai DI**, Tsay PK, Chen WT, Chu CM, Liaw YF. Relative roles of HBsAg seroclearance and mortality in the decline of HBsAg prevalence with increasing age. *Am J Gastroenterol* 2010; **105**: 1102-1109 [PMID: 20197760 DOI: 10.1038/ajg.2009.669]

63 **Su HJ**, Kao JH, Tseng TC, Yang HC, Su TH, Chen PJ, Liu CJ. Pathologic findings of patients with nonalcoholic fatty liver disease and the impact of concurrent hepatitis B virus infection in Taiwan. *J Formos Med Assoc* 2020; **119**: 1476-1482 [PMID: 32499209 DOI: 10.1016/j.jfma.2020.05.027]

64 **Chan TT**, Chan WK, Wong GL, Chan AW, Nik Mustapha NR, Chan SL, Chong CC, Mahadeva S, Shu SS, Lai PB, Chan HL, Wong VW. Positive Hepatitis B Core Antibody Is Associated With Cirrhosis and Hepatocellular Carcinoma in Nonalcoholic Fatty Liver Disease. *Am J Gastroenterol* 2020; **115**: 867-875 [PMID: 32149781 DOI: 10.14309/ajg.0000000000000588]

65 **Ceylan B**, Arslan F, Batırel A, Fincancı M, Yardımcı C, Fersan E, Paşaoğlu E, Yılmaz M, Mert A. Impact of fatty liver on hepatitis B virus replication and virologic response to tenofovir and entecavir. *Turk J Gastroenterol* 2016; **27**: 42-46 [PMID: 26674977 DOI: 10.5152/tjg.2015.150348]

66 **Li J**, Le AK, Chaung KT, Henry L, Hoang JK, Cheung R, Nguyen MH. Fatty liver is not independently associated with the rates of complete response to oral antiviral therapy in chronic hepatitis B patients. *Liver Int* 2020; **40**: 1052-1061 [PMID: 32086988 DOI: 10.1111/Liv.14415]

67 **Gong L**, Liu J, Wang J, Lou GQ, Shi JP. Hepatic Steatosis as a Predictive Factor of Antiviral Effect of Pegylated Interferon Therapy in Patients With Hepatitis B. *Transplant Proc* 2015; **47**: 2886-2891 [PMID: 26707308 DOI: 10.1016/j.transproceed.2015.10.023]

68 **Zhu Y**, Yang Q, Lv F, Yu Y. The Effect of Hepatosteatosis on Response to Antiviral Treatment in Patients with Chronic Hepatitis B: A Meta-Analysis. *Gastroenterol Res Pract* 2017; **2017**: 1096406 [PMID: 28421108 DOI: 10.1155/2017/1096406]

69 **Yao J**, Zhou L, Hua X, Kong M, Chen Y, Duan Z. Effects of nucleos(t)ide analogs on body composition in HBV-infected men: An age- and BMI-matched, cross-sectional study. *Nutrition* 2016; **32**: 1206-1210 [PMID: 27283043 DOI: 10.1016/j.nut.2016.04.001]

70 **Shaheen AA**, AlMattooq M, Yazdanfar S, Burak KW, Swain MG, Congly SE, Borman MA, Lee SS, Myers RP, Coffin CS. Tenofovir disoproxil fumarate significantly decreases serum lipoprotein levels compared with entecavir nucleos(t)ide analogue therapy in chronic hepatitis B carriers. *Aliment Pharmacol Ther* 2017; **46**: 599-604 [PMID: 28707319 DOI: 10.1111/apt.14218]

71 **Suzuki K**, Suda G, Yamamoto Y, Furuya K, Baba M, Nakamura A, Miyoshi H, Kimura M, Maehara O, Yamada R, Kitagataya T, Yamamoto K, Shigesawa T, Ohara M, Kawagishi N, Nakai M, Sho T, Natsuizaka M, Morikawa K, Ogawa K, Ohnishi S, Sakamoto N; NORTE Study Group. Tenofovir-disoproxil-fumarate modulates lipid metabolism *via* hepatic CD36/PPAR-alpha activation in hepatitis B virus infection. *J Gastroenterol* 2021; **56**: 168-180 [PMID: 33211179 DOI: 10.1007/s00535-020-01750-3]

72 **Sripongpun P**, Kim WR, Mannalithara A, Kwong A, Daugherty T, Goel A, Kwo PY. Tenofovir Alafenamide Attenuates Effects of Diabetes and Body Mass on Serum Alanine Aminotransferase Activities in Patients With Chronic Hepatitis B. *Clin Gastroenterol Hepatol* 2020 [PMID: 33285291 DOI: 10.1016/j.cgh.2020.11.047]

73 **Donkers JM**, Roscam Abbing RLP, van Weeghel M, Levels JHM, Boelen A, Schinkel AH, Oude Elferink RPJ, van de Graaf SFJ. Inhibition of Hepatic Bile Acid Uptake by Myrcludex B Promotes Glucagon-Like Peptide-1 Release and Reduces Obesity. *Cell Mol Gastroenterol Hepatol* 2020; **10**: 451-466 [PMID: 32330730 DOI: 10.1016/j.jcmgh.2020.04.009]

74 **Seto WK**, Hui RWH, Mak LY, Fung J, Cheung KS, Liu KSH, Wong DK, Lai CL, Yuen MF. Association Between Hepatic Steatosis, Measured by Controlled Attenuation Parameter, and Fibrosis

Burden in Chronic Hepatitis B. *Clin Gastroenterol Hepatol* 2018; **16**: 575-583. e2 [PMID: 28970146 DOI: 10.1016/j.cgh.2017.09.044]

75 **Fu MM**, Sun R, Tian ZG, Wei HM. Increased susceptibility to experimental steatohepatitis induced by methionine-choline deficiency in HBs-Tg mice. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 513-519 [PMID: 20943461]

76 **Krawczyk M**, Rau M, Schattenberg JM, Bantel H, Pathil A, Demir M, Kluwe J, Boettler T, Lammert F, Geier A; NAFLD Clinical Study Group. Combined effects of the PNPLA3 rs738409, TM6SF2 rs58542926, and MBOAT7 rs641738 variants on NAFLD severity: a multicenter biopsy-based study. *J Lipid Res* 2017; **58**: 247-255 [PMID: 27836992 DOI: 10.1194/jlr.P067454]

77 **Eslam M**, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. *J Hepatol* 2018; **68**: 268-279 [PMID: 29122391 DOI: 10.1016/j.jhep.2017.09.003]

78 **Pan Q**, Chen MM, Zhang RN, Wang YQ, Zheng RD, Mi YQ, Liu WB, Shen F, Su Q, Fan JG. *PNPLA3* rs1010023 Predisposes Chronic Hepatitis B to Hepatic Steatosis but Improves Insulin Resistance and Glucose Metabolism. *J Diabetes Res* 2017; **2017**: 4740124 [PMID: 28695131 DOI: 10.1155/2017/4740124]

79 **Pan Q**, Zhang RN, Wang YQ, Zheng RD, Mi YQ, Liu WB, Shen F, Chen GY, Lu JF, Zhu CY, Zhang SY, Chen YM, Sun WL, Fan JG. Linked PNPLA3 polymorphisms confer susceptibility to nonalcoholic steatohepatitis and decreased viral load in chronic hepatitis B. *World J Gastroenterol* 2015; **21**: 8605-8614 [PMID: 26229402 DOI: 10.3748/wjg.v21.i28.8605]

80 **Eslam M**, Mangia A, Berg T, Chan HL, Irving WL, Dore GJ, Abate ML, Bugianesi E, Adams LA, Najim MA, Miele L, Weltman M, Mollison L, Cheng W, Riordan S, Fischer J, Romero-Gomez M, Spengler U, Nattermann J, Rahme A, Sheridan D, Booth DR, McLeod D, Powell E, Liddle C, Douglas MW, van der Poorten D, George J; International Liver Disease Genetics Consortium. Diverse impacts of the rs58542926 E167K variant in TM6SF2 on viral and metabolic liver disease phenotypes. *Hepatology* 2016; **64**: 34-46 [PMID: 26822232 DOI: 10.1002/hep.28475]

81 **Kim KH**, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, Kim HH, Yang US, Yu DY, Cheong J. Hepatitis B virus X protein induces hepatic steatosis *via* transcriptional activation of SREBP1 and PPARgamma. *Gastroenterology* 2007; **132**: 1955-1967 [PMID: 17484888 DOI: 10.1053/j.gastro.2007.03.039]

82 **Wu YL**, Peng XE, Zhu YB, Yan XL, Chen WN, Lin X. Hepatitis B Virus X Protein Induces Hepatic Steatosis by Enhancing the Expression of Liver Fatty Acid Binding Protein. *J Virol* 2016; **90**: 1729-1740 [PMID: 26637457 DOI: 10.1128/jvi.02604-15]

83 **Zhang S**, Gao S, Zhao M, Liu Y, Bu Y, Jiang Q, Zhao Q, Ye L, Zhang X. Anti-HBV drugs suppress the growth of HBV-related hepatoma cells *via* down-regulation of hepatitis B virus X protein. *Cancer Lett* 2017; **392**: 94-104 [PMID: 28192212 DOI: 10.1016/j.canlet.2017.02.003]

84 **Hsu CS**, Liu WL, Chao YC, Lin HH, Tseng TC, Wang CC, Chen DS, Kao JH. Adipocytokines and liver fibrosis stages in patients with chronic hepatitis B virus infection. *Hepatol Int* 2015; **9**: 231-242 [PMID: 25788201 DOI: 10.1007/s12072-015-9616-2]

85 **Bedossa P**; FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. *Hepatology* 2014; **60**: 565-575 [PMID: 24753132 DOI: 10.1002/hep.27173]

86 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

87 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]

88 and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. *Hepatology* 1994; **20**: 15-20 [PMID: 8020885]

89 **Zhuang Z**, Qu H, Yang W, Liu J, Wang F, Liu Y, Ding J, Shi J. Comparing hepatic steatosis distribution patterns between non-alcoholic fatty liver disease and fatty liver disease with chronic hepatitis B by second-harmonic generation/two-photon excited fluorescence method. *Ann Hepatol* 2020; **19**: 313-319 [PMID: 31870745 DOI: 10.1016/j.aohep.2019.11.003]

90 **Worland T**, Apostolov R, Asadi K, Leung C. Hepatitis B virus activity is not associated with degree of liver steatosis in patients with hepatitis B-related chronic liver disease. *Liver Int* 2020; **40**: 1500-1502 [PMID: 31883410 DOI: 10.1111/Liv.14344]

91 **U S. Food and Drug Administration (FDA) Guidance for Industry**. Noncirrhotic nonalcoholic steatohepatitis with liver fibrosis: developing drugs for treatment. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER). 2018. [cited5 February2021] Available from:https://www.federalregister.gov/documents/2018/12/04/2018-26333/noncirrhotic-nonalcoholic-steatohepatitis-with-liver-fibrosis-developing-drugs-for-treatment-draft

92 **Anania FA**, Dimick-Santos L, Mehta R, Toerner J, Beitz J. Nonalcoholic Steatohepatitis: Current Thinking From the Division of Hepatology and Nutrition at the Food and Drug Administration. *Hepatology* 2021; **73**: 2023-2027 [PMID: 33340111 DOI: 10.1002/hep.31687]

93 **Eslam M**, Sanyal AJ, George J. Toward More Accurate Nomenclature for Fatty Liver Diseases. *Gastroenterology* 2019; **157**: 590-593 [PMID: 31158374 DOI: 10.1053/j.gastro.2019.05.064]

94 **Eslam M**, Sarin SK, Wong VW, Fan JG, Kawaguchi T, Ahn SH, Zheng MH, Shiha G, Yilmaz Y,

Gani R, Alam S, Dan YY, Kao JH, Hamid S, Cua IH, Chan WK, Payawal D, Tan SS, Tanwandee T, Adams LA, Kumar M, Omata M, George J. The Asian Pacific Association for the Study of the Liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. *Hepatol Int* 2020; **14**: 889-919 [PMID: 33006093 DOI: 10.1007/s12072-020-10094-2]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 3984-4003

DOI: 10.3748/wjg.v27.i26.3984

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Acute kidney injury and hepatorenal syndrome in cirrhosis

Kapil Gupta, Abhishek Bhurwal, Cindy Law, Scott Ventre, Carlos D Minacapelli, Savan Kabaria, You Li, Christopher Tait, Carolyn Catalano, Vinod K Rustgi

ORCID number: Kapil Gupta 0000-0002-0872-7867; Abhishek Bhurwal 0000-0002-3886-7537; Cindy Law 0000-0002-2188-1340; Scott Ventre 0000-0002-9476-1788; Carlos D Minacapelli 0000-0001-7295-0249; Savan Kabaria 0000-0002-6347-6335; You Li 0000-0001-8758-0770; Christopher Tait 0000-0002-2663-2191; Carolyn Catalano 0000-0002-7793-0332; Vinod K Rustgi 0000-0002-4105-5783.

Author contributions: All authors contributed to the research, writing, and editing of the submitted document.

Conflict-of-interest statement: There are no relevant financial disclosures or conflicts of interest for all authors.

Kapil Gupta, Abhishek Bhurwal, Cindy Law, Carlos D Minacapelli, You Li, Christopher Tait, Carolyn Catalano, Vinod K Rustgi, Division of Gastroenterology and Hepatology, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ 08901, United States

Scott Ventre, Savan Kabaria, Department of Internal Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ 08901, United States

Corresponding author: Vinod K Rustgi, MD, Professor, Division of Gastroenterology and Hepatology, Rutgers Robert Wood Johnson Medical School, 1 Robert Wood Johnson Place Medical Education Building, Rm # 479, New Brunswick, NJ 08901, United States. vr262@rwjms.rutgers.edu

Abstract

Acute kidney injury (AKI) in cirrhosis, including hepatorenal syndrome (HRS), is a common and serious complication in cirrhotic patients, leading to significant morbidity and mortality. AKI is separated into two categories, non-HRS AKI and HRS-AKI. The most recent definition and diagnostic criteria of AKI in cirrhosis and HRS have helped diagnose and prognosticate the disease. The patho-physiology behind non-HRS-AKI and HRS is more complicated than once theorized and involves more processes than just splanchnic vasodilation. The common biomarkers clinicians use to assess kidney injury have significant limitations in cirrhosis patients; novel biomarkers being studied have shown promise but require further studies in clinical settings and animal models. The overall management of non-HRS AKI and HRS-AKI requires a systematic approach. Although pharmacological treatments have shown mortality benefit, the ideal HRS treatment option is liver transplantation with or without simultaneous kidney transplantation. Further research is required to optimize pharmacologic and nonpharmacologic approaches to treatment. This article reviews the current guidelines and recommendations of AKI in cirrhosis.

Key Words: Acute kidney injury; Hepatorenal syndrome; Liver cirrhosis; Treatment; Biomarkers; Prognosis

Core Tip: This review paper is a comprehensive review of acute kidney injury in

Manuscript source: Unsolicited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: United States

Peer-review report's scientific quality classification

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

Received: January 27, 2021
Peer-review started: January 27, 2021
First decision: March 7, 2021
Revised: March 19, 2021
Accepted: June 22, 2021
Article in press: June 22, 2021
Published online: July 14, 2021

P-Reviewer: Bramhall SR, Wu ZQ
S-Editor: Fan JR
L-Editor: A
P-Editor: Liu JH

cirrhosis as well as hepatorenal syndrome. We review the most current topics including diagnosis, current definitions, pathophysiology, novel biomarkers, treatment, pharmacology, nonpharmacologic treatment, and topics of further research.

Citation: Gupta K, Bhurwal A, Law C, Ventre S, Minacapelli CD, Kabaria S, Li Y, Tait C, Catalano C, Rustgi VK. Acute kidney injury and hepatorenal syndrome in cirrhosis. *World J Gastroenterol* 2021; 27(26): 3984-4003
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/3984.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.3984

INTRODUCTION

Acute kidney injury (AKI) is a relative decrease in a kidney's glomerular kidney function (GFR) and frequently occurs in patients. The incidence of AKI ranges from 20%-50% in cirrhotic patients when hospitalized for acute decompensation[1-6]. AKI imparts significant morbidity and mortality in patients with liver cirrhosis. Hospitalized cirrhotic patients have a high mortality rate, both inpatient and post-discharge [7]. Cirrhosis itself is a complex disease process that causes significant morbidity due to substantial volume shifts and increased vasodilation. Renal dysfunction, therefore, imparts another layer of complexity to those with cirrhosis and must be considered when a patient is being evaluated for liver transplantation (LT)[8].

Renal function is a weighted parameter in the Model for End-Stage Liver disease (MELD) score[9,10]. By accounting for creatinine, the MELD score allows patients with renal failure (acute or chronic) to receive liver transplants promptly[9,10]. Renal disease is an increasing health care burden in the United States as there has been a rise in the prevalence and incidence of type II DM and obesity along with chronic liver disease. Rustgi *et al*[11] calculated the additional cost of chronic kidney disease (CKD) in chronic liver disease patients by stage[11].

In the 1960s, Hecker and Sherlock described the process of renal dysfunction with the presence of ascites in advanced cirrhosis and defined it as hepatorenal syndrome (HRS)[12,13]. HRS is renal dysfunction resulting from systemic hemodynamic effects of portal hypertension secondary to liver cirrhosis[12], AKI in liver cirrhosis has been separated into non-HRS-AKI and HRS. The latter has been subdivided into type 1 HRS, known more recently as HRS-AKI, or type 2 HRS, known as HRS-CKD. The current recommendations and literature involving AKI and HRS in patients with liver cirrhosis are reviewed here.

DIAGNOSIS (NON-HRS-AKI)

The definition of HRS relies first and foremost on the definition of AKI. The definition of AKI has evolved. The first challenge has been determining the most accurate and available renal function measurement, which is the calculation of GFR. There is, however, no consensus on the most accurate method to measure GFR. Traditionally, the definition of AKI has been based on urine output and serum creatinine (sCr). The diagnosis of AKI is dependent on the patient's baseline sCr. The International Club of Ascites (ICA) defines a baseline sCr as the last sCr within three months of current sCr [14].

The definition of AKI historically has gone through many updates as enumerated in Table 1[14-17]: Given the complexity of cirrhosis, AKI in cirrhosis needed its definition with specific criteria. In 2004, AKI was defined by the Acute Dialysis Quality Initiative (ADQI) group using the RIFLE criteria and divided into the three stages (stage 1 or R, stage 2 or I, or stage 3 or F)[15]. Further updates by the AKI Network (AKIN) and Kidney Disease Improving Global Outcomes (KDIGO), which labeled the stages 1-3 [14-17]. Numerous consensus definitions have defined AKI. KDIGO is the most recent consensus definition for AKI that was updated in 2012[17]. In 2010, the ADQI with the ICA defined criteria for AKI in liver cirrhosis as shown in Table 2[18-20].

The guidelines were again updated in 2015 by the ICA to adopt the 2012 KDIGO definition of AKI. The benefit of the KDIGO criteria over the AKIN criteria for AKI is removing the absolute creatinine value of at least 1.5 mg/dL as a requirement, sCr in

Table 1 A brief overview of the consensus definitions of acute kidney injury

Criteria	Stage	Definition
RIFLE criteria/ADQI in 2004[15]		At least 1.5 × baseline serum creatinine within 7 d, decrease in urine output of 0.5 mL/kg/h for 6 h, decrease in GFR of at least 25%
	Stage 1 (R)	1.5 × baseline Cr, GFR decrease of 25%, UOP < 0.5 mL/kg/h for 6-12 h.
	Stage 2 (I)	2 × baseline serum creatinine, decrease of GFR < 50%, UOP < 0.5 mL/kg/h for 12 h
	Stage 3 (F)	3 × baseline serum creatinine, decrease of GFR of 75%, UOP < 0.3 mL/kg/h for 24 h, anuria for 12 h, or on RRT acutely
Acute Kidney Injury Network (AKIN) in 2007 [16]		Definition: increase of at least 0.3 mg/dL in last 48 h, 1.5 × baseline creatinine in last 48 h, or UOP < 0.5 mL/kg/h for at least 6 h
	Stage 1	Increase of 0.3 mg/dL w/in 2 d, 1.5-2 × baseline serum creatinine within 2 d, or UOP < 0.5 mL/kg/h for 6-12 h
	Stage 2	2-3 × baseline serum Cr, UOP < 0.5 mL/kg/h for at least 12 h
	Stage 3	3 × baseline serum Cr, UOP < 0.3 mL/kg/h for 24 h, anuria for 12 h, on RRT
Kidney Disease Improving Global Outcomes (KDIGO) in 2012[17]		Increase in sCr of at least 0.3 mg/dL within 48 h, increase of at least 1.5 × baseline in the last 7 d, or urine output < 0.5 mL/kg/h for at least 6 h
	Stage 1	Increase of 0.3 mg/dL, 1.5-2 × baseline Cr, UOP < 0.5 mL/kg/h for 6-12 h
	Stage 2	2-3 × baseline serum Cr or UOP < 0.5 mL/kg/h for at least 12 h
	Stage 3	3 × baseline serum Cr, increase of 0.5 mg/dL above absolute level of 4.0 mg/dL, on RRT, UOP < 0.3 mL/kg/h for 24 h, or 12 h of anuria

GFR: Glomerular kidney function; UOP: Urine output; RRT: Renal replacement therapy; ADQI: Acute Dialysis Quality Initiative; Cr: Creatinine.

Table 2 The current and past consensus definitions of acute kidney injury in cirrhosis

Criteria	Stage	Definition
ADQI/ICA in 2010[19]		The absolute increase in serum Cr of at least 0.3 mg/dL or 1.5 × baseline serum creatinine
	Stage 1	Increase of 0.3 mg/dL within 48 h or 1.5-2 × baseline serum creatinine
	Stage 2	Increase of 2-3 × baseline serum Cr
	Stage 3	At least 3 × baseline serum Cr with an increase of 0.5 mg/dL or currently on RRT
ICA-AKI in 2015[14]		An absolute increase in serum Cr of at least 0.3 mg/dL within 48 h or 1.5 × baseline Cr level within the last 7 d
	Stage 1A	Increase of 0.3 mg/dL from baseline in 48 h, 1.5-2 × baseline serum creatine. Absolute value of serum Cr < 1.5 mg/dL
	Stage 1B	Increase of 0.3 mg/dL from baseline in 48 h, 1.5-2 × baseline serum creatine. Absolute value of serum Cr > 1.5 mg/dL
	Stage 2	Increase of 2-3 × baseline
	Stage 3	Greater than 3 × baseline Cr, Cr > 4 mg/dL with rise of > 0.5, or on RRT

RRT: Renal replacement therapy; ADQI: Acute Dialysis Quality Initiative; ICA: International Club of Ascites; AKI: Acute kidney injury; Cr: Creatinine.

patients with cirrhosis may underestimate renal dysfunction due to low baseline muscle mass[14]. However, in staging AKI, as stressed by Angeli *et al*[14], the absolute level of 1.5 mg/dL was used to differentiate between stage 1-A and stage 1-B[14], as shown in Table 2. The new ICA criteria emphasize the importance of having a baseline sCr for making the diagnosis and allow for a prior sCr within three months to be considered a baseline[14].

DIAGNOSIS (HRS)

HRS is defined as renal dysfunction in chronic liver disease (usually severe or advanced cirrhosis) or acute liver failure[1,8,14]. HRS has primarily considered a

diagnosis of exclusion with specific criteria explained in Table 3, and its two types are generally differentiated by disease course. However, it may be challenging to differentiate from acute tubular necrosis (ATN). Table 3 lists the definitions of HRS types 1 and 2[21]. Type 1 and 2 HRS were renamed HRS-AKI and HRS-CKD in 2015. The most significant difference between the prior diagnosis of HRS type 1 and HRS-AKI has been eliminating an absolute sCr level of 2.5 mg/dL[21-23].

PATHOPHYSIOLOGY OF HRS

HRS has been theorized to be caused by various mechanisms. The most well-understood hypothesis evokes splanchnic vasodilation changes, leading to increased peripheral vasoconstriction[24,25]. Additionally, there is evidence for other processes. Hepatocytes and stellate cells are known to produce vasodilatory mediators, including nitric oxide, prostacyclin, carbon monoxide, endogenous cannabinoids, adrenomedullin[1,8,26-28]. The destruction of hepatocytes leads to an increased release of these products into the splanchnic circulation, resulting in significant arterial vasodilation. This, in turn, decreases the systemic mean arterial pressure, causing compensatory activation of the sympathetic nervous system resulting in the consistent release of norepinephrine, angiotensin II and antidiuretic hormone[8,26-31]. These processes trigger unopposed vasoconstriction in the renal arteries *via* multiple physiologic mechanisms to counteract the splanchnic vasodilation and preserve renal function. As cirrhosis progresses, the systemic vascular resistance is decreased to the point that an increase in cardiac output cannot compensate adequately to maintain adequate organ perfusion[8,25] (Figure 1). This phenomenon is described as cirrhotic cardiomyopathy, directly related to sustained portal hypertension[1,32,33]. The possibility of spontaneous bacterial peritonitis (SBP) must be accounted for every time a patient is treated for AKI[2,34,35].

PATHOPHYSIOLOGY OF NON-HRS AKI

The typical forms of non-HRS-AKI include prerenal azotemia (PRA), parenchymal renal disease, and drug-induced kidney injury. Prerenal AKI accounts for up to 60% of all AKI cases in patients with cirrhosis[2,34]. The most common causes of AKI in cirrhosis are hypovolemia, SBP, bacterial infections (other than SBP), sepsis, upper gastrointestinal bleeding, and shock. Infections and sepsis (urinary tract infections, pneumonia, skin infections, or SBP) cause decreased blood flow to the renal vasculature and cause kidney injury for cirrhosis patients who are already susceptible to volume shifts[3-5,36,37]. Frequent large-volume paracentesis can cause hypovolemia, exacerbated by increased third spacing and hemodynamic instability[7]. Gastrointestinal bleeding also causes hypovolemia and is commonly implicated in renal dysfunction[3-5,36,37]. Common drugs which can contribute to AKI in cirrhosis are diuretics and laxatives, particularly lactulose. Intrinsic renal dysfunction is present in around 30% of AKI cases in cirrhosis[34,35]. Intrinsic renal disease plays a role in AKI as well. Many of the insults that affect liver function and are common etiologies in cirrhosis can lead to acute and chronic kidney disease. These can include autoimmune disease, medications, hepatitis B infection, and hepatitis C infection[7].

There are cirrhosis-specific mechanisms that also contribute to non-HRS AKI. Hepatic inflammation has been well-described in the literature for contributing to non-HRS AKI[12,38]. In the setting of cirrhosis or chronic liver disease, inflammation may be the result of damage-associated molecular patterns (DAMPs) in hepatocytes and gut immunity weakening from pathogen-associated molecular patterns (PAMPs)[12,39]. DAMPs specific to the liver include interleukin (IL)-1, IL-33, and bile acids recognized by the Kupfer cells' toll-like receptors[12,40]. Gut bacterial translocation has been associated with the release of PAMPs (*e.g.*, lipopolysaccharide), or DAMPs (*e.g.*, heat shock proteins), from a cirrhotic liver leading to a systemic inflammatory response which can lead to the development of non-HRS AKI[12,41-45] (Figure 1).

Adrenal insufficiency is also frequently present in patients with cirrhosis. A retrospective study by Moini *et al*[46] evaluated 105 cirrhotic patients and reported that 15% of cirrhotic patients had some degree of adrenal insufficiency and identified hyponatremia and elevated international normalized ratio as risk factors for its development[46,47]. These processes can decrease glucocorticoids' synthesis and result in adrenal insufficiency[48]. Inadequate adrenal response subsequently alters cardiovascular hemodynamics through vascular tone changes and cardiac output

Table 3 The previous and current definition and nomenclature of hepatorenal syndrome[14,19,21-23]

	Previous and current definition and nomenclature
Criteria to confirm of HRS *vs* other etiology of renal dysfunction	To diagnose HRS, patients must have: (1) The presence of ascites; (2) No improvement of creatinine after holding diuretics; (3) No improvement after 48 h of albumin supplementation (1 g/kg/d); (4) No signs of shock; (5) No recent nephrotoxic medications (antibiotics, contrast, NSAIDs); and (6) No signs of kidney disease (proteinuria, microhematuria, no findings on renal ultrasound)
HRS type 1 (most recent definition in 2007)	Rapid renal injury (within two weeks) defined by 2 × baseline serum creatinine to a value > 2.5 mg/dL or 50% reduction in creatinine clearance
HRS type 2	Moderate renal failure with creatinine ranging from 1.5 to 2.5 mg/dL that occurs progressively
Definition of HRS-AKI	Patients with the criteria above and ICA-AKI 2015 definition for AKI
Definition of HRS-CKD	Patients who meet the criteria in row 1 and the rise of serum creatinine and changes in urine output are all progressive (> 1 wk)
	Patients with HRS-CKD are known to have decreased urine output over weeks to months

ICA: International Club of Ascites; AKI: Acute kidney injury; HRS: Hepatorenal syndrome; CKD: Chronic kidney disease; NSAID: Non-steroidal anti-inflammatory drug.

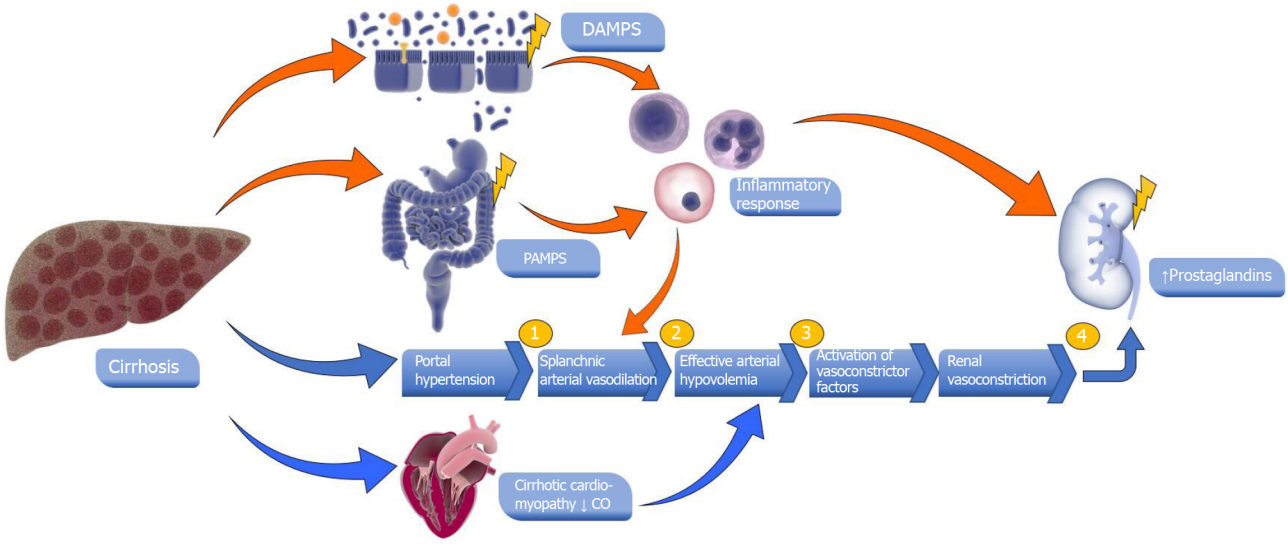

Figure 1 Pathogenesis of hepatorenal syndrome and acute kidney injury in cirrhosis. (1) Patients with cirrhosis present with a marked splanchnic arterial vasodilation due to portal hypertension; (2) Splanchnic vasodilation causes a decrease in systemic vascular resistance leading to effective arterial hypovolemia; (3) There is activation of endogenous vasoconstrictors such as the renin-angiotensin-aldosterone system, sympathetic nervous system and arginine vasopressin; and (4) The activation of these systems leads to renal vasoconstriction inducing a decrease in glomerular filtration rate and development of hepatorenal syndrome. A decrease in cardiac output may contribute to a decrease in effective arterial blood volume. Pathogen-associated molecular patterns and damage-associated molecular patterns, derived from bacterial translocation and from injured liver, may activate circulating innate immune cells, leading to an inflammatory response. The Inflammatory mediators may lead to impairment of circulatory dysfunction and consequently, kidney tissue damage. Library of Science & Medical Illustrations were utilized in part to create this figure (https://creativecommons.org/Licenses/by-nc-sa/4.0/). DAMPs: Damage-associated molecular patterns; PAMPs: Pathogen-associated molecular patterns.

leading to decreased renal perfusion[46].

In patients with nonalcoholic steatohepatitis (NASH), studies have shown that around 28% have worsened renal function[46,49]. Patients with NASH/nonalcoholic fatty liver disease (NAFLD) and CKD have been shown to alter the renin-angiotensin system[46,50]. In patients with metabolic syndrome and NAFLD, alterations in the renin-angiotensin system with increased renin/angiotensin II receptor activation (from increased activation of angiotensin-converting enzyme-2) have been linked to hepatic steatosis, fibrosis and leading to NASH cirrhosis. This same process is well established to cause physiologic changes in the kidney, such as efferent artery vasoconstriction, which initially causes glomerular hyperfiltration and leads to hypertrophy with eventual scarring[46,50]. Other mechanisms in patients with NASH cirrhosis include 5′AMP-activated protein kinase activation, lipoprotein dysmetabolism, and oxidative damage through downregulation of sirtuin-1[46,51,52]. Patients with NAFLD/NASH will have comorbidities such as hypertension and diabetes mellitus and are highly

susceptible to AKI[34,35].

In viral hepatitis, the most common kidney injury mechanism involves creating immune complexes with the virus, antibodies against infected hepatocytes, or direct cytopathic impact[46,53]. Hepatitis B infection is associated with polyarteritis nodosa (PAN), membranous nephropathy, and membranoproliferative glomerulonephritis[54,55]. Pathologically, renal biopsies generally reveal immune complex deposition, particularly hepatitis B envelope antigen in membranous nephropathy[55]. Chronic hepatitis C infections are also often linked with glomerular disease. The most common renal dysfunction causes include mixed cryoglobulinemia, PAN, and membranous nephropathy[56].

BIOMARKERS

Early recognition of AKI and accurate measurement of renal function in cirrhosis is crucial when treating patients. Still, AKI can often be missed due to the baseline abnormalities present in patients with cirrhosis. Urine output is not an accurate measurement of a patient's renal function or GFR in cirrhosis. Third-spacing causes urine output to drop, which underestimates renal function. At the same time, diuretic use may lead to an overestimation of renal function.

The most frequently used laboratory value to measure GFR is sCr because it is readily available, inexpensive, and accurate[57-60]. However, sCr has many factors that influence its value, such as race, age, gender, and muscle mass[18,60]. In cirrhosis, patients are malnourished, cachectic, and sarcopenic, leading to a deficiency in protein intake and is associated with muscle wasting[61]. These patient-specific factors are why creatinine may be lower in cirrhotic patients leading to an overestimation of GFR and renal function. Another factor leading to inaccuracy in creatinine correlating with GFR is that hyperbilirubinemia affects Jaffe's kinetic assay that measures sCr and leads to an inaccurately low measurement[18,59].

sCr remains the primary measurement of renal function in cirrhosis because the use of novel biomarkers remains experimental[59]. Urinary sodium and the fractional excretion of sodium (FeNa) have only been used as an adjunct to sCr to help diagnose HRS and PRA[23].

NOVEL BIOMARKERS

Given that sCr may not evaluate the degree or the timing of AKI promptly, novel biomarkers with promise are being evaluated[59,62]. Cystatin C is a low-molecular-weight protein that is produced by all nucleated cells. It is filtered by the glomerulus and mainly reabsorbed by the proximal tubule[63]. Cystatin C testing is less readily available and is more expensive. Despite the limitations, cystatin C is not affected by age, muscle mass, malignancy, or inflammation[64,65]. The assay, unlike sCr, is not affected by high levels of serum bilirubin[66]. Prior studies have not had sufficient evidence of superiority for cystatin C in comparison to Cr. However, combination equations of Cr and cystatin C are superior to sCr[64,65]. Cystatin C is an independent predictor of AKI and outcomes, including mortality[67,68]. Other biomarkers of interest include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), IL-18, and liver-type fatty acid-binding protein (L-FABP)[18,59,69]. The biomarkers' clinical benefits and limitations are described in Table 4.

NGAL is a small protein made by the kidney, lung, stomach, and colon[70,71]. Using mouse and rat models, Mishra *et al*[70] in 2003 demonstrated that NGAL was upregulated in prerenal AKI and ATN setting and that increased urinary NGAL could be detected within 2 h of initial renal injury[70]. Multiple studies have evaluated the efficacy and utility of urinary NGAL in cirrhotic patients with AKI. When urinary NGAL was used to define and predict morbidity in AKI, the authors concluded that urinary NGAL levels were elevated in ATN compared to PRA or HRS-AKI. However, the most significant confounder in its utility is the overlap between ATN's lower values and HRS's upper values or PRA[18,72-75]. Two studies had found that urinary NGAL was superior to cystatin C in utility for diagnosis of AKI or ATN[75,76]. In contrast, Barreto *et al*[74] studied 132 cirrhotic patients hospitalized with infections. The authors found that among patients with persistent AKI, HRS-AKI could be accurately predicted with urinary NGAL values lower than 86 μg/g creatinine in 88% of patients[74]. In a study with 55 patients, Lee *et al*[77] found that urinary NGAL levels were significantly higher in ATN than HRS and PRA. Also, median urinary

Table 4 The most well-known novel biomarkers being studied for acute kidney injury in cirrhosis

Novel biomarker	Source	Benefits/Clinical uses	Limitations
Cystatin C[62-68]	Plasma, urine	Early biomarker of AKI, potential benefit with severity of disease. Unaffected with age, sarcopenia, gender, or sepsis. Unaffected by malignancy and serum bilirubin level. Multiple studies found it to be an independent risk factor of AKI and mortality	Increased levels in CKD. Influenced by low levels of albumin. Potentially influenced by elevated WBC and CRP. Takes longer time to result when compared to sCr
NGAL[18,67-79]	Urine	Found in kidney tubular cell that is released during damage or injury. Elevated in AKI in cirrhosis and potential predictor of mortality. Markedly elevated in ATN, mildly elevated in prerenal azotemia/CKD/HRS-AKI	Increased levels in CKD. Increased levels in infections, particularly urinary tract infections. Overlap with values in PRA, HRS, and other AKI types of AKI. Small quantities are made in the liver
IL-18[75,78,82-84]	Urine	Very similar to urinary NGAL. Markedly elevated in cirrhotic patients with ATN, in comparison to other AKI types. Found in monocytes and macrophages. A notable proinflammatory marker. Not confounded by CKD, sepsis or UTI	There are increased levels in PRA and HRS but significant overlap in values with limited clinical utility. Levels are increased in levels of inflammation in the kidney other than AKI
Kidney Injury Molecule-1[18,73,84-86]	Urine	Originally found in kidney tubular transmembrane protein. Not expressed in normal kidney tissue. Noted with increased levels in ATN in cirrhosis when compared to the other types of AKI in cirrhosis. High specificity for ischemic or nephrotoxic kidney injury	Elevated from inflammatory conditions. Found to have overlap between different forms of AKI. Confounded by presence of infection
L-FABP[87-93]	Urine	Found in kidney proximal tubule. Levels may be increased in AKI or AKI 2/2 sepsis. Potential utility in predictor in adverse outcomes including AKI in patients with chronic liver disease and other liver disease	Limited studies in cirrhosis. Found to be increased in CKD. Increased in acute liver injury and liver failure as well

AKI: Acute kidney injury; HRS: Hepatorenal syndrome; CKD: Chronic kidney disease; ATN: Acute tubular necrosis; UTI: Urinary tract infection; NGAL: Neutrophil gelatinase-associated lipocalin; PRA: Prerenal azotemia; CRP: C-reactive protein; WBC: White blood cell; sCr: Serum creatinine; IL: Interleukin.

NGAL levels in HRS were markedly different from PRA levels, and the authors found that NGAL was an independent risk factor for mortality with AKI[77]. Jaques *et al*[67] studied multiple biomarkers in AKI in 55 decompensated cirrhosis patients. Compared to the non-AKI patients, they found that urinary NGAL levels are higher in ATN than PRA and HRS. However, HRS urinary NGAL levels had an intermediate pattern[67]. Urinary NGAL predicted poor outcomes in patients as well[67]. Kim *et al*[68] studied urinary NGAL and cystatin C in 328 decompensated cirrhosis patients (41 patients with AKI). The authors found that urinary NGAL is a predictor of AKI and outcomes (including mortality)[68]. Recently, Huelin *et al*[78] studied urinary NGAL and IL-18 on 320 cirrhosis patients with AKI. Urinary NGAL was elevated in AKI progression during hospitalization and was predictive of AKI progression in conjunction with MELD score. Urinary NGAL was significantly elevated in ATN when compared to hypovolemia-induced AKI and HRS-AKI[78]. Currently, there are no definitive diagnostic thresholds for differentiation between these types of AKI[79-81]. Urinary NGAL does not have an established role in the diagnosis, prediction, or prognosis of AKI in cirrhosis, but more promising results in extensive studies may change that. Another significant limitation is the expense of the test.

IL-18 is a proinflammatory cytokine expressed in the proximal tubule. It is released in urine when the cells are damaged in AKI[75]. Urinary IL-18 is elevated in patients with AKI, especially from ischemic injury, but urinary IL-18 is not elevated in conditions such as urinary tract infections, nephrotoxic injury, and CKD[75,82,83]. Tsai *et al*[84] in 2013 evaluated the clinical outcomes of 168 cirrhotic patients with AKI and severe sepsis. They found that urinary IL-18 was significantly higher in patients with ATN than patients with functional AKI, proposing a cutoff of 708.5 pg/mg creatinine to differentiate between the two groups. Urinary IL-18 was found to be a stronger predictor of ATN than serum IL-18. However, the authors were unable to conclude if urinary IL-18 could distinguish ATN from HRS-AKI. Clinically, they found that elevated urinary IL-18 was associated with higher hospital mortality[84]. Huelin *et al*[78], a study previously mentioned, studied IL-18 compared to urinary NGAL and found that it had a lower accuracy to predict ATN *vs* other forms of AKI[78].

KIM-1 is elevated in AKI from ischemic injury to the proximal tubule[83,84]. Belcher *et al*[73] evaluated KIM-1 in patients with AKI with other etiologies (PRA, ATN, and HRS) and found that ATN was the most elevated with overlap with HRS[73]. Other studies found that in patients with cirrhosis, elevations in urinary KIM-1 levels were increased mainly in ATN compared to other AKI presentations and could serve as a prognostic indicator[73,85,86].

L-FABP is a small protein found in the proximal tubular epithelium and binds to free fatty acids when reabsorbed in the proximal tubule[87]. L-FABP may be elevated in sepsis and specific etiologies of CKD (diabetic nephropathy or glomerulonephritis) [88]. Yamamoto *et al*[89] studied L-FABP in animal and human models (12 kidney transplant patients) in response to AKI[89]. The authors reported an increase in levels of L-FABP in mice models with prolonged exposure to ischemia to the kidneys, particularly during ischemic reperfusion injury. Doi *et al*[90] evaluated urinary L-FABP in 145 mice and 145 septic shock patients with AKI. L-FABP was high in septic shock patients with AKI and higher in the patients who did not survive[90]. L-FABP has been studied in acute liver failure and chronic liver disease and not just HRS and AKI in cirrhosis[91]. In patients with acetaminophen included acute liver failure, serum L-FABP levels were lower in survivors when compared to patients who passed away [92]. Eguchi *et al*[93] studied L-FABP in 242 chronic liver disease patients (chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma). The authors found that serum L-FABP increased in liver cirrhosis compared to chronic hepatitis and is higher in the presence of hepatocellular carcinoma. L-FABP correlates with kidney function markers, especially BUN, creatinine, and GFR[93]. This study does show the potential for L-FABP in chronic liver disease and other complications, including AKI. Serum L-FABP may have many clinical utilities in acute and chronic liver disease, including AKI; however, more large-scale studies should be performed to ascertain exact clinical utility.

Two new biomarkers being studied for potential benefits are insulin-like growth factor binding protein-7 and tissue matrix metalloproteinase inhibitor-2. However, there is not enough evidence to note potential utility. They are only approved for evaluating AKI in patients with intensive care unit (ICU) and need further evaluation [94]. Novel biomarkers can differentiate both the degree of renal dysfunction and possible etiology, but the data are not substantial enough to currently recommend utility. Additionally, these tests are not readily available and are expensive methods to evaluate renal function.

TREATMENT (INITIAL TREATMENT OF AKI IN CIRRHOSIS)

In AKI injury, clinicians must recognize and intervene as soon as possible. In patients with cirrhosis, all factors possibly contributing to AKI must be recognized promptly [14,20,37,95]. All unnecessary nephrotoxic medications such as Non-steroidal anti-inflammatory drugs should be discontinued and avoided altogether. Beta-blockers for variceal prophylaxis or other comorbidities should be evaluated for risk *vs* benefits[96, 97]. In patients with PRA or dehydration, diuretics should first be discontinued as excessive diuresis is a common cause of kidney dysfunction in cirrhosis patients[20]. Excessive diarrhea from high doses of lactulose is another potential cause[20]. Patients with gastrointestinal bleeding should be transfused if indicated. Patients should have screening for infectious etiology, and patients should be placed on antibiotics immediately along with appropriate volume supplementation if an infection is diagnosed[98-100].

Clinicians should attempt a trial of volume expansion for the patients, but crystalloid, colloid, or blood products are dependent on etiology and clinical judgment. If a patient requires large-volume paracentesis, 6-8 g of albumin *per* liter of fluid removed after 5 L should be administered.

Therapeutic response is defined as improving serum creatine to at least 0.3 mg/dL near the baseline. However, even with adequate improvement, patients should be screened frequently to prevent a recurrence. Recommendations currently include an initial screen 2 to 4 d after discharge with a 2-4 wk follow-up for the first six months after discharge[14,36]. Patients with stage 2 or 3 AKI should be suspected of HRS-AKI, and HRS-AKI management should be initiated. Figure 2 provides a brief algorithm that can be used when first approaching AKI in a cirrhotic patient.

TREATMENT (HRS-PHARMACOTHERAPY)

The patient meets the HRS criteria if there is no creatinine improvement after the withdrawal of all nephrotoxic agents and volume expansion with 1 g/kg/24 h for 48 h [14]. The patient should receive prompt pharmacologic therapy, which entails starting vasoconstrictor therapy with albumin supplementation to avoid cardiac output loss or loss of effective circulating volume[1,101]. The vasoconstrictors utilized for treatment

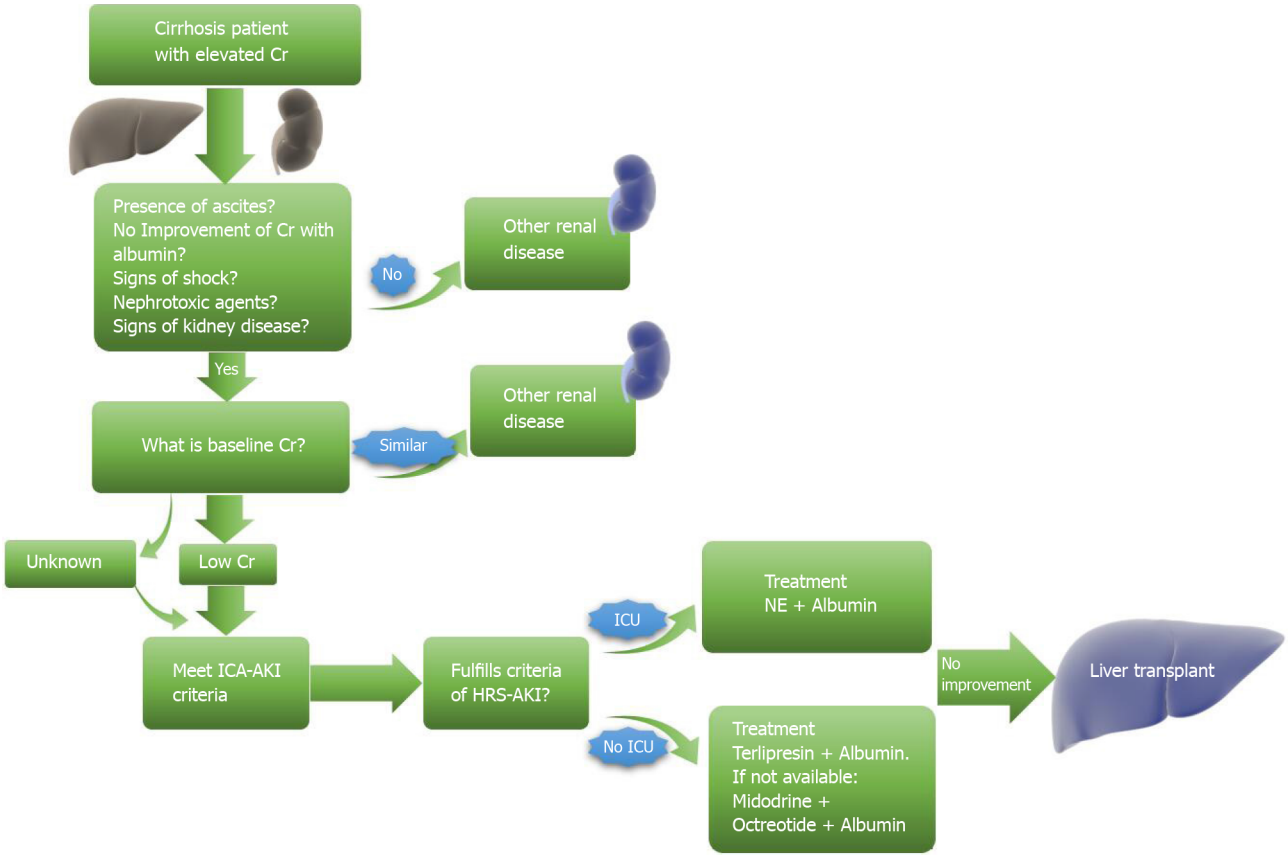

Figure 2 Algorithm of the diagnosis and treatment of hepatorenal syndrome. The algorithm indicates differential diagnosis, diagnosis of hepatorenal syndrome (HRS) and HRS treatment. Library of Science & Medical Illustrations were utilized in part to create this figure (https://creativecommons.org/Licenses/by-nc-sa/4.0/). Cr: Creatinine; ICA: International club ascites; AKI: Acute kidney injury; HRS: Hepatorenal syndrome; ICU: Intensive care unit; NE: Norepinephrine.

are terlipressin, noradrenaline, octreotide, and midodrine[102-106]. The treatment goal is cited to be a goal sCr of 1.5 mg/dL or less with a reduction of at least 50%.

Terlipressin has been the most extensively studied and has the most robust evidence of efficacy in treating HRS-AKI of the three vasoconstrictor therapies with known superiority to octreotide and midodrine[101-108]. Terlipressin is more effective with fewer adverse effects when given in continuous infusions than bolus administration [99-108]. Over the years, multiple trials proved the efficacy of terlipressin with albumin as an effective treatment of HRS type 1[101,103-105,108-113]. A recent phase 3 trial by Wong *et al*[114] studied 300 patients using terlipressin and albumin compared to the placebo group. They found a significant improvement of HRS versal and renal function but was significantly associated with adverse events, including respiratory failure[114]. Serious adverse effects include angina, dysrhythmia, hypertension, and peripheral ischemia (intestines, fingers, scrotum). Patients with ischemic cardiomyopathy or peripheral vascular disease should not be treated with terlipressin[110]. Currently, it is not available in the Unoited States.

Noradrenaline has alpha-adrenergic properties that promote vasoconstriction with fewer effects on contractility[111,115]. Patients treated with noradrenaline require central venous access and require close, frequent monitoring in the ICU[116]. In their prospective study, Gupta *et al*[117] found norepinephrine to be an effective treatment for HRS reversal in 30 patients[117]. Multiple randomized controlled trials (RCTs) have compared noradrenaline to terlipressin[102,111,118-121]. Alessandria *et al*[118], in their pilot unblinded RCT, evaluated 22 patients comparing terlipressin and noradrenaline. The difference in HRS reversal was 83% and 70%, respectively, but there was no mortality difference[118]. Singh *et al*[119], Sharma *et al*[102], and Goyal *et al*[121] evaluated noradrenaline *vs* terlipressin and found them to have comparable efficacy and safety to improve HRS renal function[102,119,121]. Liu *et al*[122], in a randomized, double-blinded trial with 617 patients with septic shock found no significant difference in 28-d mortality between terlipressin compared to noradrenaline[122]. These studies have bolstered the use of noradrenaline, which is less expensive and more readily available in most countries. Consequently, Arora *et al* [123] in an open-label RCT, found that terlipressin, when compared to noradrenaline,

showed significant improvement in the reversal of HRS (40% *vs* 16.7%), day 4 response (26.1% *vs* 11.7%), day 7 response (41.7% *vs* 20%) and in 28-d survival (48.3% *vs* 20%) [123].

The third vasoconstrictor therapy that is commonly used is midodrine in conjunction with albumin and octreotide. Midodrine is an alpha-adrenergic agonist that is frequently used in patients with orthostatic hypotension, and octreotide is a somatostatin analog that physiologically is meant to antagonize the primary pathophysiology of HRS[124,125]. In a pilot study, Angeli *et al*[124] evaluated the efficacy of octreotide, and midodrine found it to reverse HRS in around 40% of the patients with type 1 HRS[124]. It is recommended to utilize the regimen if terlipressin and noradrenaline are contraindicated or unavailable[116]. In 2009, Skagen *et al*[126], in a retrospective study, evaluated the use of octreotide, midodrine, and albumin in 75 patients and found that it improved short-term renal function and survival compared to the group who did not receive them[126].

Many patients, unfortunately, do not respond appropriately to pharmacologic therapy. After 14 d, all medications should be discontinued, and further nonpharmacologic treatment options must be considered.

TREATMENT (HRS-TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT)

Transjugular intrahepatic portosystemic shunt (TIPS) has been considered for the treatment of HRS, particularly HRS-AKI. Physiologically, treating portal hypertension should improve renal function in HRS; however, in practice, TIPS can cause transient ischemia to the liver, which can lead to acute on chronic liver failure. This may precipitate and worsen renal function in HRS, leading to increased mortality[1]. While several prospective studies have shown a significant benefit in renal function and mortality, they are limited by small size, lack of control groups, selection bias, and strict inclusion/exclusion criteria. The most extensive prospective study compared 31 transplant-ineligible patients with HRS (14 with HRS-AKI and 17 with HRS-NAKI) who underwent TIPS to 10 transplant-ineligible patients who did not undergo TIPS. The 3-mo survival rates were 81% for the group undergoing TIPS and 10% for the TIPS-ineligible group[127]. A 2018 meta-analysis of studies including 128 patients with HRS who underwent TIPS showed pooled 1-year survival rates of 47% in HRS-AKI patients and 64% in HRS-NAKI and renal improvement in 83% of patients[128]. While these results are certainly encouraging, randomized trials with adequate control groups are still lacking. Therefore, TIPS may be appropriate in specific clinical contexts but, at this time, is not routinely recommended in the treatment of HRS.

TREATMENT (HRS-RENAL REPLACEMENT THERAPY)

Renal replacement therapy (RRT) (hemodialysis) is not a treatment for HRS-AKI and is only meant to be a bridge for recovery of liver function or LT. RRT recommendations for cirrhosis patients are the same as for the general population (refractory volume overload, refractory electrolyte imbalance, refractory acidosis, uremia, or intoxication) [116]. Zhang *et al*[129], in a retrospective study, evaluated RRT in patients with HRS type 1 who did not respond to pharmacologic therapy. The study concluded that it did not improve mortality (30-d or 180-d survival)[129]. Patients who are not deemed transplant candidates are not considered candidates for RRT[130].

TREATMENT [HRS-LIVER REPLACEMENT THERAPY (ALBUMIN DIALYSIS)-MOLECULAR ADSORBENT RECYCLING SYSTEM]

A molecular adsorbent recirculating system (MARS) is a form of albumin dialysis which circulates albumin to remove cytokines and bacterial products to combat vasodilation[12]. A 2010 RCT with 189 patients with acute-on-chronic liver failure (50% had HRS AKI) revealed a statistically significant reduction in sCr compared to medical management. However, overall mortality in 28 d was not significantly different in patients with HRS AKI[131]. In 2013, a trial by Lavayssière *et al*[132] studied MARS and found that compared to a control, MARS was able to lower

bilirubin and sCr compared to the control group[132]. However, many studies did not show any significant improvement in creatinine or GFR after MARS. The RELIEF trial failed to show a statistically significant improvement in mortality compared to medical therapy[131]. Due to the equivocal results of all the trials evaluating MARS, the European Association for the Study of the Liver (EASL) does not recommend MARS for HRS treatment but suggested a further investigation into its potential benefits.

TREATMENT [HRS-LIVER REPLACEMENT THERAPY (ALBUMIN DIALYSIS)-BIOARTIFICIAL LIVER SUPPORT SYSTEMS]

Another approach studied to bridge patients with cirrhosis to transplant or recovery includes bioartificial liver support systems. Several types exist, but all generally involve integrating animal or human hepatocytes into a bioreactor to filter toxins. These technologies continue to be studied in both clinical and preclinical trials, showing some promise in acute liver failure[133]. However, large-controlled trials are needed to understand better their role in the treatment of AKI in patients with acute on chronic liver failure.

TREATMENT (HRS-PREVENTION)

Multiple studies have evaluated possible mechanisms to prevent HRS in patients from common causes. When treating infections in cirrhotic patients, there is evidence that albumin administration may have a protective role against HRS. The current recommendation to prevent HRS in SBP is albumin administration at a dosage of 1.5 g *per* kg on day 1 and 1 g *per* kg on day 3[134,135]. This albumin administration regimen has been found to reduce the incidence of HRS and overall mortality in SBP[134,136]. However, these results have not been replicated in other infections[136-138]. An RCT by Guevara *et al*[137] reported that renal function and circulatory function were significantly improved in the treatment group compared to the control with fewer cases of HRS type 1[137]. Another RCT by Thévenot *et al*[138] reported that albumin therapy delayed renal failure, but the 3-mo renal failure rate was not significantly improved. The authors cautioned using large amounts of albumin in critically ill cirrhotic patients[138]. SBP prophylaxis with norfloxacin has been studied and found to lower HRS incidence and improve survival[136,139].

TREATMENT (HRS-TRANSPLANTATION)

The only definitive treatment of HRS refractory to pharmacologic therapy is LT. The use of creatinine in the MELD score has demonstrated the increased importance for patients with renal dysfunction (HRS-AKI or HRS-CKD) to undergo LT. In the setting of HRS, Boyer *et al*[140] reported a survival advantage of 100% *vs* 34% in patients with HRS treated with terlipressin and LT compared to patients treated with terlipressin alone[140]. Although LT remains the only definitive treatment of HRS-AKI, the role of the liver and even simultaneous liver-kidney transplant (SLK) remains unclear in the setting of non-HRS-AKI. In a large retrospective study comparing survival in HRS-AKI patients after undergoing SLK *vs* cirrhotic patients with non-HRS-AKI undergoing the same, HRS-AKI patients' survival post-transplant was significantly superior to those in the non-HRS-AKI group[141].

The percentage of liver transplant recipients undergoing SLKs has substantially increased over the last 18 years. The increase in SLK is likely partly due to the adoption of the MELD score by the Unified Network for Organ Sharing in 2002. The MELD score places significant weight on sCr and imparts a high and increasingly higher transplant priority to progressive renal dysfunction patients. Guidelines for SLK, developed in 2012, were modified in 2017. For patients with cirrhosis and CKD, SLK was recommended for patients with epidermal GFR (eGFR) less than 60 mL/min for at least 90 d before listing or eGFR less than 35 mL/min during the time of listing or inherited metabolic disease[142]. In patients with cirrhosis and AKI, there must be a combination of dialysis and eGFR < 25 mL/min for six weeks[143].

PROGNOSIS

AKI in cirrhosis has a high mortality rate, with 26% of patients dying before discharge [7]. Multiple studies show that the disease course and prognosis of AKI in cirrhosis depend on numerous factors-etiology of kidney injury, multiorgan dysfunction, stage of AKI upon diagnosis and progression of AKI, and lack of response to treatment[7]. Jenq *et al*[144], using the RIFLE criteria, found mortality of 134 cirrhotic patients admitted to the ICU to be 32.1% without AKI, 68.8% with RIFLE-R, 71.4% with RIFLE-I, and 94.8% with RIFLE-F[144]. However, the results were not reliable as patients admitted to the ICU usually have multiorgan dysfunction. The AKI stage directly correlates with in-hospital mortality and post-transplant mortality. Wong *et al*[145] found that the 30-d mortality of patients who do not recover from AKI was 80% *vs* 15% for those who recover[145]. Huelin *et al*[146] in a cohort of 547 patients, found a 90-d transplant-free survival to be 84% with stage 1A AKI, 58% with stage 1B AKI, 48% with stage 2 AKI, and 43% with stage 3 AKI compared to 89% with patients without AKI[1,146]. Bucsics *et al*[147], in a 239-patient retrospective study in 2015, also found that the 30-d mortality increased with increased stage of AKI on diagnosis or progression[147]. Mortality with AKI is markedly increased with complications of cirrhosis, including hepatic encephalopathy and ascites. In a retrospective study, Mindikoglu *et al*[148] reviewed 6917 cirrhotic patients between 2004 to 2014 who developed AKI during hospitalization and were subsequently discharged, and the authors calculated a 32% 90-d mortality and 48% 1-year mortality with higher rates in patients with pre-existing renal disease[148]. Although their study population was primarily male, this was one of the very few studies that studied post-discharge outcomes for patients, as most studies involved inpatient mortality only. Makar *et al* [149] studied the National Inpatient Sample data of 2016 and concluded that of the 6733 hospitalized cirrhosis patients who had AKI that patients with AKI had increased risk of mortality (OR: 8.09; 95%CI: 6.68-9.79; $P < 0.0001$) and prolonged hospital stay by 3.68 d (95%CI: 3.42-3.93; $P < 0.0001$)[149]. Another study found that community-acquired AKI had increased morbidity (progression to CKD) and mortality rates compared to hospital-acquired AKI[150]. In 2020, Tariq *et al*[151], in a meta-analysis of 18747 patients with cirrhosis (from 30 selected studies), found an in-hospital morality up to 6-fold higher in patients with AKI. Important risk factors were noted to be MELD score, Child-Pugh Turcotte stage C, presence of ascites, and sepsis (with or without shock)[151].

Once HRS of either type is diagnosed, it imparts a grave prognosis with median survival for HRS-AKI and HRS-NAKI determined to be about 1 and 6.7 mo, respectively[152]. Importantly, in all the studies evaluating AKI mortality in cirrhosis, the two types of AKI with the highest mortality were AKI-HRS and ATN[4,6,146,153]. Piano *et al*[6] also studied hospitalized patients with cirrhosis and ascites and AKI using the AKIN stage and found that patients who met the ICA criteria for HRS-AKI had the highest mortality[6]. Fagundes *et al*[4] found that patients with HRS or infection-related AKI had the highest mortality[4].

CONCLUSION

Regardless of type, AKI remains a severe complication to cirrhosis patients and a significant challenge for physicians tasked with treating it. Its incidence has increased as definitions shift to recognize and account for the unique clinical and laboratory abnormalities present in cirrhosis. Differentiating HRS-AKI from non-HRS-AKI is essential as the treatments vary, and early interventions may improve outcomes. Transplantation continues to be the only definitive therapy for HRS-AKI as more data are needed to support the use of less invasive strategies such as TIPS and liver replacement therapy. As our understanding of these diseases' pathophysiology and progression evolve, novel biomarkers and directed therapies will hopefully evolve as well.

REFERENCES

1 **Ginès P**, Solà E, Angeli P, Wong F, Nadim MK, Kamath PS. Hepatorenal syndrome. *Nat Rev Dis Primers* 2018; **4**: 23 [PMID: 30213943 DOI: 10.1038/s41572-018-0022-7]

2 **Garcia-Tsao G**, Parikh CR, Viola A. Acute kidney injury in cirrhosis. *Hepatology* 2008; **48**: 2064-2077 [PMID: 19003880 DOI: 10.1002/hep.22605]

3 **de Carvalho JR**, Villela-Nogueira CA, Luiz RR, Guzzo PL, da Silva Rosa JM, Rocha E, Moraes Coelho HS, de Mello Perez R. Acute kidney injury network criteria as a predictor of hospital mortality in cirrhotic patients with ascites. *J Clin Gastroenterol* 2012; **46**: e21-e26 [PMID: 21934526 DOI: 10.1097/MCG.0b013e31822e8e12]

4 **Fagundes C**, Barreto R, Guevara M, Garcia E, Solà E, Rodríguez E, Graupera I, Ariza X, Pereira G, Alfaro I, Cárdenas A, Fernández J, Poch E, Ginès P. A modified acute kidney injury classification for diagnosis and risk stratification of impairment of kidney function in cirrhosis. *J Hepatol* 2013; **59**: 474-481 [PMID: 23669284 DOI: 10.1016/j.jhep.2013.04.036]

5 **Warner NS**, Cuthbert JA, Bhore R, Rockey DC. Acute kidney injury and chronic kidney disease in hospitalized patients with cirrhosis. *J Investig Med* 2011; **59**: 1244-1251 [PMID: 21941210 DOI: 10.2130/JIM.0b013e3182321471]

6 **Piano S**, Rosi S, Maresio G, Fasolato S, Cavallin M, Romano A, Morando F, Gola E, Frigo AC, Gatta A, Angeli P. Evaluation of the Acute Kidney Injury Network criteria in hospitalized patients with cirrhosis and ascites. *J Hepatol* 2013; **59**: 482-489 [PMID: 23665185 DOI: 10.1016/j.jhep.2013.03.039]

7 **DellaVolpe J**, Al-Khafaji A. Acute Kidney Injury Before and After Liver Transplant. *J Intensive Care Med* 2019; **34**: 687-695 [PMID: 30060683 DOI: 10.1177/0885066618790558]

8 **Ginès P**, Schrier RW. Renal failure in cirrhosis. *N Engl J Med* 2009; **361**: 1279-1290 [PMID: 19776409 DOI: 10.1056/NEJMra0809139]

9 **Wiesner R**, Edwards E, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R; United Network for Organ Sharing Liver Disease Severity Score Committee. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology* 2003; **124**: 91-96 [PMID: 12512033 DOI: 10.1053/gast.2003.50016]

10 **Gonwa TA**, McBride MA, Anderson K, Mai ML, Wadei H, Ahsan N. Continued influence of preoperative renal function on outcome of orthotopic liver transplant (OLTX) in the US: where will MELD lead us? *Am J Transplant* 2006; **6**: 2651-2659 [PMID: 16939515 DOI: 10.1111/j.1600-6143.2006.01526.x]

11 **Rustgi VK**, Li Y, John T, Catalano C, Elsaid MI. Health Care Resource Use and Cost Burden of Chronic Kidney Disease in Patients With Chronic Liver Disease: A Real-World Claims Analysis. *Hepatol Commun* 2020; **4**: 1404-1418 [PMID: 33024912 DOI: 10.1002/hep4.1573]

12 **Chancharoenthana W**, Leelahavanichkul A. Acute kidney injury spectrum in patients with chronic liver disease: Where do we stand? *World J Gastroenterol* 2019; **25**: 3684-3703 [PMID: 31391766 DOI: 10.3748/wjg.v25.i28.3684]

13 **Jalan R**, Williams R. Acute-on-chronic liver failure: pathophysiological basis of therapeutic options. *Blood Purif* 2002; **20**: 252-261 [PMID: 11867872 DOI: 10.1159/000047017]

14 **Angeli P**, Ginès P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. *J Hepatol* 2015; **62**: 968-974 [PMID: 25638527 DOI: 10.1016/j.jhep.2014.12.029]

15 **Bellomo R**, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative workgroup. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; **8**: R204-R212 [PMID: 15312219 DOI: 10.1186/cc2872]

16 **Mehta RL**, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A; Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; **11**: R31 [PMID: 17331245 DOI: 10.1186/cc5713]

17 **Kellum JA**, Lameire N; KDIGO AKI Guideline Work Group. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care* 2013; **17**: 204 [PMID: 23394211 DOI: 10.1186/cc11454]

18 **MacDonald AJ**, Nadim MK, Durand F, Karvellas CJ. Acute kidney injury in cirrhosis: implications for liver transplantation. *Curr Opin Crit Care* 2019; **25**: 171-178 [PMID: 30676329 DOI: 10.1097/MCC.0000000000000590]

19 **Nadim MK**, Kellum JA, Davenport A, Wong F, Davis C, Pannu N, Tolwani A, Bellomo R, Genyk YS; ADQI Workgroup. Hepatorenal syndrome: the 8th International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2012; **16**: R23 [PMID: 22322077 DOI: 10.1186/cc11188]

20 **Wong F**. Acute kidney injury in liver cirrhosis: new definition and application. *Clin Mol Hepatol* 2016; **22**: 415-422 [PMID: 27987536 DOI: 10.3350/cmh.2016.0056]

21 **Salerno F**, Gerbes A, Ginès P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Postgrad Med J* 2008; **84**: 662-670 [PMID: 19201943 DOI: 10.1136/gut.2006.107789]

22 **Wong F**. The evolving concept of acute kidney injury in patients with cirrhosis. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 711-719 [PMID: 26484442 DOI: 10.1038/nrgastro.2015.174]

23 **Arroyo V**, Ginès P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, Reynolds TB, Ring-Larsen H, Schölmerich J. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology* 1996; **23**: 164-176 [PMID: 8550036 DOI: 10.1002/hep.510230122]

24 **Martín-Llahí M**, Guevara M, Torre A, Fagundes C, Restuccia T, Gilabert R, Solá E, Pereira G, Marinelli M, Pavesi M, Fernández J, Rodés J, Arroyo V, Ginès P. Prognostic importance of the cause of renal failure in patients with cirrhosis. *Gastroenterology* 2011; **140**: 488-496. e4 [PMID: 20682324 DOI: 10.1053/j.gastro.2010.07.043]

25 **Fagundes C**, Ginès P. Hepatorenal syndrome: a severe, but treatable, cause of kidney failure in cirrhosis. *Am J Kidney Dis* 2012; **59**: 874-885 [PMID: 22480795 DOI: 10.1053/j.ajkd.2011.12.032]

26 **Martin PY**, Ginès P, Schrier RW. Nitric oxide as a mediator of hemodynamic abnormalities and sodium and water retention in cirrhosis. *N Engl J Med* 1998; **339**: 533-541 [PMID: 9709047 DOI: 10.1056/NEJM199808203390807]

27 **Iwakiri Y**, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006; **43**: S121-S131 [PMID: 16447289 DOI: 10.1002/hep.20993]

28 **Ros J**, Clària J, To-Figueras J, Planagumà A, Cejudo-Martín P, Fernández-Varo G, Martín-Ruiz R, Arroyo V, Rivera F, Rodés J, Jiménez W. Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology* 2002; **122**: 85-93 [PMID: 11781284 DOI: 10.1053/gast.2002.30305]

29 **Acevedo JG**, Cramp ME. Hepatorenal syndrome: Update on diagnosis and therapy. *World J Hepatol* 2017; **9**: 293-299 [PMID: 28293378 DOI: 10.4254/wjh.v9.i6.293]

30 **Henriksen JH**, Møller S, Ring-Larsen H, Christensen NJ. The sympathetic nervous system in liver disease. *J Hepatol* 1998; **29**: 328-341 [PMID: 9722218 DOI: 10.1016/s0168-8278(98)80022-6]

31 **Bernardi M**, Trevisani F, Gasbarrini A, Gasbarrini G. Hepatorenal disorders: role of the renin-angiotensin-aldosterone system. *Semin Liver Dis* 1994; **14**: 23-34 [PMID: 8016659 DOI: 10.1055/s-2007-1007295]

32 **Wong F**. Cirrhotic cardiomyopathy. *Hepatol Int* 2009; **3**: 294-304 [PMID: 19669380 DOI: 10.1007/s12072-008-9109-7]

33 **Ruiz-del-Arbol L**, Monescillo A, Arocena C, Valer P, Ginès P, Moreira V, Milicua JM, Jiménez W, Arroyo V. Circulatory function and hepatorenal syndrome in cirrhosis. *Hepatology* 2005; **42**: 439-447 [PMID: 15977202 DOI: 10.1002/hep.20766]

34 **Choi YJ**, Kim JH, Koo JK, Lee CI, Lee JY, Yang JH, Ko SY, Choe WH, Kwon SY, Lee CH. Prevalence of renal dysfunction in patients with cirrhosis according to ADQI-IAC working party proposal. *Clin Mol Hepatol* 2014; **20**: 185-191 [PMID: 25032185 DOI: 10.3350/cmh.2014.20.2.185]

35 **Fang JT**, Tsai MH, Tian YC, Jenq CC, Lin CY, Chen YC, Lien JM, Chen PC, Yang CW. Outcome predictors and new score of critically ill cirrhotic patients with acute renal failure. *Nephrol Dial Transplant* 2008; **23**: 1961-1969 [PMID: 18187499 DOI: 10.1093/ndt/gfm914]

36 **Tsien CD**, Rabie R, Wong F. Acute kidney injury in decompensated cirrhosis. *Gut* 2013; **62**: 131-137 [PMID: 22637695 DOI: 10.1136/gutjnl-2011-301255]

37 **Bucsics T**, Krones E. Renal dysfunction in cirrhosis: acute kidney injury and the hepatorenal syndrome. *Gastroenterol Rep (Oxf)* 2017; **5**: 127-137 [PMID: 28533910 DOI: 10.1093/gastro/gox009]

38 **Rolando N**, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. *Hepatology* 2000; **32**: 734-739 [PMID: 11003617 DOI: 10.1053/jhep.2000.17687]

39 **Strnad P**, Tacke F, Koch A, Trautwein C. Liver - guardian, modifier and target of sepsis. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 55-66 [PMID: 27924081 DOI: 10.1038/nrgastro.2016.168]

40 **Woolbright BL**, Jaeschke H. The impact of sterile inflammation in acute liver injury. *J Clin Transl Res* 2017; **3**: 170-188 [PMID: 28670626 DOI: 10.18053/jctres.03.2017S1.003]

41 **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]

42 **Bernardi M**, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis. *J Hepatol* 2015; **63**: 1272-1284 [PMID: 26192220 DOI: 10.1016/j.jhep.2015.07.004]

43 **Cazzaniga M**, Dionigi E, Gobbo G, Fioretti A, Monti V, Salerno F. The systemic inflammatory response syndrome in cirrhotic patients: relationship with their in-hospital outcome. *J Hepatol* 2009; **51**: 475-482 [PMID: 19560225 DOI: 10.1016/j.jhep.2009.04.017]

44 **Cervoni JP**, Thévenot T, Weil D, Muel E, Barbot O, Sheppard F, Monnet E, Di Martino V. C-reactive protein predicts short-term mortality in patients with cirrhosis. *J Hepatol* 2012; **56**: 1299-1304 [PMID: 22314431 DOI: 10.1016/j.jhep.2011.12.030]

45 **Wiest R**, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014; **60**: 197-209 [PMID: 23993913 DOI: 10.1016/j.jhep.2013.07.044]

46 **Moini M**, Yazdani Sarvestani M, Shams M, Nomovi M. Evaluation of Adrenal Function in Nonhospitalized Patients with Cirrhosis. *Can J Gastroenterol Hepatol* 2017; **2017**: 2354253 [PMID: 28812008 DOI: 10.1155/2017/2354253]

47 **Muciño-Bermejo MJ**. Mechanisms of kidney dysfunction in the cirrhotic patient: Non-hepatorenal acute-on-chronic kidney damage considerations. *Ann Hepatol* 2020; **19**: 145-152 [PMID: 31594758 DOI: 10.1016/j.aohep.2019.06.022]

48 **Acevedo J**, Fernández J, Prado V, Silva A, Castro M, Pavesi M, Roca D, Jimenez W, Ginès P, Arroyo V. Relative adrenal insufficiency in decompensated cirrhosis: Relationship to short-term risk of severe sepsis, hepatorenal syndrome, and death. *Hepatology* 2013; **58**: 1757-1765 [PMID: 23728792 DOI: 10.1002/hep.26535]

49 **Nampoothiri RV**, Duseja A, Rathi M, Agrawal S, Sachdeva N, Mehta M, Dhaliwal HS, Dhiman RK, Chawla Y. Renal Dysfunction in Patients With Nonalcoholic Fatty Liver Disease is Related to the Presence of Diabetes Mellitus and Severity of Liver Disease. *J Clin Exp Hepatol* 2019; **9**: 22-28 [PMID: 30765935 DOI: 10.1016/j.jceh.2017.12.005]

50 **Mizuiri S**, Ohashi Y. ACE and ACE2 in kidney disease. *World J Nephrol* 2015; **4**: 74-82 [PMID: 25664248 DOI: 10.5527/wjn.v4.i1.74]

51 **Musso G**, Gambino R, Cassader M. Emerging molecular targets for the treatment of nonalcoholic fatty liver disease. *Annu Rev Med* 2010; **61**: 375-392 [PMID: 20059344 DOI: 10.1146/annurev.med.60.101107.134820]

52 **Ramis MR**, Esteban S, Miralles A, Tan DX, Reiter RJ. Caloric restriction, resveratrol and melatonin: Role of SIRT1 and implications for aging and related-diseases. *Mech Ageing Dev* 2015; **146-148**: 28-41 [PMID: 25824609 DOI: 10.1016/j.mad.2015.03.008]

53 **Lhotta K**. Beyond hepatorenal syndrome: glomerulonephritis in patients with liver disease. *Semin Nephrol* 2002; **22**: 302-308 [PMID: 12118395]

54 **Johnson RJ**, Couser WG. Hepatitis B infection and renal disease: clinical, immunopathogenetic and therapeutic considerations. *Kidney Int* 1990; **37**: 663-676 [PMID: 1968522 DOI: 10.1038/ki.1990.32]

55 **Lai KN**, Ho RT, Tam JS, Lai FM. Detection of hepatitis B virus DNA and RNA in kidneys of HBV related glomerulonephritis. *Kidney Int* 1996; **50**: 1965-1977 [PMID: 8943480 DOI: 10.1038/ki.1996.519]

56 **Lai TS**, Lee MH, Yang HI, You SL, Lu SN, Wang LY, Yuan Y, L'Italien G, Chien KL, Chen CJ; REVEAL-HCV Study Group. Hepatitis C viral load, genotype, and increased risk of developing end-stage renal disease: REVEAL-HCV study. *Hepatology* 2017; **66**: 784-793 [PMID: 28370058 DOI: 10.1002/hep.29192]

57 **Cockcroft DW**, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31-41 [PMID: 1244564 DOI: 10.1159/000180580]

58 **Levey AS**, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470 [PMID: 10075613 DOI: 10.7326/0003-4819-130-6-199903160-00002]

59 **Francoz C**, Nadim MK, Durand F. Kidney biomarkers in cirrhosis. *J Hepatol* 2016; **65**: 809-824 [PMID: 27238754 DOI: 10.1016/j.jhep.2016.05.025]

60 **Levey AS**, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604-612 [PMID: 19414839 DOI: 10.7326/0003-4819-150-9-200905050-00006]

61 **Sherman DS**, Fish DN, Teitelbaum I. Assessing renal function in cirrhotic patients: problems and pitfalls. *Am J Kidney Dis* 2003; **41**: 269-278 [PMID: 12552488 DOI: 10.1053/ajkd.2003.50035]

62 **Herget-Rosenthal S**, Trabold S, Pietruck F, Holtmann M, Philipp T, Kribben A. Cystatin C: efficacy as screening test for reduced glomerular filtration rate. *Am J Nephrol* 2000; **20**: 97-102 [PMID: 10773608 DOI: 10.1159/000013564]

63 **Tenstad O**, Roald AB, Grubb A, Aukland K. Renal handling of radiolabelled human cystatin C in the rat. *Scand J Clin Lab Invest* 1996; **56**: 409-414 [PMID: 8869663 DOI: 10.3109/00365519609088795]

64 **Shlipak MG**, Coresh J, Gansevoort RT. Cystatin C versus creatinine for kidney function-based risk. *N Engl J Med* 2013; **369**: 2459 [PMID: 24350959 DOI: 10.1056/NEJMc1312801]

65 **Inker LA**, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS; CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; **367**: 20-29 [PMID: 22762315 DOI: 10.1056/NEJMoa1114248]

66 **Gerbes AL**, Gülberg V, Bilzer M, Vogeser M. Evaluation of serum cystatin C concentration as a marker of renal function in patients with cirrhosis of the liver. *Gut* 2002; **50**: 106-110 [PMID: 11772976 DOI: 10.1136/gut.50.1.106]

67 **Jaques DA**, Spahr L, Berra G, Poffet V, Lescuyer P, Gerstel E, Garin N, Martin PY, Ponte B. Biomarkers for acute kidney injury in decompensated cirrhosis: A prospective study. *Nephrology (Carlton)* 2019; **24**: 170-180 [PMID: 29369449 DOI: 10.1111/nep.13226]

68 **Kim TH**, Seo YS, Kang SH, Kim MY, Kim SG, Lee HY, Lee JH, Lee YS, Kim JH, Jeong SW, Jang JY, Suk KT, Jung YK, An H, Yim HJ, Kim YS, Um SH; Korean Study Group of Portal Hypertension. Prognosis predictability of serum and urine renal markers in patients with decompensated cirrhosis: A multicentre prospective study. *Liver Int* 2020; **40**: 3083-3092 [PMID: 32750739 DOI: 10.1111/liv.14631]

69 **Puthumana J**, Ariza X, Belcher JM, Graupera I, Ginès P, Parikh CR. Urine Interleukin 18 and Lipocalin 2 Are Biomarkers of Acute Tubular Necrosis in Patients With Cirrhosis: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2017; **15**: 1003-1013. e3 [PMID: 28013112 DOI: 10.1016/j.cgh.2016.11.035]

70 **Mishra J**, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol* 2003; **14**: 2534-2543 [PMID: 14514731 DOI: 10.1097/01.asn.0000088027.54400.c6]

71 **Mishra J**, Mori K, Ma Q, Kelly C, Yang J, Mitsnefes M, Barasch J, Devarajan P. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol* 2004; **15**: 3073-3082 [PMID: 15579510 DOI: 10.1097/01.ASN.0000145013.44578.45]

72 **Fagundes C**, Pépin MN, Guevara M, Barreto R, Casals G, Solà E, Pereira G, Rodríguez E, Garcia E, Prado V, Poch E, Jiménez W, Fernández J, Arroyo V, Ginès P. Urinary neutrophil gelatinase-associated lipocalin as biomarker in the differential diagnosis of impairment of kidney function in cirrhosis. *J Hepatol* 2012; **57**: 267-273 [PMID: 22521351 DOI: 10.1016/j.jhep.2012.03.015]

73 **Belcher JM**, Sanyal AJ, Peixoto AJ, Perazella MA, Lim J, Thiessen-Philbrook H, Ansari N, Coca SG, Garcia-Tsao G, Parikh CR; TRIBE-AKI Consortium. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. *Hepatology* 2014; **60**: 622-632 [PMID: 24375576 DOI: 10.1002/hep.26980]

74 **Barreto R**, Elia C, Solà E, Moreira R, Ariza X, Rodríguez E, Graupera I, Alfaro I, Morales-Ruiz M, Poch E, Guevara M, Fernández J, Jiménez W, Arroyo V, Ginès P. Urinary neutrophil gelatinase-associated lipocalin predicts kidney outcome and death in patients with cirrhosis and bacterial infections. *J Hepatol* 2014; **61**: 35-42 [PMID: 24613364 DOI: 10.1016/j.jhep.2014.02.023]

75 **Ariza X**, Solà E, Elia C, Barreto R, Moreira R, Morales-Ruiz M, Graupera I, Rodríguez E, Huelin P, Solé C, Fernández J, Jiménez W, Arroyo V, Ginès P. Analysis of a urinary biomarker panel for clinical outcomes assessment in cirrhosis. *PLoS One* 2015; **10**: e0128145 [PMID: 26042740 DOI: 10.1371/journal.pone.0128145]

76 **Jo SK**, Yang J, Hwang SM, Lee MS, Park SH. Role of biomarkers as predictors of acute kidney injury and mortality in decompensated cirrhosis. *Sci Rep* 2019; **9**: 14508 [PMID: 31601879 DOI: 10.1038/s41598-019-51053-8]

77 **Lee JH**, Yoon EL, Park SE, Park JY, Choi JM, Jeon TJ, Shin WC, Choi WC. Clinical Significance of Urinary Neutrophil Gelatinase-associated Lipocalin Levels in Defining the Various Etiologies of Acute Kidney Injury in Liver Cirrhosis Patients. *Korean J Gastroenterol* 2019; **74**: 212-218 [PMID: 31650797 DOI: 10.4166/kjg.2019.74.4.212]

78 **Huelin P**, Solà E, Elia C, Solé C, Risso A, Moreira R, Carol M, Fabrellas N, Bassegoda O, Juanola A, de Prada G, Albertos S, Piano S, Graupera I, Ariza X, Napoleone L, Pose E, Filella X, Morales-Ruiz M, Rios J, Fernández J, Jiménez W, Poch E, Torres F, Ginès P. Neutrophil Gelatinase-Associated Lipocalin for Assessment of Acute Kidney Injury in Cirrhosis: A Prospective Study. *Hepatology* 2019; **70**: 319-333 [PMID: 30810244 DOI: 10.1002/hep.30592]

79 **Davenport A**, Sheikh MF, Lamb E, Agarwal B, Jalan R. Acute kidney injury in acute-on-chronic liver failure: where does hepatorenal syndrome fit? *Kidney Int* 2017; **92**: 1058-1070 [PMID: 28844314 DOI: 10.1016/j.kint.2017.04.048]

80 **Ostermann M**, Joannidis M. Biomarkers for AKI improve clinical practice: no. *Intensive Care Med* 2015; **41**: 618-622 [PMID: 25387818 DOI: 10.1007/s00134-014-3540-0]

81 **Glassford NJ**, Schneider AG, Xu S, Eastwood GM, Young H, Peck L, Venge P, Bellomo R. The nature and discriminatory value of urinary neutrophil gelatinase-associated lipocalin in critically ill patients at risk of acute kidney injury. *Intensive Care Med* 2013; **39**: 1714-1724 [PMID: 23917325 DOI: 10.1007/s00134-013-3040-7]

82 **Liu Y**, Guo W, Zhang J, Xu C, Yu S, Mao Z, Wu J, Ye C, Mei C, Dai B. Urinary interleukin 18 for detection of acute kidney injury: a meta-analysis. *Am J Kidney Dis* 2013; **62**: 1058-1067 [PMID: 23830182 DOI: 10.1053/j.ajkd.2013.05.014]

83 **Wu H**, Craft ML, Wang P, Wyburn KR, Chen G, Ma J, Hambly B, Chadban SJ. IL-18 contributes to renal damage after ischemia-reperfusion. *J Am Soc Nephrol* 2008; **19**: 2331-2341 [PMID: 18815244 DOI: 10.1681/ASN.2008020170]

84 **Tsai MH**, Chen YC, Yang CW, Jenq CC, Fang JT, Lien JM, Hung CC, Weng HH, Wu CS, Peng YS, Shen CH, Tung SY, Tian YC. Acute renal failure in cirrhotic patients with severe sepsis: value of urinary interleukin-18. *J Gastroenterol Hepatol* 2013; **28**: 135-141 [PMID: 23034155 DOI: 10.1111/j.1440-1746.2012.07288.x]

85 **Qasem AA**, Farag SE, Hamed E, Emara M, Bihery A, Pasha H. Urinary biomarkers of acute kidney injury in patients with liver cirrhosis. *ISRN Nephrol* 2014; **2014**: 376795 [PMID: 24967242 DOI: 10.1155/2014/376795]

86 **Ichimura T**, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, Sanicola M. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J Biol Chem* 1998; **273**: 4135-4142 [PMID: 9461608 DOI: 10.1074/jbc.273.7.4135]

87 **Furuhashi M**, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov* 2008; **7**: 489-503 [PMID: 18511927 DOI: 10.1038/nrd2589]

88 **Xu Y**, Xie Y, Shao X, Ni Z, Mou S. L-FABP: A novel biomarker of kidney disease. *Clin Chim Acta* 2015; **445**: 85-90 [PMID: 25797895 DOI: 10.1016/j.cca.2015.03.017]

89 **Yamamoto T**, Noiri E, Ono Y, Doi K, Negishi K, Kamijo A, Kimura K, Fujita T, Kinukawa T, Taniguchi H, Nakamura K, Goto M, Shinozaki N, Ohshima S, Sugaya T. Renal L-type fatty acid--binding protein in acute ischemic injury. *J Am Soc Nephrol* 2007; **18**: 2894-2902 [PMID: 17942962 DOI: 10.1681/ASN.2007010097]

90 **Doi K**, Noiri E, Maeda-Mamiya R, Ishii T, Negishi K, Hamasaki Y, Fujita T, Yahagi N, Koide H, Sugaya T, Nakamura T. Urinary L-type fatty acid-binding protein as a new biomarker of sepsis complicated with acute kidney injury. *Crit Care Med* 2010; **38**: 2037-2042 [PMID: 20657273 DOI: 10.1097/CCM.0b013e3181eedac0]

91 **Eguchi A**, Iwasa M. The Role of Elevated Liver-Type Fatty Acid-Binding Proteins in Liver Diseases. *Pharm Res* 2021; **38**: 89-95 [PMID: 33534129 DOI: 10.1007/s11095-021-02998-x]

92 **Karvellas CJ**, Speiser JL, Tremblay M, Lee WM, Rose CF; US Acute Liver Failure Study Group. Elevated FABP1 serum levels are associated with poorer survival in acetaminophen-induced acute liver failure. *Hepatology* 2017; **65**: 938-949 [PMID: 27859489 DOI: 10.1002/hep.28945]

93 **Eguchi A**, Hasegawa H, Iwasa M, Tamai Y, Ohata K, Oikawa T, Sugaya T, Takei Y. Serum Liver-Type Fatty Acid-Binding Protein Is a Possible Prognostic Factor in Human Chronic Liver Diseases From Chronic Hepatitis to Liver Cirrhosis and Hepatocellular Carcinoma. *Hepatol Commun* 2019; **3**: 825-837 [PMID: 31168516 DOI: 10.1002/hep4.1350]

94 **Allegretti AS**, Solà E, Ginès P. Clinical Application of Kidney Biomarkers in Cirrhosis. *Am J Kidney Dis* 2020; **76**: 710-719 [PMID: 32622560 DOI: 10.1053/j.ajkd.2020.03.016]

95 **Wong F**. Diagnosing and treating renal disease in cirrhotic patients. *Minerva Gastroenterol Dietol* 2016; **62**: 253-266 [PMID: 27096702]

96 **Mandorfer M**, Reiberger T. Beta blockers and cirrhosis, 2016. *Dig Liver Dis* 2017; **49**: 3-10 [PMID: 27717792 DOI: 10.1016/j.dld.2016.09.013]

97 **Mandorfer M**, Bota S, Schwabl P, Bucsics T, Pfisterer N, Kruzik M, Hagmann M, Blacky A, Ferlitsch A, Sieghart W, Trauner M, Peck-Radosavljevic M, Reiberger T. Nonselective β blockers increase risk for hepatorenal syndrome and death in patients with cirrhosis and spontaneous bacterial peritonitis. *Gastroenterology* 2014; **146**: 1680-90. e1 [PMID: 24631577 DOI: 10.1053/j.gastro.2014.03.005]

98 **Schwabl P**, Bucsics T, Soucek K, Mandorfer M, Bota S, Blacky A, Hirschl AM, Ferlitsch A, Trauner M, Peck-Radosavljevic M, Reiberger T. Risk factors for development of spontaneous bacterial peritonitis and subsequent mortality in cirrhotic patients with ascites. *Liver Int* 2015; **35**: 2121-2128 [PMID: 25644943 DOI: 10.1111/liv.12795]

99 **Jalan R**, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, Stadlbauer V, Gustot T, Bernardi M, Canton R, Albillos A, Lammert F, Wilmer A, Mookerjee R, Vila J, Garcia-Martinez R, Wendon J, Such J, Cordoba J, Sanyal A, Garcia-Tsao G, Arroyo V, Burroughs A, Ginès P. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. *J Hepatol* 2014; **60**: 1310-1324 [PMID: 24530646 DOI: 10.1016/j.jhep.2014.01.024]

100 **Arabi YM**, Dara SI, Memish Z, Al Abdulkareem A, Tamim HM, Al-Shirawi N, Parrillo JE, Dodek P, Lapinsky S, Feinstein D, Wood G, Dial S, Zanotti S, Kumar A; Cooperative Antimicrobial Therapy of Septic Shock (CATSS) Database Research Group. Antimicrobial therapeutic determinants of outcomes from septic shock among patients with cirrhosis. *Hepatology* 2012; **56**: 2305-2315 [PMID: 22753144 DOI: 10.1002/hep.25931]

101 **Sanyal AJ**, Boyer T, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Blei A, Gülberg V, Sigal S, Teuber P; Terlipressin Study Group. A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. *Gastroenterology* 2008; **134**: 1360-1368 [PMID: 18471513 DOI: 10.1053/j.gastro.2008.02.014]

102 **Sharma P**, Kumar A, Shrama BC, Sarin SK. An open label, pilot, randomized controlled trial of noradrenaline versus terlipressin in the treatment of type 1 hepatorenal syndrome and predictors of response. *Am J Gastroenterol* 2008; **103**: 1689-1697 [PMID: 18557715 DOI: 10.1111/j.1572-0241.2008.01828.x]

103 **Martín-Llahí M**, Pépin MN, Guevara M, Díaz F, Torre A, Monescillo A, Soriano G, Terra C, Fábrega E, Arroyo V, Rodés J, Ginès P; TAHRS Investigators. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology* 2008; **134**: 1352-1359 [PMID: 18471512 DOI: 10.1053/j.gastro.2008.02.024]

104 **Cavallin M**, Kamath PS, Merli M, Fasolato S, Toniutto P, Salerno F, Bernardi M, Romanelli RG, Colletta C, Salinas F, Di Giacomo A, Ridola L, Fornasiere E, Caraceni P, Morando F, Piano S, Gatta A, Angeli P; Italian Association for the Study of the Liver Study Group on Hepatorenal Syndrome. Terlipressin plus albumin versus midodrine and octreotide plus albumin in the treatment of hepatorenal syndrome: A randomized trial. *Hepatology* 2015; **62**: 567-574 [PMID: 25644760 DOI: 10.1002/hep.27709]

105 **Boyer TD**, Sanyal AJ, Wong F, Frederick RT, Lake JR, O'Leary JG, Ganger D, Jamil K, Pappas SC; REVERSE Study Investigators. Terlipressin Plus Albumin Is More Effective Than Albumin Alone in Improving Renal Function in Patients With Cirrhosis and Hepatorenal Syndrome Type 1. *Gastroenterology* 2016; **150**: 1579-1589. e2 [PMID: 26896734 DOI: 10.1053/j.gastro.2016.02.026]

106 **Cavallin M**, Piano S, Romano A, Fasolato S, Frigo AC, Benetti G, Gola E, Morando F, Stanco M, Rosi S, Sticca A, Cillo U, Angeli P. Terlipressin given by continuous intravenous infusion versus intravenous boluses in the treatment of hepatorenal syndrome: A randomized controlled study. *Hepatology* 2016; **63**: 983-992 [PMID: 26659927 DOI: 10.1002/hep.28396]

107 **Gifford FJ**, Morling JR, Fallowfield JA. Systematic review with meta-analysis: vasoactive drugs for the treatment of hepatorenal syndrome type 1. *Aliment Pharmacol Ther* 2017; **45**: 593-603 [PMID: 28052382 DOI: 10.1111/apt.13912]

108 **Solanki P**, Chawla A, Garg R, Gupta R, Jain M, Sarin SK. Beneficial effects of terlipressin in hepatorenal syndrome: a prospective, randomized placebo-controlled clinical trial. *J Gastroenterol Hepatol* 2003; **18**: 152-156 [PMID: 12542598 DOI: 10.1046/j.1440-1746.2003.02934.x]

109 **Neri S**, Pulvirenti D, Malaguarnera M, Cosimo BM, Bertino G, Ignaccolo L, Siringo S, Castellino P. Terlipressin and albumin in patients with cirrhosis and type I hepatorenal syndrome. *Dig Dis Sci* 2008; **53**: 830-835 [PMID: 17939047 DOI: 10.1007/s10620-007-9919-9]

110 **European Association for the Study of the Liver**. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]

111 **Mattos ÂZ**, Schacher FC, Mattos AA. Vasoconstrictors in hepatorenal syndrome - A critical review. *Ann Hepatol* 2019; **18**: 287-290 [PMID: 31023616 DOI: 10.1016/j.aohep.2018.12.002]

112 **Krishna R**, Raj J, Dev D, Prasad SC, Reghu R, V SO. A study on clinical outcomes of combination of terlipressin and albumin in Hepatorenal Syndrome. *Scand J Gastroenterol* 2020; **55**: 860-864 [PMID: 32634332 DOI: 10.1080/00365521.2020.1786851]

113 **Kalambokis GN**, Christaki M, Tsiakas I, Despotis G, Milionis HJ. Efficacy of treatment with terlipressin plus albumin in hepatorenal syndrome diagnosed with the new acute kidney injury versus the conventional criteria. *Eur J Gastroenterol Hepatol* 2019; **31**: 1292-1294 [PMID: 31498285 DOI: 10.1097/MEG.0000000000001460]

114 **Wong F**, Pappas SC, Curry MP, Reddy KR, Rubin RA, Porayko MK, Gonzalez SA, Mumtaz K, Lim N, Simonetto DA, Sharma P, Sanyal AJ, Mayo MJ, Frederick RT, Escalante S, Jamil K; CONFIRM Study Investigators. Terlipressin plus Albumin for the Treatment of Type 1 Hepatorenal Syndrome. *N Engl J Med* 2021; **384**: 818-828 [PMID: 33657294 DOI: 10.1056/NEJMoa2008290]

115 **Duvoux C**, Zanditenas D, Hézode C, Chauvat A, Monin JL, Roudot-Thoraval F, Mallat A, Dhumeaux D. Effects of noradrenalin and albumin in patients with type I hepatorenal syndrome: a pilot study. *Hepatology* 2002; **36**: 374-380 [PMID: 12143045 DOI: 10.1053/jhep.2002.34343]

116 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018; **69**: 406-460 [PMID: 29653741 DOI: 10.1016/j.jhep.2018.03.024]

117 **Gupta K**, Rani P, Rohatgi A, Verma M, Handa S, Dalal K, Jain A. Noradrenaline for reverting hepatorenal syndrome: a prospective, observational, single-center study. *Clin Exp Gastroenterol* 2018; **11**: 317-324 [PMID: 30271187 DOI: 10.2147/CEG.S153858]

118 **Alessandria C**, Ottobrelli A, Debernardi-Venon W, Todros L, Cerenzia MT, Martini S, Balzola F, Morgando A, Rizzetto M, Marzano A. Noradrenalin vs terlipressin in patients with hepatorenal syndrome: a prospective, randomized, unblinded, pilot study. *J Hepatol* 2007; **47**: 499-505 [PMID: 17560680 DOI: 10.1016/j.jhep.2007.04.010]

119 **Singh V**, Ghosh S, Singh B, Kumar P, Sharma N, Bhalla A, Sharma AK, Choudhary NS, Chawla Y, Nain CK. Noradrenaline vs. terlipressin in the treatment of hepatorenal syndrome: a randomized study. *J Hepatol* 2012; **56**: 1293-1298 [PMID: 22322237 DOI: 10.1016/j.jhep.2012.01.012]

120 **Ghosh S**, Choudhary NS, Sharma AK, Singh B, Kumar P, Agarwal R, Sharma N, Bhalla A, Chawla YK, Singh V. Noradrenaline vs terlipressin in the treatment of type 2 hepatorenal syndrome: a randomized pilot study. *Liver Int* 2013; **33**: 1187-1193 [PMID: 23601499 DOI: 10.1111/liv.12179]

121 **Goyal O**, Sidhu SS, Sehgal N, Puri S. Noradrenaline is as Effective as Terlipressin in Hepatorenal Syndrome Type 1: A Prospective, Randomized Trial. *J Assoc Physicians India* 2016; **64**: 30-35 [PMID: 27762512]

122 **Liu ZM**, Chen J, Kou Q, Lin Q, Huang X, Tang Z, Kang Y, Li K, Zhou L, Song Q, Sun T, Zhao L, Wang X, He X, Wang C, Wu B, Lin J, Yuan S, Gu Q, Qian K, Shi X, Feng Y, Lin A; Study Group of investigators, Guan XD. Terlipressin versus norepinephrine as infusion in patients with septic shock: a multicentre, randomised, double-blinded trial. *Intensive Care Med* 2018; **44**: 1816-1825 [PMID: 29971593 DOI: 10.1007/s00134-018-5267-9]

123 **Arora V**, Maiwall R, Rajan V, Jindal A, Muralikrishna Shasthry S, Kumar G, Jain P, Sarin SK. Terlipressin Is Superior to Noradrenaline in the Management of Acute Kidney Injury in Acute on Chronic Liver Failure. *Hepatology* 2020; **71**: 600-610 [PMID: 30076614 DOI: 10.1002/hep.30208]

124 **Angeli P**, Volpin R, Gerunda G, Craighero R, Roner P, Merenda R, Amodio P, Sticca A, Caregaro L, Maffei-Faccioli A, Gatta A. Reversal of type 1 hepatorenal syndrome with the administration of midodrine and octreotide. *Hepatology* 1999; **29**: 1690-1697 [PMID: 10347109 DOI: 10.1002/hep.510290629]

125 **Wong F**, Pantea L, Sniderman K. Midodrine, octreotide, albumin, and TIPS in selected patients with cirrhosis and type 1 hepatorenal syndrome. *Hepatology* 2004; **40**: 55-64 [PMID: 15239086 DOI: 10.1002/hep.20262]

126 **Skagen C**, Einstein M, Lucey MR, Said A. Combination treatment with octreotide, midodrine, and albumin improves survival in patients with type 1 and type 2 hepatorenal syndrome. *J Clin Gastroenterol* 2009; **43**: 680-685 [PMID: 19238094 DOI: 10.1097/MCG.0b013e318188947c]

127 **Brensing KA**, Textor J, Perz J, Schiedermaier P, Raab P, Strunk H, Klehr HU, Kramer HJ, Spengler U, Schild H, Sauerbruch T. Long term outcome after transjugular intrahepatic portosystemic stent-shunt in non-transplant cirrhotics with hepatorenal syndrome: a phase II study. *Gut* 2000; **47**: 288-295 [PMID: 10896924 DOI: 10.1136/gut.47.2.288]

128 **Song T**, Rössle M, He F, Liu F, Guo X, Qi X. Transjugular intrahepatic portosystemic shunt for hepatorenal syndrome: A systematic review and meta-analysis. *Dig Liver Dis* 2018; **50**: 323-330 [PMID: 29422242 DOI: 10.1016/j.dld.2018.01.123]

129 **Zhang Z**, Maddukuri G, Jaipaul N, Cai CX. Role of renal replacement therapy in patients with type 1 hepatorenal syndrome receiving combination treatment of vasoconstrictor plus albumin. *J Crit Care* 2015; **30**: 969-974 [PMID: 26051980 DOI: 10.1016/j.jcrc.2015.05.006]

130 **Keller F**, Heinze H, Jochimsen F, Passfall J, Schuppan D, Büttner P. Risk factors and outcome of 107 patients with decompensated liver disease and acute renal failure (including 26 patients with hepatorenal syndrome): the role of hemodialysis. *Ren Fail* 1995; **17**: 135-146 [PMID: 7644764 DOI:

10.3109/08860229509026250]
131 **Bañares R**, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, Saliba F, Sauerbruch T, Klammt S, Ockenga J, Pares A, Wendon J, Brünnler T, Kramer L, Mathurin P, de la Mata M, Gasbarrini A, Müllhaupt B, Wilmer A, Laleman W, Eefsen M, Sen S, Zipprich A, Tenorio T, Pavesi M, Schmidt HH, Mitzner S, Williams R, Arroyo V; RELIEF study group. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. *Hepatology* 2013; **57**: 1153-1162 [PMID: 23213075 DOI: 10.1002/hep.26185]
132 **Lavayssière L**, Kallab S, Cardeau-Desangles I, Nogier MB, Cointault O, Barange K, Muscari F, Rostaing L, Kamar N. Impact of molecular adsorbent recirculating system on renal recovery in type-1 hepatorenal syndrome patients with chronic liver failure. *J Gastroenterol Hepatol* 2013; **28**: 1019-1024 [PMID: 23425070 DOI: 10.1111/jgh.12159]
133 **He YT**, Qi YN, Zhang BQ, Li JB, Bao J. Bioartificial liver support systems for acute liver failure: A systematic review and meta-analysis of the clinical and preclinical literature. *World J Gastroenterol* 2019; **25**: 3634-3648 [PMID: 31367162 DOI: 10.3748/wjg.v25.i27.3634]
134 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325 DOI: 10.1056/NEJM199908053410603]
135 **Terg R**, Gadano A, Cartier M, Casciato P, Lucero R, Muñoz A, Romero G, Levi D, Terg G, Miguez C, Abecasis R. Serum creatinine and bilirubin predict renal failure and mortality in patients with spontaneous bacterial peritonitis: a retrospective study. *Liver Int* 2009; **29**: 415-419 [PMID: 18803587 DOI: 10.1111/j.1478-3231.2008.01877.x]
136 **de Mattos ÁZ**, de Mattos AA, Méndez-Sánchez N. Hepatorenal syndrome: Current concepts related to diagnosis and management. *Ann Hepatol* 2016; **15**: 474-481 [PMID: 27236146]
137 **Guevara M**, Terra C, Nazar A, Solà E, Fernández J, Pavesi M, Arroyo V, Ginès P. Albumin for bacterial infections other than spontaneous bacterial peritonitis in cirrhosis. A randomized, controlled study. *J Hepatol* 2012; **57**: 759-765 [PMID: 22732511 DOI: 10.1016/j.jhep.2012.06.013]
138 **Thévenot T**, Bureau C, Oberti F, Anty R, Louvet A, Plessier A, Rudler M, Heurgué-Berlot A, Rosa I, Talbodec N, Dao T, Ozenne V, Carbonell N, Causse X, Goria O, Minello A, De Ledinghen V, Amathieu R, Barraud H, Nguyen-Khac E, Becker C, Paupard T, Botta-Fridlung D, Abdelli N, Guillemot F, Monnet E, Di Martino V. Effect of albumin in cirrhotic patients with infection other than spontaneous bacterial peritonitis. A randomized trial. *J Hepatol* 2015; **62**: 822-830 [PMID: 25463545 DOI: 10.1016/j.jhep.2014.11.017]
139 **Fernández J**, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, Vila C, Pardo A, Quintero E, Vargas V, Such J, Ginès P, Arroyo V. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology* 2007; **133**: 818-824 [PMID: 17854593 DOI: 10.1053/j.gastro.2007.06.065]
140 **Boyer TD**, Sanyal AJ, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Gülberg V, Sigal S, Bexon AS, Teuber P; Terlipressin Study Group. Impact of liver transplantation on the survival of patients treated for hepatorenal syndrome type 1. *Liver Transpl* 2011; **17**: 1328-1332 [PMID: 21837734 DOI: 10.1002/lt.22395]
141 **Cannon RM**, Jones CM, Davis EG, Eckhoff DE. Effect of Renal Diagnosis on Survival in Simultaneous Liver-Kidney Transplantation. *J Am Coll Surg* 2019; **228**: 536-544. e3 [PMID: 30586642 DOI: 10.1016/j.jamcollsurg.2018.12.003]
142 **Nadim MK**, Sung RS, Davis CL, Andreoni KA, Biggins SW, Danovitch GM, Feng S, Friedewald JJ, Hong JC, Kellum JA, Kim WR, Lake JR, Melton LB, Pomfret EA, Saab S, Genyk YS. Simultaneous liver-kidney transplantation summit: current state and future directions. *Am J Transplant* 2012; **12**: 2901-2908 [PMID: 22822723 DOI: 10.1111/j.1600-6143.2012.04190.x]
143 **Hussain SM**, Sureshkumar KK. Refining the Role of Simultaneous Liver Kidney Transplantation. *J Clin Transl Hepatol* 2018; **6**: 289-295 [PMID: 30271741 DOI: 10.14218/JCTH.2017.00065]
144 **Jenq CC**, Tsai MH, Tian YC, Lin CY, Yang C, Liu NJ, Lien JM, Chen YC, Fang JT, Chen PC, Yang CW. RIFLE classification can predict short-term prognosis in critically ill cirrhotic patients. *Intensive Care Med* 2007; **33**: 1921-1930 [PMID: 17605129 DOI: 10.1007/s00134-007-0760-6]
145 **Wong F**, O'Leary JG, Reddy KR, Patton H, Kamath PS, Fallon MB, Garcia-Tsao G, Subramanian RM, Malik R, Maliakkal B, Thacker LR, Bajaj JS; North American Consortium for Study of End-Stage Liver Disease. New consensus definition of acute kidney injury accurately predicts 30-day mortality in patients with cirrhosis and infection. *Gastroenterology* 2013; **145**: 1280-8. e1 [PMID: 23999172 DOI: 10.1053/j.gastro.2013.08.051]
146 **Huelin P**, Piano S, Solà E, Stanco M, Solé C, Moreira R, Pose E, Fasolato S, Fabrellas N, de Prada G, Pilutti C, Graupera I, Ariza X, Romano A, Elia C, Cárdenas A, Fernández J, Angeli P, Ginès P. Validation of a Staging System for Acute Kidney Injury in Patients With Cirrhosis and Association With Acute-on-Chronic Liver Failure. *Clin Gastroenterol Hepatol* 2017; **15**: 438-445. e5 [PMID: 27720915 DOI: 10.1016/j.cgh.2016.09.156]
147 **Bucsics T**, Mandorfer M, Schwabl P, Bota S, Sieghart W, Ferlitsch A, Trauner M, Peck-Radosavljevic M, Reiberger T. Impact of acute kidney injury on prognosis of patients with liver cirrhosis and ascites: A retrospective cohort study. *J Gastroenterol Hepatol* 2015; **30**: 1657-1665 [PMID: 25967931 DOI: 10.1111/jgh.13002]
148 **Mindikoglu AL**, Hernaez R, Liu Y, Kramer JR, Taylor T, Rana A, Kanwal F. Renal Trajectory Patterns Are Associated With Postdischarge Mortality in Patients With Cirrhosis and Acute Kidney

Injury. *Clin Gastroenterol Hepatol* 2020; **18**: 1858-1866. e6 [PMID: 31778807 DOI: 10.1016/j.cgh.2019.11.035]

149 **Makar M**, Reja D, Chouthai A, Kabaria S, Patel AV. The impact of acute kidney injury on mortality and clinical outcomes in patients with alcoholic cirrhosis in the USA. *Eur J Gastroenterol Hepatol* 2021; **33**: 905-910 [PMID: 32976187 DOI: 10.1097/MEG.0000000000001947]

150 **Patidar KR**, Shamseddeen H, Xu C, Ghabril MS, Nephew LD, Desai AP, Anderson M, El-Achkar TM, Ginès P, Chalasani NP, Orman ES. Hospital-Acquired Versus Community-Acquired Acute Kidney Injury in Patients With Cirrhosis: A Prospective Study. *Am J Gastroenterol* 2020; **115**: 1505-1512 [PMID: 32371628 DOI: 10.14309/ajg.0000000000000670]

151 **Tariq R**, Hadi Y, Chahal K, Reddy S, Salameh H, Singal AK. Incidence, Mortality and Predictors of Acute Kidney Injury in Patients with Cirrhosis: A Systematic Review and Meta-analysis. *J Clin Transl Hepatol* 2020; **8**: 135-142 [PMID: 32832393 DOI: 10.14218/JCTH.2019.00060]

152 **Alessandria C**, Ozdogan O, Guevara M, Restuccia T, Jiménez W, Arroyo V, Rodés J, Ginès P. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology* 2005; **41**: 1282-1289 [PMID: 15834937 DOI: 10.1002/hep.20687]

153 **Belcher JM**, Garcia-Tsao G, Sanyal AJ, Bhogal H, Lim JK, Ansari N, Coca SG, Parikh CR; TRIBE-AKI Consortium. Association of AKI with mortality and complications in hospitalized patients with cirrhosis. *Hepatology* 2013; **57**: 753-762 [PMID: 22454364 DOI: 10.1002/hep.25735]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4004-4017

DOI: 10.3748/wjg.v27.i26.4004

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Progress and challenges in the comprehensive management of chronic viral hepatitis: Key ways to achieve the elimination

Fátima Higuera-de la Tijera, Alfredo Servín-Caamaño, Luis Servín-Abad

ORCID number: Fátima Higuera-de la Tijera 0000-0003-3870-0478; Alfredo Servín-Caamaño 0000-0002-7214-0868; Luis Servín-Abad 0000-0002-1880-8505.

Author contributions: Higuera-de la Tijera F designed the overall concept of the manuscript; Higuera-de la Tijera F and Servín-Caamaño A performed the literature review and wrote the manuscript; Servín-Abad L translated and edited the manuscript to its English version.

Conflict-of-interest statement: Fátima Higuera-de-la-Tijera is speaker for Gilead Sciences Inc. Alfredo Servín-Caamaño and Luis Servín-Abad have not conflict of interests.

Fátima Higuera-de la Tijera, Department of Gastroenterology and Hepatology, Hospital General de México, Mexico City 06726, Mexico

Alfredo Servín-Caamaño, Department of Internal Medicine, Hospital General de México, Mexico City 06726, Mexico

Luis Servín-Abad, Department of Gastroenterology, Saint Cloud Hospital, Saint Cloud, MN 56303, United States

Corresponding author: Fátima Higuera-de la Tijera, MD, MSc, PhD, Academic Research, Professor, Department of Gastroenterology and Hepatology, Hospital General de México, Dr. Balmis 148 Col. Doctores, Del. Cuauhtémoc, Departamento de Gastroenterología 310-D., Mexico City 06726, Mexico. fatimahiguera@yahoo.com.mx

Abstract

Chronic viral hepatitis is a significant health problem throughout the world, which already represents high annual mortality. By 2040, chronic viral hepatitis due to virus B and virus C and their complications cirrhosis and hepatocellular carcinoma will be more deadly than malaria, vitellogenesis-inhibiting hormone, and tuberculosis altogether. In this review, we analyze the global impact of chronic viral hepatitis with a focus on the most vulnerable groups, the goals set by the World Health Organization for the year 2030, and the key points to achieve them, such as timely access to antiviral treatment of direct-acting antiviral, which represents the key to achieving hepatitis C virus elimination. Likewise, we review the strategies to prevent transmission and achieve control of hepatitis B virus. Finally, we address the impact that the coronavirus disease 2019 pandemic has had on implementing elimination strategies and the advantages of implementing telemedicine programs.

Key Words: Hepatitis C; Hepatitis B; Vaccination; Elimination program; Telemedicine; Direct antiviral agents

Core Tip: The World Health Organization proposes eliminating hepatitis infection as a

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Mexico

Peer-review report's scientific quality classification

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

Received: January 22, 2021
Peer-review started: January 22, 2021
First decision: February 28, 2021
Revised: March 4, 2021
Accepted: June 17, 2021
Article in press: June 17, 2021
Published online: July 14, 2021

P-Reviewer: Kumar R, Martin Mateos RM
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Ma YJ

threat to public health by 2030. Despite notable advances reached to achieve those goals, many challenges persist, such as guarantee access to complete vaccination schemes for hepatitis B virus and universal screening for all adults at least once in life to screen for hepatitis C virus. Those non-vaccinated against hepatitis B virus guarantee access to effective therapies programs to all patients who need it, emphasizing risk groups like prison inmates, sex workers, injecting drug users, and men who have sex with men, trying to reduce the high incidence of viral hepatitis in these groups. Telemedicine and telementoring approaches are valuable strategies to facilitate more patients access to healthcare systems and should be encouraged. Coronavirus disease 2019 pandemic affects all strategies significantly to eliminate viral hepatitis, particularly in low-income and middle-income countries. With available effective vaccines for anti-severe acute respiratory syndrome-coronavirus-2, strategies to immunize most people are crucial to restarting the viral hepatitis elimination programs throughout the world as soon as possible.

Citation: Higuera-de la Tijera F, Servín-Caamaño A, Servín-Abad L. Progress and challenges in the comprehensive management of chronic viral hepatitis: Key ways to achieve the elimination. *World J Gastroenterol* 2021; 27(26): 4004-4017
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4004.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4004

INTRODUCTION

More than 320 million people worldwide have chronic viral hepatitis. Around 248 million people are living with hepatitis B virus (HBV) chronic infection, which represents 3.2% of the global population[1-3]; and an estimated 71 to 80 million individuals (1.1%) are living with hepatitis C virus (HCV) chronic infection[3,4].

Chronic viral hepatitis and its related complications, cirrhosis and hepatocellular carcinoma (HCC), have been regarded as the leading causes of death for decades[1], causing globally more than 1 million deaths each year[5]. In fact, by 2040, deaths from chronic viral hepatitis are expected to exceed the related mortality as a whole from human immunodeficiency virus infection (HIV), tuberculosis, and malaria[5,6]. Liver disease due to viral hepatitis represents a substantial burden in the Asia-Pacific region. This region lives 1.8 billion people, which means around 25% of the world's population; a third of global deaths occur due to viral hepatitis, mainly driven by cirrhosis and HCC. Asia-Pacific represents 40% of the global burden of chronic hepatitis, where 115 million people in the Western Pacific are chronically infected with HBV and 14 million with HCV. Al least 58.6% of deaths due to cirrhosis and HCC in the Asia-Pacific region are related to HBV or HCV[7]. In 2013, China was the country that reported the most significant absolute number of deaths and disability-adjusted life-years attributable to viral hepatitis[1].

The North of Africa and the Middle East are also geographic regions extensively affected by viral hepatitis. They have a wide range of viral hepatitis causes, viremic prevalence, and diversity in HBV and HCV genotype distributions. Vaccination and treatment policies, socioeconomic conditions, and migration are responsible factors for the high prevalence of viral hepatitis in these particular regions. Here, elimination strategies might be challenging to implement because of a scarcity of reliable and profitable quality epidemiological data on hepatitis[8].

SEARCH METHODS

It is a narrative review. We searched PubMed, EMBASE, MEDLINE, and Web of Science from January 2015 to January 2021 to identify all studies documenting achievements and challenges on vaccination, diagnosis, access to healthcare systems, therapy, and elimination programs on hepatitis B and hepatitis C viral infections. The following search terms alone or matched with the Boolean operators "AND" or "OR" were used: "Hepatitis C," "hepatitis B," "World Health Organization (WHO)'s goals," "vaccination," "detection," "access to diagnosis," "access to healthcare system," "direct

antiviral agents," "sofosbuvir-velpatasvir (SOF-VEL)," "glecaprevir-pibrentasvir (G-P)," "entecavir (ETV)," "tenofovir disoproxil fumarate (TDF)," "tenofovir alafenamide (TAF)," "elimination program," "telemedicine," "coronavirus disease 2019 (COVID-19)". Using these terms, we found a total of 13497 articles; no study design or language restrictions were applied. We focused on full-text articles, but abstracts were considered if relevant. Finally, we selected the those with the most relevant content.

WHO GOALS FOR 2030

WHO goals are to achieve a 65% reduction in liver-related deaths, which means preventing more than 7 million related deaths by 2030, achieving a 90% reduction in viral hepatitis incidence, and reaching 90% of patients living with viral hepatitis diagnosed by 2030[9-12]. Specifically, in the case of HCV infection, the reduction in liver-related deaths is today achievable since the disponibility of direct-acting antivirals (DAAs), which have a high rate of sustained viral response (SVR). Nevertheless, an increase in harm reduction programs and treatment among populations at risk of transmission is undoubtedly still needed to reduce new infections[9].

For HBV infection, the WHO aims are divided into two main categories: First, prevention of new HBV cases through vaccination and blood safety; second, identification, linkage to care, and treatment of persons living with HBV who need it[10].

THE EFFECTIVE AND SAFE CURE FOR HEPATITIS C

In the absence of an effective vaccine, the cornerstone to achieving HCV elimination worldwide is treatment with DAAs[2], which have excellent efficacy and good tolerability profiles, offering a unique opportunity[13,14]. Currently, pan-genotypic regimens are available, which allows them to simplify decisions when initiating HCV therapy and ensuring universal access for these patients[15].

SOF-VEL is a pan-genotypic regimen that allows achieving the SVR in more than 95%. It can be prescribed even in decompensated cirrhosis because SOF-VEL is a protease inhibitor-free regimen proven effective and safe in this clinical scenario; HCV-infected liver posttransplant recipients are also effectively and safely treated with it SOF-VEL[16-29]. Several cohort studies also have validated the efficacy and safety of SOF-VEL in the real world[30-33]. Despite nearly 80% of SOF being renally excreted[4], the treatment with SOF-VEL is safe. It can be prescribed, achieving a SVR rate greater than 95% in patients with hepatitis C and end-stage renal disease, even in those requiring dialysis[34].

G-P, also a pan-genotypic regimen, is effective and safe in those without cirrhosis and with compensated cirrhosis[35-50] but is contraindicated in decompensated cirrhosis since glecaprevir is a protease inhibitor[4,15]. G-P is effective and safe in patients with end-stage renal disease[51-53]. The study MAGELLAN-2 validated that G-P is a safe and effective therapy to treat HCV infection in those patients who received a liver or kidney transplant[54].

Both pan-genotypic regimens, SOF-VEL, and G-P are also effective and safe in patients coinfected with HIV[55-57].

Around 5% of patients with chronic HCV infection treated with the first line DAAs do not achieve SVR; for this group of patients, sofosbuvir-velpatasvir-voxilaprevir (SOF-VEL-VOX) for 12 wk is the current option of rescue[4,15]. In a study including 137 patients who failed a previous combination of DAAs, a SVR of 95% was reached with SOF-VEL-VOX. Factors related to the reduced rate of SVR were genotype 3 and cirrhosis[58]. Even in those coinfected HIV-HCV patients who failed a previous combination of DAAs, the RESOLVE study demonstrated that 12 wk of SOF-VEL-VOX was safe and effective. The treatment response was not diminished by HIV coinfection [59].

Sixteen weeks of G-P treatment is an effective and safe option for those who failed NS5A or NS3-protease inhibitors[50,60,61]. In a randomized study including genotype 1 patients who failed previous treatment with SOF plus an NS5A inhibitor, retreatment with G-P achieved the SVR in greater than 90% of cases, including patients with compensated cirrhosis[60].

DAAS AND THE LIVER TRANSPLANT PROGRAMS

Since DAAs represent a highly effective and safe therapy, livers from HCV-infected donors can now be used to transplant, optimizing the transplant opportunity for more patients. After transplantation from an HCV-positive donor, the occurrence of HCV infection in HCV-negative recipients is practically universal, requiring post-transplant antiviral treatment[62].

Some interesting strategies are being studied to reduce HCV infection likelihood in organ recipients from HCV-infected donors. Feld *et al*[62] found that ezetimibe (10 mg; an HCV entry inhibitor) plus G-P (300 mg/120 mg) given previous and during 7 d after transplant avoided the occurrence of chronic hepatitis C in 30 (100%) recipients of different organs from HCV-positive donors.

Although patients with HCV infection had a higher risk of post-liver transplant (LT) graft failure and death in the pre-DAA era, this issue seems to be solved in the post-DAA era[63]. The burden of HCV-related LT waitlist and LT is declining in the DAA era, with improved post-transplant outcomes[64]. It probably reflects the impact of DAAs on bettering post-LT results in patients with hepatitis C and maybe also a better patient selection for a LT after 2014[63]. After the availability of DAAs, HCV as an indication for LT has reduced, patients exhibit a less severe disease at transplantation, and there is a trend towards better patient survival[65,66].

Overall listing rates for decompensated HCV cirrhosis have decreased in the DAA era. According to Bittermann and Reddy[67], waitlist recovery is more frequent for HCV patients post-DAAs [adjusted survival hazard ratio 1.78 *vs* pre-DAAs, 95% confidence interval (95%CI): 1.58-2.02; $P < 0.001$], while improvements in waitlist mortality by era are similar to non-HCV candidates [adjusted survival hazard ratio 0.74 (95%CI: 0.7-0.78; $P < 0.001$) and 0.77 (95%CI: 0.74-0.8; $P < 0.001$), respectively][67].

THE STRATEGIES TO CONTROL HEPATITIS B TRANSMISSION AND TO CONTROL THE BURDEN OF DISEASE

Universal vaccination is the essential strategy to prevent HBV transmission. Already in 1992, WHO recommended introducing universal childhood vaccination all around the world. Nowadays, at least 180 countries have adopted this recommendation[68]. The efficacy of universal vaccination programs has been demonstrated in several countries all around the world. In Taiwan, the prevalence of hepatitis B surface antigen (HBsAg) decreased notably from 14.3% in 1995 to 1.1% in 2009, and the seroprevalence of hepatitis B e-antigen (HBeAg) reduced from 5.9% in 1995 to 0.3% in 2009[69]. Furthermore, in Taiwan, the HCC incidence reduced from 0.57 to 0.17 *per* 100000 person-years following mass anti-HBV vaccination[70].

Before the HBV vaccination program, Korea was considered an area of high endemicity. Studies from the 1980s and 1990s revealed that chronic HBV carriage prevalence ranged from 8%-10% before introducing the anti-HBV vaccination in Korea. Since 1990, the percentage of vaccinated infants has surpassed 98.9%, and after 25 years of active vaccination, the HBsAg carrier rate in the general population decreased to 3.7% in 2007. Also, the administration of the anti-HBV vaccine reduced the risk of HCC among adults[71].

However, continuous efforts are needed to ensure timely access to be vaccinated with comprehensive schemes[72]. In spite of the success of vaccination and therapy, chronic hepatitis B (CHB) infection remains a major concern due to many patients ignoring their clinical status. The troubles in diagnosis and screening may be overcome by lifting awareness, favoring partnerships, and allocating resources[73]. In a meta-analysis of 26 studies, the prevalence of HBV infection in non-vaccinated and vaccinated cohorts went from 0.6% to 16.3% and from 0.3% to 8.5%, respectively. The relative prevalence, comparing vaccinated *vs* non-vaccinated, was 0.24 (95%CI: 0.16-0.35) for HBsAg and 0.23 (95%CI: 0.17-0.32) for antibody anti-hepatitis B core antigen. For populations with targeted vaccination, relative prevalence was 0.32 (95%CI: 0.24-0.43) and 0.33 (95%CI: 0.23-0.45), respectively. The residual burden of infection in cohorts offered vaccination suggests that longer-term evaluations of vaccination coverage, timeliness, and other program quality aspects are needed. As HBV-vaccinated infant cohorts reach adulthood, ongoing analysis of prevalence in adolescents and young adults will ensure that elimination efforts are on track[72].

Notwithstanding guidelines suggest screening in high-risk groups like immigrants, these recommendations have not been adopted everywhere[73]. Also, there is a need to improve the uptake of vaccination for household contacts of HBV carriers[74].

The second important strategy to avoid the transmission and control the disease's burden in people living with CHB infection is to guarantee access to medical care and treatment[75,76]. However, most people with CHB live in resource-constrained countries where effective drugs are not always widely available[73]. First-choice drugs in patients with CHB, who meet the criteria for initiating treatment, include nucleoside analogs (ETV) and nucleotide analogs (TDF and TAF)[77-79]. After 10 years of follow-up, TDF and ETV showed effective suppression of the HBV viral load, between 94% and 99%, both in HBeAg-positive and HBeAg-negative patients. HBeAg seroconversion in HBeAg-positive patients with TDF or ETV has been reported in 49%-53% of cases. Alanine aminotransferase normalization has been achieved between 77% and 83% of patients with CHB treated with any of these regimens. However, the annual frequency of HBsAg seroconversion is rare (< 1% annually)[80]. TAF is as effective as TDF but with a better bone and renal safety profile[81-84]. However, some disparities in the opportunity to access hepatitis B therapy have been reported. Miquel *et al*[85] found that a minor proportion of non-immigrants with the indication of effectively receiving hepatitis B therapy got it, compared with non-immigrants (57.8 *vs* 83.2%, P < 0.001)[85]. Similarly, other studies also have reported that immigrants are lost more frequently during the 1st year of follow-up[86]. Immigrants constitute a vulnerable group that would benefit from a more active approach to recognize timely HBV infection and access treatment programs[87].

THE EFFORTS TO CONSTRUCT MICRO AND MACRO-ELIMINATION PROGRAMS THROUGHOUT THE WORLD

The high chronic hepatitis prevalence groups should be recognized and prioritized for detection and linkage to healthcare to reduce the risk of transmitting these infectious diseases. The most vulnerable groups are prison inmates, homosexual men, intravenous drug users (IDU), and sex workers[88]. According to the study by Alonso *et al* [88], in Latin America and the Caribbean, the estimated pooled regional anti-HCV prevalence for IDU was 49% (95%CI: 22.6%-76.3 %); for homosexual men was 3% (95%CI: 1.7%-4.5%); for sex workers was 2% (95%CI: 1.0%-3.4%)[88].

In Canada, penitentiary test-and-treat programs could achieve the most significant decreases in incidence (48%; 95% crude incidence: 38%-57%) over 2018-2030 and prevent the newest first chronic infections (22%; 95% crude incidence: 16%-28%) within those who never exposed to HCV[89]. The project HIPPOCRATES is an example of a micro-elimination program conducted in prison inmates, a vulnerable population to receive treatment less frequently due to many obstacles in healthcare access. The onsite evaluation and treatment of HCV-infected prison inmates achieved an unprecedented effective success rate (SVR was 99%). This type of integral program should be replicated to favor hepatitis C elimination[90].

More attention should be paid to the risk group of homosexual men since HCV incidence in this high-risk group seems to be increasing. In France, a recently important change in HCV epidemiology was reported within HIV-infected patients since the higher rate of HCV transmission occurs in 2018 among homosexual men. From 2012 to 2018, the HCV prevalence among new HIV cases increased from 1.9% to 3.5% in homosexual men. Recently acquired HCV incidence increased from 0.36/100 person-years to 1.25/100 person-years in homosexual men. If well, the proportion of all viremic patients reduced from 67.0% to 8.9%, homosexual men became the first group of viremic patients in 2018 (37.9%), and recently acquired hepatitis represented 59.2% of viremic homosexual men in 2018. Global DAA treatment prescription went from 11.4% to 61.5%. More treatments were initiated in homosexual men in 2018 (41.2%). In homosexual men, treatment at the acute phase represented 30.0% of treatments in 2018[91]. In Spain, a very close to HCV elimination country, homosexual men also carry the highest HCV acquisition risk. The identified main risk factors contributing to new cases of HCV infection in Spain are history of sexually acquired infections [incidence rate ratio (IRR) = 18.2, 95%CI: 1.9-172.1; P = 0.01)], male gender (IRR = 8.3, 95%CI: 1.4-54.2; P = 0.03)] and sharing chem-sex drugs (IRR: 4.9, 95%CI: 1.2-20.8; P = 0.03)[92]. In the Netherlands, homosexual men also have the highest incidence and the highest HCV reinfection rate despite universal and unrestricted access to DAAs, stressing the need for additional preventive measures[93,94].

However, other risk factors should not be minimized either; for example, the unapparent parenteral transmission, through shared nail clippers, rakes, and manicure scissors can also be the primary source of viral infection[95]. Therefore, it is now recommended to perform universal one-time in-life routine HCV screening for all

adults[15].

Likewise, the telemedicine programs and telementoring approaches are outstanding options that may help reduce urban-rural disparities, facilitate access to healthcare systems to receive timely therapy to all kinds of patients who need it, and save costs [96-104]. In Mexico, with the aid of a telemedicine approach, significant savings were achieved by minimizing costs since nearly half of the patients were outsiders. Coverage reached 86%, and treatment with DAAs achieved 99% of SVR[100] (see Table 1).

HOW HAS THE COVID-19 PANDEMIC AFFECTED THE WHO'S GOALS TO ELIMINATE CHRONIC VIRAL HEPATITIS?

Quarantine and social distancing for COVID-19 can drastically affect some parts of the HBV[105] and HCV elimination programs, such as diagnosis, treatment, and harm reduction programs. Therefore, the rate of diagnosis has decreased as voluntary activities such as the NoHep program have been reduced. Furthermore, the incidence of viral hepatitis may increase due to the closure of harm reduction centers[106]. According to the World Hepatitis Alliance global survey to evaluate the collateral damage of the pandemic on viral hepatitis elimination programs, civil society organizations are a vital contributor to the success of the elimination programs; of them, 123 of 131 (94%) reported that the effect of the COVID-19 pandemic altered their activities. A participant from the United States reported that collateral effects from the COVID-19 pandemic included the limitation or even the stop of presential interventions, also affecting community education and detection programs. As a negative outcome, fewer people living with viral hepatitis are expected to be diagnosed during 2020[107]. The World Hepatitis Alliance survey data show that treatment access has been significantly deteriorated by COVID-19 in low-income and middle-income countries (LMICs), with 15 (52%) of 29 respondents from those countries described that the patients could not timely access treatments. However, in high-income countries, like the United Kingdom, the impact of COVID-19 on HCV treatment will be lesser, partly due to telemedicine and home delivery of medicines, conditions that are not very feasible in LMICs[108]. Sperring *et al*[109] explored the impact of the COVID-19 pandemic on screening HCV testing, finding a comprehensive hospital-wide HCV testing reduced by 49.6%, and new HCV+ patient identification reduced by 42.1%. In ambulatory clinics, testing reduced by 71.9%, and new HCV+ identification reduced by 63.3%[109].

According to the mathematical model projection by Blach *et al*[110], a 1-year delay in viral hepatitis elimination programs will result in 44800 [95% uncertainty interval (UI): 43800-49300] excess HCC cases and 72300 (95%UI: 70600-79400) excess liver-related deaths, relative to the no-delay scenario globally, from 2020 to 2030. Most missed treatments would be in LMICs, whereas most excess HCC and liver-related deaths would be among high-income countries. Authorities should privilege hepatitis programs as soon as safe to attenuate the negative impact on elimination programs and reduce excess mortality from delayed treatment[110].

With the approval of a severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) vaccine, most of the possibility to reactivate elimination viral hepatitis programs throughout the world will rely on SARS-CoV-2 effective vaccination strategies that gradually allow restarting the function of viral hepatitis detection campaigns, safe-needle programs, and outpatient clinics to dispenser antiviral medication. According to mathematical modeling analyses, a vaccine with efficacy (VE) ≥ 70% can prevent the infection. A vaccine with VE < 70% may still control the infection transmission if it reduces infectiousness or infection duration among those vaccinated who acquire the infection if it is supplemented with a < 20% reduction in contact rate complemented with herd immunity. The probability of a significant outbreak is zero at VE ≥ 70% regardless of the number of virus introductions. However, an increase in the social contact rate among those vaccinated (behavior compensation) can undermine vaccine impact[111]. Existing reports of currently approved SARS-CoV-2 vaccines indicate their effectiveness at around 95%, making it very plausible to achieve collective herd-acquired immunity based on the mass implementation of vaccination programs against COVID-19 soon[112].

Table 1 World Health Organization's goals to achieve viral hepatitis elimination and strategies to make it

Goal to 2030	Existing resources	Barriers	Strategies that should be improved
Hepatitis C			
90% reduction of new viral hepatitis infections	Harm reduction programs: Safe-sex, safe-needles, and safe-syringes	If well, programs exist in the real-life world are not always sufficiently implemented	Target high-risk population such as MSM, prison inmates, sexual workers, patients with HIV, IDU, immigrants, children born from an HCV+ mother
To reach 90% of patients with viral hepatitis infections being diagnosed	Tests with high sensitivity	If well, detection campaigns exist, it is not enough to reach all people in a real-life setting	Once in life, universal screening for all adults. Also target high-risk population such as immigrants, MSM, prison inmates, sexual workers, patients with HIV, IDU, children born from an HCV+ mother
65% reduction in liver-related deaths	DAAs. Telemedicine and telementoring programs	Still, there is limited access to therapy. More restrained access in LMICs. Vulnerable groups with high prevalence and incidence of viral hepatitis have restricted access to therapy	Flexible policies that guarantee timely access to treatment to all who need it, including vulnerable groups such as immigrants, prison inmates, sexual workers, patients with HIV, IDU, children born from an HCV+ mother when appropriate. Consider including those without healthcare insurance to cover their medication. Encourage telemedicine programs to access communities of difficult access
Hepatitis B			
Prevention of new HBV infections through vaccination and blood safety	Effective and safe vaccine	In the real-life world they are not always available or schemes are applied incompletely	Programs that effectively ensure universal and complete schemes of vaccination at birth for infants and later for those who did not receive the vaccination in childhood. Coverage should be extended and also prioritized for vulnerable groups
Identification, linkage to care, and treatment of persons with chronic HBV	Serologic HBV panels. Nucleos(t)ide analogs with a highly effective and high barrier to resistance Telemedicine and telementoring programs	Serologic HBV panels for diagnosis sometimes are restricted to specialists. Still, there is limited access to therapy, more restrained in LMICs. Vulnerable groups with high prevalence and incidence of viral hepatitis have restricted access to therapy	Basic diagnostic tests (HBsAg and anti-HBc) should be available at primary healthcare. More flexible policies that guarantee timely access to treatment to all who need it, including vulnerable groups such as immigrants, prison inmates, sexual workers, IDU, children born from an HCV+ mother when appropriate. Consider including those without healthcare insurance to cover their medication. Encourage telemedicine programs to access communities of difficult access

anti-HBc: Antibody against hepatitis B core antigen; DAAs: Direct antiviral agents; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCV+: Positive to hepatitis C virus; HIV: Human immunodeficiency virus; IDU: Injecting drug users; LMICs: Low and middle-income countries; MSM: Men who have sex with men.

CONCLUSION

Chronic viral hepatitis and its complications, cirrhosis, and HCC affect many people worldwide. Without a plan of action, the projection to 2040 will exceed the related mortality as a whole from other significant infectious healthcare problems. Asia-Pacific, Middle East, and North Africa regions have the highest prevalence, representing a substantial burden of the disease. Hopefully, notable advances have been made to achieve WHO goals to 2030 regarding eliminating hepatitis infection better adaptable to actual reality. In that case, actions need to continue being implemented, which must include more harm limitation programs and timely therapy access for those at risk of transmission are certainly needed to reach an incidence decrease. Since universal vaccination is the essential strategy to prevent HBV transmission, continuous efforts are needed to ensure timely access to be vaccinated with comprehensive schemes. Strategies to find positive contacts ensuing a timely screening and diagnosis must be continuously promoted. To avoid viral hepatitis transmission and control the burden of the disease, guarantee access to medical care and effective therapies must include all people who need it, with more emphasis on including vulnerable groups with currently limited access like immigrants, prison inmates, and sex workers. More attention should be paid to the risk group of men who have sex with men since HCV incidence in this high-risk group seems to be increasing. Telemedicine and telementoring approaches facilitate access to healthcare systems and save costs; therefore, this kind of program should be implemented. Finally, the COVID-19 pandemic is currently a significant challenge to achieve viral hepatitis elimination; with the recent approval of a SARS-CoV-2 vaccine, most of the possibility to reactivate elimination viral hepatitis programs throughout the world will rely on SARS-CoV-2 effective vaccination strategies that gradually allows restarting the operativity of liver clinics and services.

REFERENCES

1 **The Lancet**. Towards elimination of viral hepatitis by 2030. *Lancet* 2016; **388**: 308 [PMID: 27477148 DOI: 10.1016/S0140-6736(16)31144-8]

2 **Lazarus JV**, Wiktor S, Colombo M, Thursz M; EASL International Liver Foundation. Micro-elimination - A path to global elimination of hepatitis C. *J Hepatol* 2017; **67**: 665-666 [PMID: 28760329 DOI: 10.1016/j.jhep.2017.06.033]

3 **Howell J**, Pedrana A, Cowie BC, Doyle J, Getahun A, Ward J, Gane E, Cunningham C, Wallace J, Lee A, Malani J, Thompson A, Hellard ME. Aiming for the elimination of viral hepatitis in Australia, New Zealand, and the Pacific Islands and Territories: Where are we now and barriers to meeting World Health Organization targets by 2030. *J Gastroenterol Hepatol* 2019; **34**: 40-48 [PMID: 30151932 DOI: 10.1111/jgh.14457]

4 **European Association for the Study of the Liver**. Clinical Practice Guidelines Panel: Chair:; EASL Governing Board representative; Panel members. EASL recommendations on treatment of hepatitis C: Final update of the series☆. *J Hepatol* 2020; **73**: 1170-1218 [PMID: 32956768 DOI: 10.1016/j.jhep.2020.08.018]

5 **Thomas DL**. Global Elimination of Chronic Hepatitis. *N Engl J Med* 2019; **380**: 2041-2050 [PMID: 31116920 DOI: 10.1056/NEJMra1810477]

6 **Foreman KJ**, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, Pletcher MA, Smith AE, Tang K, Yuan CW, Brown JC, Friedman J, He J, Heuton KR, Holmberg M, Patel DJ, Reidy P, Carter A, Cercy K, Chapin A, Douwes-Schultz D, Frank T, Goettsch F, Liu PY, Nandakumar V, Reitsma MB, Reuter V, Sadat N, Sorensen RJD, Srinivasan V, Updike RL, York H, Lopez AD, Lozano R, Lim SS, Mokdad AH, Vollset SE, Murray CJL. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet* 2018; **392**: 2052-2090 [PMID: 30340847 DOI: 10.1016/S0140-6736(18)31694-5]

7 **Kasai T**. Time to act to make elimination of viral hepatitis a reality. *Lancet Gastroenterol Hepatol* 2020; **5**: 102-103 [PMID: 31852633 DOI: 10.1016/S2468-1253(19)30303-6]

8 **Poortahmasebi V**, Baghi HB. Living in the shadows of hepatitis. *Lancet Infect Dis* 2019; **19**: 1171-1172 [PMID: 31657779 DOI: 10.1016/S1473-3099(19)30534-1]

9 **European Union HCV Collaborators**. Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: a modelling study. *Lancet Gastroenterol Hepatol* 2017; **2**: 325-336 [PMID: 28397696 DOI: 10.1016/S2468-1253(17)30045-6]

10 **McMahon BJ**. Meeting the WHO and US Goals to Eliminate Hepatitis B Infection by 2030: Opportunities and Challenges. *Clin Liver Dis (Hoboken)* 2018; **12**: 29-32 [PMID: 30988906 DOI: 10.1002/cld.733]

11 **Ward JW**, Hinman AR. What Is Needed to Eliminate Hepatitis B Virus and Hepatitis C Virus as Global Health Threats. *Gastroenterology* 2019; **156**: 297-310 [PMID: 30391470 DOI: 10.1053/j.gastro.2018.10.048]

12 **Popping S**, El-Sayed M, Feld J, Hatzakis A, Hellard M, Lesi O, Ninburg M, Ward J, Boucher C. Report from the International Viral Hepatitis Elimination Meeting (IVHEM), 17-18 November 2017, Amsterdam, the Netherlands: gaps and challenges in the WHO 2030 hepatitis C elimination framework. *J Virus Erad* 2018; **4**: 193-195 [PMID: 30050685]

13 **Asselah T**, Marcellin P, Schinazi RF. Treatment of hepatitis C virus infection with direct-acting antiviral agents: 100% cure? *Liver Int* 2018; **38** Suppl 1: 7-13 [PMID: 29427484 DOI: 10.1111/liv.13673]

14 **Asselah T**. A village without hepatitis C in Egypt: will micro-elimination lead to macro-elimination? *Lancet Gastroenterol Hepatol* 2018; **3**: 734-736 [PMID: 30030069 DOI: 10.1016/S2468-1253(18)30178-X]

15 **Ghany MG**, Morgan TR; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* 2020; **71**: 686-721 [PMID: 31816111 DOI: 10.1002/hep.31060]

16 **Feld JJ**, Jacobson IM, Hézode C, Asselah T, Ruane PJ, Gruener N, Abergel A, Mangia A, Lai CL, Chan HL, Mazzotta F, Moreno C, Yoshida E, Shafran SD, Towner WJ, Tran TT, McNally J, Osinusi A, Svarovskaia E, Zhu Y, Brainard DM, McHutchison JG, Agarwal K, Zeuzem S; ASTRAL-1 Investigators. Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. *N Engl J Med* 2015; **373**: 2599-2607 [PMID: 26571066 DOI: 10.1056/NEJMoa1512610]

17 **Foster GR**, Afdhal N, Roberts SK, Bräu N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C, Towner WJ, Conway B, Ruane P, Bourlière M, Asselah T, Berg T, Zeuzem S, Rosenberg W, Agarwal K, Stedman CA, Mo H, Dvory-Sobol H, Han L, Wang J, McNally J, Osinusi A, Brainard DM, McHutchison JG, Mazzotta F, Tran TT, Gordon SC, Patel K, Reau N, Mangia A, Sulkowski M; ASTRAL-2 Investigators; ASTRAL-3 Investigators. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N Engl J Med* 2015; **373**: 2608-2617 [PMID: 26575258 DOI: 10.1056/NEJMoa1512612]

18 **Curry MP**, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, Reddy KR, Lawitz E, Flamm SL, Schiano T, Teperman L, Fontana R, Schiff E, Fried M, Doehle B, An D, McNally J, Osinusi A, Brainard DM, McHutchison JG, Brown RS Jr, Charlton M; ASTRAL-4 Investigators.

Sofosbuvir and Velpatasvir for HCV in Patients with Decompensated Cirrhosis. *N Engl J Med* 2015; **373**: 2618-2628 [PMID: 26569658 DOI: 10.1056/NEJMoa1512614]

19 **Grebely J**, Dalgard O, Conway B, Cunningham EB, Bruggmann P, Hajarizadeh B, Amin J, Bruneau J, Hellard M, Litwin AH, Marks P, Quiene S, Siriragavan S, Applegate TL, Swan T, Byrne J, Lacalamita M, Dunlop A, Matthews GV, Powis J, Shaw D, Thurnheer MC, Weltman M, Kronborg I, Cooper C, Feld JJ, Fraser C, Dillon JF, Read P, Gane E, Dore GJ; SIMPLIFY Study Group. Sofosbuvir and velpatasvir for hepatitis C virus infection in people with recent injection drug use (SIMPLIFY): an open-label, single-arm, phase 4, multicentre trial. *Lancet Gastroenterol Hepatol* 2018; **3**: 153-161 [PMID: 29310928 DOI: 10.1016/S2468-1253(17)30404-1]

20 **Isakov V**, Chulanov V, Abdurakhmanov D, Burnevich E, Nurmukhametova E, Kozhevnikova G, Gankina N, Zhuravel S, Romanova S, Hyland RH, Lu S, Svarovskaia ES, McNally J, Brainard DM, Ivashkin V, Morozov V, Bakulin I, Lagging M, Zhdanov K, Weiland O. Sofosbuvir/velpatasvir for the treatment of HCV: excellent results from a phase-3, open-label study in Russia and Sweden. *Infect Dis (Lond)* 2019; **51**: 131-139 [PMID: 30499360 DOI: 10.1080/23744235.2018.1535186]

21 **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]

22 **Agarwal K**, Castells L, Müllhaupt B, Rosenberg WMC, McNabb B, Arterburn S, Camus G, McNally J, Stamm LM, Brainard DM, Mani Subramanian G, Mariño Z, Dufour JF FX. Sofosbuvir / velpatasvir for 12 wk in genotype 1 – 4 HCV-infected liver transplant recipients. *J Hepatol* 2018; **69**: 603-607 [DOI: 10.1016/j.jhep.2018.05.039]

23 **Asselah T**, Shafran SD, Bourgeois S, Lai CL, Mathurin P, Willems B, Nguyen MH, Davis MN, Huang KC, Svarovskaia E, Osinusi A, McNally J, Brainard DM, Shaikh OS, Tran TT. Deferred treatment with a fixed-dose combination of sofosbuvir-velpatasvir for chronic hepatitis C virus genotype 1, 2, 4 and 6 infection. *J Viral Hepat* 2019; **26**: 1229-1232 [PMID: 31216086 DOI: 10.1111/jvh.13159]

24 **Sood A**, Duseja A, Kabrawala M, Amrose P, Goswami B, Chowdhury A, Sarin SK, Koshy A, Hyland RH, Lu S, Camus G, Stamm LM, Brainard DM, Subramanian GM, Prasad M, Bhatia S, Shah SR, Kapoor D, Shalimar, Saraswat V. Sofosbuvir-velpatasvir single-tablet regimen administered for 12 weeks in a phase 3 study with minimal monitoring in India. *Hepatol Int* 2019; **13**: 173-179 [PMID: 30790229 DOI: 10.1007/s12072-019-09927-6]

25 **Wei L**, Lim SG, Xie Q, Văn KN, Piratvisuth T, Huang Y, Wu S, Xu M, Tang H, Cheng J, Le Manh H, Gao Y, Mou Z, Sobhonslidsuk A, Dou X, Thongsawat S, Nan Y, Tan CK, Ning Q, Tee HP, Mao Y, Stamm LM, Lu S, Dvory-Sobol H, Mo H, Brainard DM, Yang YF, Dao L, Wang GQ, Tanwandee T, Hu P, Tangkijvanich P, Zhang L, Gao ZL, Lin F, Le TTP, Shang J, Gong G, Li J, Su M, Duan Z, Mohamed R, Hou JL, Jia J. Sofosbuvir-velpatasvir for treatment of chronic hepatitis C virus infection in Asia: a single-arm, open-label, phase 3 trial. *Lancet Gastroenterol Hepatol* 2019; **4**: 127-134 [PMID: 30555048 DOI: 10.1016/S2468-1253(18)30343-1]

26 **Takehara T**, Sakamoto N, Nishiguchi S, Ikeda F, Tatsumi T, Ueno Y, Yatsuhashi H, Takikawa Y, Kanda T, Sakamoto M, Tamori A, Mita E, Chayama K, Zhang G, De-Oertel S, Dvory-Sobol H, Matsuda T, Stamm LM, Brainard DM, Tanaka Y, Kurosaki M. Efficacy and safety of sofosbuvir-velpatasvir with or without ribavirin in HCV-infected Japanese patients with decompensated cirrhosis: an open-label phase 3 trial. *J Gastroenterol* 2019; **54**: 87-95 [PMID: 30203225 DOI: 10.1007/s00535-018-1503-x]

27 **Esteban R**, Pineda JA, Calleja JL, Casado M, Rodríguez M, Turnes J, Morano Amado LE, Morillas RM, Forns X, Pascasio Acevedo JM, Andrade RJ, Rivero A, Carrión JA, Lens S, Riveiro-Barciela M, McNabb B, Zhang G, Camus G, Stamm LM, Brainard DM, Subramanian GM, Buti M. Efficacy of Sofosbuvir and Velpatasvir, With and Without Ribavirin, in Patients With Hepatitis C Virus Genotype 3 Infection and Cirrhosis. *Gastroenterology* 2018; **155**: 1120-1127. e4 [PMID: 29958855 DOI: 10.1053/j.gastro.2018.06.042]

28 **Hezode C**, Reau N, Svarovskaia ES, Doehle BP, Shanmugam R, Dvory-Sobol H, Hedskog C, McNally J, Osinusi A, Brainard DM, Miller MD, Mo H, Roberts SK, O'Leary JG, Shafran SD, Zeuzem S. Resistance analysis in patients with genotype 1-6 HCV infection treated with sofosbuvir/velpatasvir in the phase III studies. *J Hepatol* 2018; **68**: 895-903 [PMID: 29221887 DOI: 10.1016/j.jhep.2017.11.032]

29 **Grebely J**, Dore GJ, Zeuzem S, Aspinall RJ, Fox R, Han L, McNally J, Osinusi A, Brainard DM, Subramanian GM, Natha M, Foster GR, Mangia A, Sulkowski M, Feld JJ. Efficacy and Safety of Sofosbuvir/Velpatasvir in Patients With Chronic Hepatitis C Virus Infection Receiving Opioid Substitution Therapy: Analysis of Phase 3 ASTRAL Trials. *Clin Infect Dis* 2016; **63**: 1479-1481 [PMID: 27553377 DOI: 10.1093/cid/ciw579]

30 **Mangia A**, Milligan S, Khalili M, Fagiuoli S, Shafran SD, Carrat F, Ouzan D, Papatheodoridis G, Ramji A, Borgia SM, Wedemeyer H, Losappio R, Pérez-Hernandez F, Wick N, Brown RS Jr, Lampertico P, Doucette K, Ntalla I, Ramroth H, Mertens M, Vanstraelen K, Turnes J. Global real-world evidence of sofosbuvir/velpatasvir as simple, effective HCV treatment: Analysis of 5552 patients from 12 cohorts. *Liver Int* 2020; **40**: 1841-1852 [PMID: 32449966 DOI: 10.1111/liv.14537]

31 **Mangia A**, Piazzolla V, Giannelli A, Visaggi E, Minerva N, Palmieri V, Carraturo I, Potenza D,

Napoli N, Lauletta G, Tagarielli V, Santoro R, Piccigallo E, De Gioia S, Chimenti A, Cuccorese G, Metrangolo A, Mazzola M, Agostinacchio E, Mennea G, Sabbà C, Cela M, Copetti M, Losappio R. SVR12 rates higher than 99% after sofosbuvir/velpatasvir combination in HCV infected patients with F0-F1 fibrosis stage: A real world experience. *PLoS One* 2019; **14**: e0215783 [PMID: 31091254 DOI: 10.1371/journal.pone.0215783]

32 **von Felden J**, Vermehren J, Ingiliz P, Mauss S, Lutz T, Simon KG, Busch HW, Baumgarten A, Schewe K, Hueppe D, Boesecke C, Rockstroh JK, Daeumer M, Luebke N, Timm J, Schulze Zur Wiesch J, Sarrazin C, Christensen S. High efficacy of sofosbuvir/velpatasvir and impact of baseline resistance-associated substitutions in hepatitis C genotype 3 infection. *Aliment Pharmacol Ther* 2018; **47**: 1288-1295 [PMID: 29536554 DOI: 10.1111/apt.14592]

33 **Mangia A**, Cenderello G, Copetti M, Verucchi G, Piazzolla V, Lorusso C, Santoro R, Squillante MM, Orlandini A, Minisini R, Ciancio A. SVR12 Higher than 97% in GT3 Cirrhotic Patients with Evidence of Portal Hypertension Treated with SOF/VEL without Ribavirin: A Nation-Wide Cohort Study. *Cells* 2019; **8** [PMID: 30987413 DOI: 10.3390/cells8040313]

34 **Borgia SM**, Dearden J, Yoshida EM, Shafran SD, Brown A, Ben-Ari Z, Cramp ME, Cooper C, Foxton M, Rodriguez CF, Esteban R, Hyland R, Lu S, Kirby BJ, Meng A, Markova S, Dvory-Sobol H, Osinusi AO, Bruck R, Ampuero J, Ryder SD, Agarwal K, Fox R, Shaw D, Haider S, Willems B, Lurie Y, Calleja JL, Gane EJ. Sofosbuvir/velpatasvir for 12 weeks in hepatitis C virus-infected patients with end-stage renal disease undergoing dialysis. *J Hepatol* 2019; **71**: 660-665 [PMID: 31195062 DOI: 10.1016/j.jhep.2019.05.028]

35 **Wei L**, Wang G, Alami NN, Xie W, Heo J, Xie Q, Zhang M, Kim YJ, Lim SG, Fredrick LM, Lu W, Liu W, Kalluri HV, Krishnan P, Tripathi R, Mobashery N, Burroughs M, Asatryan A, Jia J, Hou J. Glecaprevir-pibrentasvir to treat chronic hepatitis C virus infection in Asia: two multicentre, phase 3 studies- a randomised, double-blind study (VOYAGE-1) and an open-label, single-arm study (VOYAGE-2). *Lancet Gastroenterol Hepatol* 2020; **5**: 839-849 [PMID: 32682494 DOI: 10.1016/S2468-1253(20)30086-8]

36 **Toyoda H**, Atsukawa M, Watanabe T, Nakamuta M, Uojima H, Nozaki A, Takaguchi K, Fujioka S, Iio E, Shima T, Akahane T, Fukunishi S, Asano T, Michitaka K, Tsuji K, Abe H, Mikami S, Okubo H, Okubo T, Shimada N, Ishikawa T, Moriya A, Tani J, Morishita A, Ogawa C, Tachi Y, Ikeda H, Yamashita N, Yasuda S, Chuma M, Tsutsui A, Hiraoka A, Ikegami T, Genda T, Tsubota A, Masaki T, Tanaka Y, Iwakiri K, Kumada T. Real-world experience of 12-week direct-acting antiviral regimen of glecaprevir and pibrentasvir in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2020; **35**: 855-861 [PMID: 31609495 DOI: 10.1111/jgh.14874]

37 **Gane E**, Poordad F, Zadeikis N, Valdes J, Lin CW, Liu W, Asatryan A, Wang S, Stedman C, Greenbloom S, Nguyen T, Elkhashab M, Wörns MA, Tran A, Mulkay JP, Setze C, Yu Y, Pilot-Matias T, Porcalla A, Mensa FJ. Safety and Pharmacokinetics of Glecaprevir/Pibrentasvir in Adults With Chronic Genotype 1-6 Hepatitis C Virus Infections and Compensated Liver Disease. *Clin Infect Dis* 2019; **69**: 1657-1664 [PMID: 30923816 DOI: 10.1093/cid/ciz022]

38 **Foster GR**, Asselah T, Kopecky-Bromberg S, Lei Y, Asatryan A, Trinh R, Zadeikis N, Mensa FJ. Safety and efficacy of glecaprevir/pibrentasvir for the treatment of chronic hepatitis C in patients aged 65 years or older. *PLoS One* 2019; **14**: e0208506 [PMID: 30601818 DOI: 10.1371/journal.pone.0208506]

39 **Flamm S**, Mutimer D, Asatryan A, Wang S, Rockstroh J, Horsmans Y, Kwo PY, Weiland O, Villa E, Heo J, Gane E, Ryder SD, Welzel TM, Ruane PJ, Agarwal K, Ng TI, Xue Z, Lovell SS, Krishnan P, Kopecky-Bromberg S, Trinh R, Mensa FJ, Wyles DL. Glecaprevir/Pibrentasvir in patients with chronic HCV genotype 3 infection: An integrated phase 2/3 analysis. *J Viral Hepat* 2019; **26**: 337-349 [PMID: 30421537 DOI: 10.1111/jvh.13038]

40 **Asselah T**, Lee SS, Yao BB, Nguyen T, Wong F, Mahomed A, Lim SG, Abergel A, Sasadeusz J, Gane E, Zadeikis N, Schnell G, Zhang Z, Porcalla A, Mensa FJ, Nguyen K. Efficacy and safety of glecaprevir/pibrentasvir in patients with chronic hepatitis C virus genotype 5 or 6 infection (ENDURANCE-5,6): an open-label, multicentre, phase 3b trial. *Lancet Gastroenterol Hepatol* 2019; **4**: 45-51 [PMID: 30393106 DOI: 10.1016/S2468-1253(18)30341-8]

41 **Krishnan P**, Pilot-Matias T, Schnell G, Tripathi R, Ng TI, Reisch T, Beyer J, Dekhtyar T, Irvin M, Xie W, Larsen L, Mensa FJ, Collins C. Pooled Resistance Analysis in Patients with Hepatitis C Virus Genotype 1 to 6 Infection Treated with Glecaprevir-Pibrentasvir in Phase 2 and 3 Clinical Trials. *Antimicrob Agents Chemother* 2018; **62** [PMID: 30061289 DOI: 10.1128/AAC.01249-18]

42 **Puoti M**, Foster GR, Wang S, Mutimer D, Gane E, Moreno C, Chang TT, Lee SS, Marinho R, Dufour JF, Pol S, Hezode C, Gordon SC, Strasser SI, Thuluvath PJ, Zhang Z, Lovell S, Pilot-Matias T, Mensa FJ. High SVR12 with 8-week and 12-week glecaprevir/pibrentasvir therapy: An integrated analysis of HCV genotype 1-6 patients without cirrhosis. *J Hepatol* 2018; **69**: 293-300 [PMID: 29551706 DOI: 10.1016/j.jhep.2018.03.007]

43 **Zeuzem S**, Foster GR, Wang S, Asatryan A, Gane E, Feld JJ, Asselah T, Bourlière M, Ruane PJ, Wedemeyer H, Pol S, Flisiak R, Poordad F, Chuang WL, Stedman CA, Flamm S, Kwo P, Dore GJ, Sepulveda-Arzola G, Roberts SK, Soto-Malave R, Kaita K, Puoti M, Vierling J, Tam E, Vargas HE, Bruck R, Fuster F, Paik SW, Felizarta F, Kort J, Fu B, Liu R, Ng TI, Pilot-Matias T, Lin CW, Trinh R, Mensa FJ. Glecaprevir-Pibrentasvir for 8 or 12 Weeks in HCV Genotype 1 or 3 Infection. *N Engl J Med* 2018; **378**: 354-369 [PMID: 29365309 DOI: 10.1056/NEJMoa1702417]

44 **Asselah T**, Kowdley KV, Zadeikis N, Wang S, Hassanein T, Horsmans Y, Colombo M, Calinas F, Aguilar H, de Ledinghen V, Mantry PS, Hezode C, Marinho RT, Agarwal K, Nevens F, Elkhashab

M, Kort J, Liu R, Ng TI, Krishnan P, Lin CW, Mensa FJ. Efficacy of Glecaprevir/Pibrentasvir for 8 or 12 Weeks in Patients With Hepatitis C Virus Genotype 2, 4, 5, or 6 Infection Without Cirrhosis. *Clin Gastroenterol Hepatol* 2018; **16**: 417-426 [PMID: 28951228 DOI: 10.1016/j.cgh.2017.09.027]

45 **Chayama K**, Suzuki F, Karino Y, Kawakami Y, Sato K, Atarashi T, Naganuma A, Watanabe T, Eguchi Y, Yoshiji H, Seike M, Takei Y, Kato K, Alves K, Burroughs M, Redman R, Pugatch DL, Pilot-Matias TJ, Krishnan P, Oberoi RK, Xie W, Kumada H. Efficacy and safety of glecaprevir/pibrentasvir in Japanese patients with chronic genotype 1 hepatitis C virus infection with and without cirrhosis. *J Gastroenterol* 2018; **53**: 557-565 [PMID: 28948366 DOI: 10.1007/s00535-017-1391-5]

46 **Wyles D**, Poordad F, Wang S, Alric L, Felizarta F, Kwo PY, Maliakkal B, Agarwal K, Hassanein T, Weilert F, Lee SS, Kort J, Lovell SS, Liu R, Lin CW, Pilot-Matias T, Krishnan P, Mensa FJ. Glecaprevir/pibrentasvir for hepatitis C virus genotype 3 patients with cirrhosis and/or prior treatment experience: A partially randomized phase 3 clinical trial. *Hepatology* 2018; **67**: 514-523 [PMID: 28926120 DOI: 10.1002/hep.29541]

47 **Toyoda H**, Chayama K, Suzuki F, Sato K, Atarashi T, Watanabe T, Atsukawa M, Naganuma A, Notsumata K, Osaki Y, Nakamuta M, Takaguchi K, Saito S, Kato K, Pugatch D, Burroughs M, Redman R, Alves K, Pilot-Matias TJ, Oberoi RK, Fu B, Kumada H. Efficacy and safety of glecaprevir/pibrentasvir in Japanese patients with chronic genotype 2 hepatitis C virus infection. *Hepatology* 2018; **67**: 505-513 [PMID: 28865152 DOI: 10.1002/hep.29510]

48 **Forns X**, Lee SS, Valdes J, Lens S, Ghalib R, Aguilar H, Felizarta F, Hassanein T, Hinrichsen H, Rincon D, Morillas R, Zeuzem S, Horsmans Y, Nelson DR, Yu Y, Krishnan P, Lin CW, Kort JJ, Mensa FJ. Glecaprevir plus pibrentasvir for chronic hepatitis C virus genotype 1, 2, 4, 5, or 6 infection in adults with compensated cirrhosis (EXPEDITION-1): a single-arm, open-label, multicentre phase 3 trial. *Lancet Infect Dis* 2017; **17**: 1062-1068 [PMID: 28818546 DOI: 10.1016/S1473-3099(17)30496-6]

49 **Kwo PY**, Poordad F, Asatryan A, Wang S, Wyles DL, Hassanein T, Felizarta F, Sulkowski MS, Gane E, Maliakkal B, Overcash JS, Gordon SC, Muir AJ, Aguilar H, Agarwal K, Dore GJ, Lin CW, Liu R, Lovell SS, Ng TI, Kort J, Mensa FJ. Glecaprevir and pibrentasvir yield high response rates in patients with HCV genotype 1-6 without cirrhosis. *J Hepatol* 2017; **67**: 263-271 [PMID: 28412293 DOI: 10.1016/j.jhep.2017.03.039]

50 **Poordad F**, Felizarta F, Asatryan A, Sulkowski MS, Reindollar RW, Landis CS, Gordon SC, Flamm SL, Fried MW, Bernstein DE, Lin CW, Liu R, Lovell SS, Ng TI, Kort J, Mensa FJ. Glecaprevir and pibrentasvir for 12 weeks for hepatitis C virus genotype 1 infection and prior direct-acting antiviral treatment. *Hepatology* 2017; **66**: 389-397 [PMID: 28128852 DOI: 10.1002/hep.29081]

51 **Atsukawa M**, Tsubota A, Toyoda H, Takaguchi K, Nakamuta M, Watanabe T, Michitaka K, Ikegami T, Nozaki A, Uojima H, Fukunishi S, Genda T, Abe H, Hotta N, Tsuji K, Ogawa C, Tachi Y, Shima T, Shimada N, Kondo C, Akahane T, Aizawa Y, Tanaka Y, Kumada T, Iwakiri K. The efficacy and safety of glecaprevir plus pibrentasvir in 141 patients with severe renal impairment: a prospective, multicenter study. *Aliment Pharmacol Ther* 2019; **49**: 1230-1241 [PMID: 30873651 DOI: 10.1111/apt.15218]

52 **Kumada H**, Watanabe T, Suzuki F, Ikeda K, Sato K, Toyoda H, Atsukawa M, Ido A, Takaki A, Enomoto N, Kato K, Alves K, Burroughs M, Redman R, Pugatch D, Pilot-Matias TJ, Krishnan P, Oberoi RK, Xie W, Chayama K. Efficacy and safety of glecaprevir/pibrentasvir in HCV-infected Japanese patients with prior DAA experience, severe renal impairment, or genotype 3 infection. *J Gastroenterol* 2018; **53**: 566-575 [PMID: 29052790 DOI: 10.1007/s00535-017-1396-0]

53 **Gane E**, Lawitz E, Pugatch D, Papatheodoridis G, Bräu N, Brown A, Pol S, Leroy V, Persico M, Moreno C, Colombo M, Yoshida EM, Nelson DR, Collins C, Lei Y, Kosloski M, Mensa FJ. Glecaprevir and Pibrentasvir in Patients with HCV and Severe Renal Impairment. *N Engl J Med* 2017; **377**: 1448-1455 [PMID: 29020583 DOI: 10.1056/NEJMoa1704053]

54 **Reau N**, Kwo PY, Rhee S, Brown RS Jr, Agarwal K, Angus P, Gane E, Kao JH, Mantry PS, Mutimer D, Reddy KR, Tran TT, Hu YB, Gulati A, Krishnan P, Dumas EO, Porcalla A, Shulman NS, Liu W, Samanta S, Trinh R, Forns X. Glecaprevir/Pibrentasvir Treatment in Liver or Kidney Transplant Patients With Hepatitis C Virus Infection. *Hepatology* 2018; **68**: 1298-1307 [PMID: 29672891 DOI: 10.1002/hep.30046]

55 **Younossi ZM**, Stepanova M, Sulkowski M, Wyles D, Kottilil S, Hunt S. Patient-reported outcomes in patients co-infected with hepatitis C virus and human immunodeficiency virus treated with sofosbuvir and velpatasvir: The ASTRAL-5 study. *Liver Int* 2017; **37**: 1796-1804 [PMID: 28470938 DOI: 10.1111/liv.13462]

56 **Wyles D**, Bräu N, Kottilil S, Daar ES, Ruane P, Workowski K, Luetkemeyer A, Adeyemi O, Kim AY, Doehle B, Huang KC, Mogalian E, Osinusi A, McNally J, Brainard DM, McHutchison JG, Naggie S, Sulkowski M; ASTRAL-5 Investigators. Sofosbuvir and Velpatasvir for the Treatment of Hepatitis C Virus in Patients Coinfected With Human Immunodeficiency Virus Type 1: An Open-Label, Phase 3 Study. *Clin Infect Dis* 2017; **65**: 6-12 [PMID: 28369210 DOI: 10.1093/cid/cix260]

57 **Rockstroh JK**, Lacombe K, Viani RM, Orkin C, Wyles D, Luetkemeyer AF, Soto-Malave R, Flisiak R, Bhagani S, Sherman KE, Shimonova T, Ruane P, Sasadeusz J, Slim J, Zhang Z, Samanta S, Ng TI, Gulati A, Kosloski MP, Shulman NS, Trinh R, Sulkowski M. Efficacy and Safety of Glecaprevir/Pibrentasvir in Patients Coinfected With Hepatitis C Virus and Human Immunodeficiency Virus Type 1: The EXPEDITION-2 Study. *Clin Infect Dis* 2018; **67**: 1010-1017 [PMID: 29566246 DOI: 10.1093/cid/ciy220]

58 **Llaneras J**, Riveiro-Barciela M, Lens S, Diago M, Cachero A, García-Samaniego J, Conde I, Arencibia A, Arenas J, Gea F, Torras X, Luis Calleja J, Antonio Carrión J, Fernández I, María Morillas R, Rosales JM, Carmona I, Fernández-Rodríguez C, Hernández-Guerra M, Llerena S, Bernal V, Turnes J, González-Santiago JM, Montoliu S, Figueruela B, Badia E, Delgado M, Fernández-Bermejo M, Iñarrairaegui M, Pascasio JM, Esteban R, Mariño Z, Buti M. Effectiveness and safety of sofosbuvir/velpatasvir/voxilaprevir in patients with chronic hepatitis C previously treated with DAAs. *J Hepatol* 2019; **71**: 666-672 [PMID: 31203153 DOI: 10.1016/j.jhep.2019.06.002]

59 **Wilson E**, Covert E, Hoffmann J, Comstock E, Emmanuel B, Tang L, Husson J, Chua J, Price A, Mathur P, Rosenthal E, Kattakuzhy S, Masur H, Kottilil S. A pilot study of safety and efficacy of HCV retreatment with sofosbuvir/velpatasvir/voxilaprevir in patients with or without HIV (RESOLVE STUDY). *J Hepatol* 2019; **71**: 498-504 [PMID: 31173815 DOI: 10.1016/j.jhep.2019.05.021]

60 **Lok AS**, Sulkowski MS, Kort JJ, Willner I, Reddy KR, Shiffman ML, Hassan MA, Pearlman BL, Hinestrosa F, Jacobson IM, Morelli G, Peter JA, Vainorius M, Michael LC, Fried MW, Wang GP, Lu W, Larsen L, Nelson DR. Efficacy of Glecaprevir and Pibrentasvir in Patients With Genotype 1 Hepatitis C Virus Infection With Treatment Failure After NS5A Inhibitor Plus Sofosbuvir Therapy. *Gastroenterology* 2019; **157**: 1506-1517. e1 [PMID: 31401140 DOI: 10.1053/j.gastro.2019.08.008]

61 **Poordad F**, Pol S, Asatryan A, Buti M, Shaw D, Hézode C, Felizarta F, Reindollar RW, Gordon SC, Pianko S, Fried MW, Bernstein DE, Gallant J, Lin CW, Lei Y, Ng TI, Krishnan P, Kopecky-Bromberg S, Kort J, Mensa FJ. Glecaprevir/Pibrentasvir in patients with hepatitis C virus genotype 1 or 4 and past direct-acting antiviral treatment failure. *Hepatology* 2018; **67**: 1253-1260 [PMID: 29152781 DOI: 10.1002/hep.29671]

62 **Feld JJ**, Cypel M, Kumar D, Dahari H, Pinto Ribeiro RV, Marks N, Kamkar N, Bahinskaya I, Onofrio FQ, Zahoor MA, Cerrochi O, Tinckam K, Kim SJ, Schiff J, Reichman TW, McDonald M, Alba C, Waddell TK, Sapisochin G, Selzner M, Keshavjee S, Janssen HLA, Hansen BE, Singer LG, Humar A. Short-course, direct-acting antivirals and ezetimibe to prevent HCV infection in recipients of organs from HCV-infected donors: a phase 3, single-centre, open-label study. *Lancet Gastroenterol Hepatol* 2020; **5**: 649-657 [PMID: 32389183 DOI: 10.1016/S2468-1253(20)30081-9]

63 **Young K**, Liu B, Bhuket T, Wong RJ. Lower Likelihood of Post-transplant Graft Failure, Death, and Retransplantation in the Era of Direct-Acting Antivirals. *J Clin Exp Hepatol* 2020; **10**: 581-589 [PMID: 33311895 DOI: 10.1016/j.jceh.2020.02.003]

64 **Arora SS**, Axley P, Ahmed Z, Satapathy SK, Wong R, Kuo YF, Singal AK. Decreasing frequency and improved outcomes of hepatitis C-related liver transplantation in the era of direct-acting antivirals - a retrospective cohort study. *Transpl Int* 2019; **32**: 854-864 [PMID: 30866110 DOI: 10.1111/tri.13424]

65 **Choudhary NS**, Saraf N, Saigal S, Rastogi A, Bhangui P, Thiagrajan S, Soin AS. Outcome of hepatitis C-related liver transplantation in direct-acting antiviral era. *Indian J Gastroenterol* 2020; **39**: 539-543 [PMID: 33230754 DOI: 10.1007/s12664-020-01105-z]

66 **Young K**, Liu B, Bhuket T, Gish RG, Wong RJ. Improved liver transplant waitlist mortality and lower risk of disease progression among chronic hepatitis C patients awaiting liver transplantation after the introduction of direct-acting antiviral therapies in the United States. *J Viral Hepat* 2019; **26**: 350-361 [PMID: 30412318 DOI: 10.1111/jvh.13039]

67 **Bittermann T**, Reddy KR. In the Era of Direct-Acting Antivirals, Liver Transplant Delisting Due to Clinical Improvement for Hepatitis C Remains Infrequent. *Clin Gastroenterol Hepatol* 2020 [PMID: 32971230 DOI: 10.1016/j.cgh.2020.09.033]

68 **Gerlich WH**. Prophylactic vaccination against hepatitis B: achievements, challenges and perspectives. *Med Microbiol Immunol* 2015; **204**: 39-55 [PMID: 25523195 DOI: 10.1007/s00430-014-0373-y]

69 **Chen SM**, Kung CM, Yang WJ, Wang HL. Efficacy of the nationwide hepatitis B infant vaccination program in Taiwan. *J Clin Virol* 2011; **52**: 11-16 [PMID: 21767983 DOI: 10.1016/j.jcv.2011.06.012]

70 **Chang MH**, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, Chu HC, Wu TC, Yang SS, Kuo HS, Chen DS; Taiwan Hepatoma Study Group. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. *J Natl Cancer Inst* 2009; **101**: 1348-1355 [PMID: 19759364 DOI: 10.1093/jnci/djp288]

71 **Park NH**, Chung YH, Lee HS. Impacts of vaccination on hepatitis B viral infections in Korea over a 25-year period. *Intervirology* 2010; **53**: 20-28 [PMID: 20068337 DOI: 10.1159/000252780]

72 **Whitford K**, Liu B, Micallef J, Yin JK, Macartney K, Van Damme P, Kaldor JM. Long-term impact of infant immunization on hepatitis B prevalence: a systematic review and meta-analysis. *Bull World Health Organ* 2018; **96**: 484-497 [PMID: 29962551 DOI: 10.2471/BLT.17.205153]

73 **Niederau C**. Chronic hepatitis B in 2014: great therapeutic progress, large diagnostic deficit. *World J Gastroenterol* 2014; **20**: 11595-11617 [PMID: 25206267 DOI: 10.3748/wjg.v20.i33.11595]

74 **Scognamiglio P**, Girardi E, Fusco M, Piselli P, Spena SR, Maione C, Pisanti FA, Serraino D; Collaborating Study Group. Lack of implementation of Hepatitis B Virus (HBV) vaccination policy in household contacts of HBV carriers in Italy. *BMC Infect Dis* 2009; **9**: 86 [PMID: 19500412 DOI: 10.1186/1471-2334-9-86]

75 **Wallace J**, Hajarizadeh B, Richmond J, McNally S. Challenges in managing patients in Australia with chronic hepatitis B: the General Practitioners' perspective. *Aust N Z J Public Health* 2013; **37**:

405-410 [PMID: 24090321 DOI: 10.1111/1753-6405.12127]
76 **Sievert K**, Liddle R, Tan A, Arachchi N, Valaydon Z, Allard N. Promoting hospital and primary care collaboration for timely and effective care for chronic hepatitis B in western Melbourne. *Aust Health Rev* 2020; **44**: 521-526 [PMID: 32718420 DOI: 10.1071/AH19135]
77 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]
78 **European Association for the Study of the Liver**. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]
79 **Yim HJ**, Kim JH, Park JY, Yoon EL, Park H, Kwon JH, Sinn DH, Lee SH, Lee JH, Lee HW. Comparison of clinical practice guidelines for the management of chronic hepatitis B: When to start, when to change, and when to stop. *Clin Mol Hepatol* 2020; **26**: 411-429 [PMID: 32854458 DOI: 10.3350/cmh.2020.0049]
80 **Tenney DJ**, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503-1514 [PMID: 19280622 DOI: 10.1002/hep.22841]
81 **Zhang Y**, Xu W, Zhu X, Li X, Li J, Shu X, Lai J, Xie J, Xie C, Peng L. The 48-week safety and therapeutic effects of tenofovir alafenamide in hbv-related acute-on-chronic liver failure: A prospective cohort study. *J Viral Hepat* 2021; **28**: 592-600 [PMID: 33423348 DOI: 10.1111/jvh.13468]
82 **Kaneko S**, Kurosaki M, Tamaki N, Itakura J, Hayashi T, Kirino S, Osawa L, Watakabe K, Okada M, Wang W, Shimizu T, Higuchi M, Takaura K, Yasui Y, Tsuchiya K, Nakanishi H, Takahashi Y, Watanabe M, Izumi N. Tenofovir alafenamide for hepatitis B virus infection including switching therapy from tenofovir disoproxil fumarate. *J Gastroenterol Hepatol* 2019; **34**: 2004-2010 [PMID: 31017689 DOI: 10.1111/jgh.14686]
83 **Seto WK**, Asahina Y, Brown TT, Peng CY, Stanciu C, Abdurakhmanov D, Tabak F, Nguyen TT, Chuang WL, Inokuma T, Ikeda F, Santantonio TA, Habersetzer F, Ramji A, Lau AH, Suri V, Flaherty JF, Wang H, Gaggar A, Subramanian GM, Mukewar S, Brunetto MR, Fung S, Chan HL. Improved Bone Safety of Tenofovir Alafenamide Compared to Tenofovir Disoproxil Fumarate Over 2 Years in Patients With Chronic HBV Infection. *Clin Gastroenterol Hepatol* 2018 [PMID: 29933096 DOI: 10.1016/j.cgh.2018.06.023]
84 **Agarwal K**, Brunetto M, Seto WK, Lim YS, Fung S, Marcellin P, Ahn SH, Izumi N, Chuang WL, Bae H, Sharma M, Janssen HLA, Pan CQ, Çelen MK, Furusyo N, Shalimar D, Yoon KT, Trinh H, Flaherty JF, Gaggar A, Lau AH, Cathcart AL, Lin L, Bhardwaj N, Suri V, Mani Subramanian G, Gane EJ, Buti M, Chan HLY; GS-US-320-0110; GS-US-320-0108 Investigators. 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. *J Hepatol* 2018; **68**: 672-681 [PMID: 29756595 DOI: 10.1016/j.jhep.2017.11.039]
85 **Miquel M**, Pardo A, Forné M, Martínez-Alpin G, Rodríguez-Castellano A, Casas M, Rosinach M, Roget M, Dalmau B, Temiño R, Quer JC, Sanchez-Delgado J, Ortiz J, Vergara M. Current trends in access to treatment for hepatitis B in immigrants vs non-immigrants. *Gastroenterol Rep (Oxf)* 2020; **8**: 362-366 [PMID: 33163191 DOI: 10.1093/gastro/goaa010]
86 **Voulgaris T**, Vlachogiannakos J, Ioannidou P, Papageorgiou MV, Zampeli E, Karagiannakis D, Georgiou A, Papazoglou A, Karamanolis G, Papatheodoridis GV. Adherence to follow-up and treatment recommendations in Greek and immigrant patients with chronic hepatitis B in Greece. *Eur J Gastroenterol Hepatol* 2017; **29**: 264-270 [PMID: 27922484 DOI: 10.1097/MEG.0000000000000788]
87 **Contini C**, Badia L, Cultrera R, Grilli A, De Togni A. Epidemiological, clinical and laboratory features of chronic hepatitis B infection in a cohort of immigrant and Italian patients from Ferrara, Italy. *Ann Hepatol* 2012; **11**: 862-869 [PMID: 23109449]
88 **Alonso M**, Gutzman A, Mazin R, Pinzon CE, Reveiz L, Ghidinelli M. Hepatitis C in key populations in Latin America and the Caribbean: systematic review and meta-analysis. *Int J Public Health* 2015; **60**: 789-798 [PMID: 26298439 DOI: 10.1007/s00038-015-0708-5]
89 **Godin A**, Kronfli N, Cox J, Alary M, Maheu-Giroux M. The role of prison-based interventions for hepatitis C virus (HCV) micro-elimination among people who inject drugs in Montréal, Canada. *Int J Drug Policy* 2020; 102738 [DOI: 10.1016/j.drugpo.2020.102738]
90 **Gaspar R**, Liberal R, Tavares J, Morgado R, Macedo G. HIPPOCRATES® project: A proof of concept of a collaborative program for hepatitis C virus micro-elimination in a prison setting. *World J Hepatol* 2020; **12**: 1314-1325 [PMID: 33442457 DOI: 10.4254/wjh.v12.i12.1314]
91 **Cotte L**, Hocqueloux L, Lefebvre M, Pradat P, Bani-Sadr F, Huleux T, Poizot-Martin I, Pugliese P, Rey D, Cabié A; Dat'AIDS study group. Microelimination or not? *Clin Infect Dis* 2021 [PMID: 33400777 DOI: 10.1093/cid/ciaa1940]
92 **Gonzalez-Serna A**, Macias J, Palacios R, Gómez-Ayerbe C, Tellez F, Rivero-Juárez A, Fernandez M, Santos J, Real LM, Gonzalez-Domenech CM, Gomez-Mateos J, Pineda JA; HEPAVIR study group. Incidence of recently acquired hepatitis C virus infection among HIV-infected patients in southern Spain. *HIV Med* 2021; **22**: 379-386 [PMID: 33369104 DOI: 10.1111/hiv.13039]
93 **Smit C**, Boyd A, Rijnders BJA, van de Laar TJW, Leyten EM, Bierman WF, Brinkman K, Claassen MAA, den Hollander J, Boerekamps A, Newsum AM, Schinkel J, Prins M, Arends JE, Op de Coul

ELM, van der Valk M, Reiss P; ATHENA observational cohort. HCV micro-elimination in individuals with HIV in the Netherlands 4 years after universal access to direct-acting antivirals: a retrospective cohort study. *Lancet HIV* 2021; **8**: e96-e105 [PMID: 33357835 DOI: 10.1016/S2352-3018(20)30301-5]

94 **Koopsen J**, Parker E, Han AX, van de Laar T, Russell C, Hoornenborg E, Prins M, van der Valk M, Schinkel J. HCV transmission among MSM in Amsterdam: external introductions may complicate micro-elimination efforts. *Clin Infect Dis* 2020 [PMID: 33289036 DOI: 10.1093/cid/ciaa1830]

95 **Stasi C**, Silvestri C, Voller F. Update on Hepatitis C Epidemiology: Unaware and Untreated Infected Population Could Be the Key to Elimination. *SN Compr Clin Med* 2020; 1-8 [PMID: 33103061 DOI: 10.1007/s42399-020-00588-3]

96 **Mohsen W**, Chan P, Whelan M, Glass A, Mouton M, Young E, Tran Q, Arora S, Davison S, Lama T, Cobrador C, Levy M. Hepatitis C treatment for difficult to access populations: can telementoring (as distinct from telemedicine) help? *Intern Med J* 2019; **49**: 351-357 [PMID: 30091164 DOI: 10.1111/imj.14072]

97 **Morey S**, Hamoodi A, Jones D, Young T, Thompson C, Dhuny J, Buchanan E, Miller C, Hewett M, Valappil M, Hunter E, McPherson S. Increased diagnosis and treatment of hepatitis C in prison by universal offer of testing and use of telemedicine. *J Viral Hepat* 2019; **26**: 101-108 [PMID: 30315691 DOI: 10.1111/jvh.13017]

98 **Sterling RK**, Cherian R, Lewis S, Genther K, Driscoll C, Martin K, Goode MB, Matherly S, Siddiqui MS, Luketic VA, Stravitz RT, Puri P, Lee H, Smith P, Patel V, Sanyal AJ. Treatment of HCV in the Department of Corrections in the Era of Oral Medications. *J Correct Health Care* 2018; **24**: 127-136 [PMID: 29566611 DOI: 10.1177/1078345818762591]

99 **Talal AH**, Andrews P, Mcleod A, Chen Y, Sylvester C, Markatou M, Brown LS. Integrated, Co-located, Telemedicine-based Treatment Approaches for Hepatitis C Virus Management in Opioid Use Disorder Patients on Methadone. *Clin Infect Dis* 2019; **69**: 323-331 [PMID: 30329042 DOI: 10.1093/cid/ciy899]

100 **Perez Hernandez JL**, Lehmann Mendoza R, Luna Martinez J, Torres Roldan JF, Chaidez Rosales PA, Martinez Arredondo HA, Rebollar Gonzalez V, De la Cruz Silva L, Santana-Vargas D, Higuera de la Tijera MF ASC. Chronic Viral Hepatitis C Micro-Elimination Program Using Telemedicine. the Mexican Experience. *Rev Española Enfermedades Dig* 2020 [DOI: 10.17235/reed.2020.7425/2020]

101 **Du P**, Wang X, Kong L, Jung J. Can Telementoring Reduce Urban-Rural Disparities in Utilization of Direct-Acting Antiviral Agents? *Telemed J E Health* 2021; **27**: 488-494 [PMID: 32882154 DOI: 10.1089/tmj.2020.0090]

102 **Cuadrado A**, Cobo C, Mateo M, Blasco AJ, Cabezas J, Llerena S, Fortea JI, Lázaro P, Crespo J. Telemedicine efficiently improves access to hepatitis C management to achieve HCV elimination in the penitentiary setting. *Int J Drug Policy* 2021; **88**: 103031 [PMID: 33221615 DOI: 10.1016/j.drugpo.2020.103031]

103 **Jiménez Galán G**, Alia Alia C, Vegue González M, García Berriguete RMª, Fernández González F, Fernández Rodríguez CM, González Fernández M, Gutiérrez García ML, Losa JE, Velasco M, Moreno L, Hervás R, Delgado-Iribarren A, Palacios García-Cervigón G. The contribution of telemedicine to hepatitis C elimination in a correctional facility. *Rev Esp Enferm Dig* 2019; **111**: 550-555 [PMID: 31215210 DOI: 10.17235/reed.2019.6152/2018]

104 **Haridy J**, Iyngkaran G, Nicoll A, Hebbard G, Tse E, Fazio T. eHealth Technologies for Screening, Diagnosis, and Management of Viral Hepatitis: A Systematic Review. *Clin Gastroenterol Hepatol* 2021; **19**: 1139-1150. e30 [PMID: 32896632 DOI: 10.1016/j.cgh.2020.09.011]

105 **Pley CM**, McNaughton AL, Matthews PC, Lourenço J. The global impact of the COVID-19 pandemic on the prevention, diagnosis and treatment of hepatitis B virus (HBV) infection. *BMJ Glob Health* 2021; **6** [PMID: 33402334 DOI: 10.1136/bmjgh-2020-004275]

106 **Karimi-Sari H**, Rezaee-Zavareh MS. COVID-19 and viral hepatitis elimination programs: Are we stepping backward? *Liver Int* 2020; **40**: 2042 [PMID: 32319207 DOI: 10.1111/liv.14486]

107 **Wingrove C**, Ferrier L, James C, Wang S. The impact of COVID-19 on hepatitis elimination. *Lancet Gastroenterol Hepatol* 2020; **5**: 792-794 [PMID: 32730783 DOI: 10.1016/S2468-1253(20)30238-7]

108 **The Lancet Gastroenterology Hepatology**. Eliminating viral hepatitis in the COVID-19 era: weighing challenge and opportunity. *Lancet Gastroenterol Hepatol* 2020; **5**: 789 [PMID: 32730787 DOI: 10.1016/S2468-1253(20)30237-5]

109 **Sperring H**, Ruiz-Mercado G, Schechter-Perkins EM. Impact of the 2020 COVID-19 Pandemic on Ambulatory Hepatitis C Testing. *J Prim Care Community Health* 2020; **11**: 2150132720969554 [PMID: 33225792 DOI: 10.1177/2150132720969554]

110 **Blach S**, Kondili LA, Aghemo A, Cai Z, Dugan E, Estes C, Gamkrelidze I, Ma S, Pawlotsky JM, Razavi-Shearer D, Razavi H, Waked I, Zeuzem S, Craxi A. Impact of COVID-19 on global HCV elimination efforts. *J Hepatol* 2021; **74**: 31-36 [PMID: 32777322 DOI: 10.1016/j.jhep.2020.07.042]

111 **Makhoul M**, Ayoub HH, Chemaitelly H, Seedat S, Mumtaz GR, Al-Omari S, Abu-Raddad LJ. Epidemiological Impact of SARS-CoV-2 Vaccination: Mathematical Modeling Analyses. *Vaccines (Basel)* 2020; **8** [PMID: 33182403 DOI: 10.3390/vaccines8040668]

112 **Feleszko W**, Lewulis P, Czarnecki A, Waszkiewicz P. Flattening the Curve of COVID-19 Vaccine Rejection-An International Overview. *Vaccines (Basel)* 2021; **9** [PMID: 33451104 DOI: 10.3390/vaccines9010044]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4018-4044

DOI: 10.3748/wjg.v27.i26.4018

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Viral hepatitis update: Progress and perspectives

María B Pisano, Cecilia G Giadans, Diego M Flichman, Viviana E Ré, María V Preciado, Pamela Valva

ORCID number: María B Pisano 0000-0002-0336-3750; Cecilia G Giadans 0000-0002-3756-5975; Diego M Flichman 0000-0001-5730-154X; Viviana E Ré 0000-0002-4106-6642; María V Preciado 0000-0003-4082-0698; Pamela Valva 0000-0003-3994-2401.

Author contributions: Pisano MB and Ré VE collected the data for HAV and HEV and wrote these sections of the manuscript; Giadans CG and Flichman DM collected the data and wrote the HBV section; Preciado MV and Valva P collected data and wrote the HCV and HDV sections; Valva P edited the article. All authors made critical revisions and approved the final version of the article.

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

María B Pisano, Viviana E Ré, Virology Institute, CONICET, School of Medical Sciences, National University of Córdoba, Cordoba X5016, Argentina

Cecilia G Giadans, María V Preciado, Pamela Valva, Multidisciplinary Institute for Investigation in Pediatric Pathologies (IMIPP) CONICET-GCBA, Laboratory of Molecular Biology, Pathology Division, Ricardo Gutiérrez Children's Hospital, CABA C1425, Buenos Aires, Argentina

Diego M Flichman, Institute of Biomedical Investigations in Retrovirus and AIDS (INBIRS), School of Medicine, University of Buenos Aires, CONICET, CABA C1121ABG, Buenos Aires, Argentina

Corresponding author: Pamela Valva, PhD, Senior Researcher, Multidisciplinary Institute for Investigation in Pediatric Pathologies (IMIPP) CONICET-GCBA, Laboratory of Molecular Biology, Pathology Division, Ricardo Gutiérrez Children's Hospital, Gallo 1330, CABA C1425, Buenos Aires, Argentina. valvapamela@yahoo.com

Abstract

Viral hepatitis, secondary to infection with hepatitis A, B, C, D, and E viruses, are a major public health problem and an important cause of morbidity and mortality. Despite the huge medical advances achieved in recent years, there are still points of conflict concerning the pathogenesis, immune response, development of new and more effective vaccines, therapies, and treatment. This review focuses on the most important research topics that deal with issues that are currently being solved, those that remain to be solved, and future research directions. For hepatitis A virus we will address epidemiology, molecular surveillance, new susceptible populations as well as environmental and food detections. In the case of hepatitis B virus, we will discuss host factors related to disease, diagnosis, therapy, and vaccine improvement. On hepatitis C virus, we will focus on pathogenesis, immune response, direct action antivirals treatment in the context of solid organ transplantation, issues related to hepatocellular carcinoma development, direct action antivirals resistance due to selection of resistance-associated variants, and vaccination. Regarding hepatitis D virus, we describe diagnostic methodology, pathogenesis, and therapy. Finally, for hepatitis E virus, we will address epidemiology (including new emerging species), diagnosis, clinical aspects, treatment, the development of a vaccine, and environmental surveillance.

Key Words: Viral hepatitis; Hepatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis D virus; Hepatitis E virus

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Argentina

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

Received: January 25, 2021
Peer-review started: January 25, 2021
First decision: March 29, 2021
Revised: April 11, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 14, 2021

P-Reviewer: Yeoh SW
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Wang LL

Core Tip: Viral hepatitis is a global public health concern that affects millions of people and causes thousands of deaths due to acute and chronic infections, cirrhosis, and liver cancer. Although clinical and epidemiological characteristics of hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, and hepatitis E virus infections are widely known, there are still other critical points that need to be discussed. This review focuses on the most important research topics, dealing unsolved issues and future research directions that can maximize practical impact in the field of viral hepatitis.

Citation: Pisano MB, Giadans CG, Flichman DM, Ré VE, Preciado MV, Valva P. Viral hepatitis update: Progress and perspectives. *World J Gastroenterol* 2021; 27(26): 4018-4044
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4018.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4018

INTRODUCTION

The term viral hepatitis refers to liver inflammation related to a viral infection. As of today, five viruses (hepatitis A, B, C, D, and E) that selectively infect the liver, usually by different routes, have been recognized. In some of these viral infections, acute hepatitis can resolved without intervention, whereas, sometimes, the process turns into a chronic infection[1]. Huge medical advances made in recent decades led to the implementation of preventive measures, the development of vaccines and passive immunization strategies, and, more recently, the development of promising and effective treatments, at least for some forms of viral hepatitis. The results obtained by basic research on viruses and on viruses-cell interaction made it possible to struggle with what a century ago seemed an insurmountable scourge on humanity. Achievements in hepatitis prevention and treatment are perhaps the paradigm of successful translational research[1]. Nonetheless, viral hepatitis is still a global public health concern that affects millions of people and causes thousands of deaths due to acute and chronic infection, cirrhosis, and liver cancer[1,2]. This review focuses on the currently most important research topics and future research directions that can maximize practical impact in the field of viral hepatitis. Table 1 summarizes the principal characteristics of these hepatotropic viruses and Tables 2-6 highlight the main topics of viral hepatitis addressed in the present review.

HEPATITIS A VIRUS

According to the World Health Organization (WHO), 1.4 million new cases of hepatitis A are reported worldwide each year, with a consequent nearly 7000 deaths[3]. Hepatitis A virus (HAV), a member of the *Picornaviridae* family and the only species from the *Hepatovirus* genus that infects humans, is a non-enveloped single-stranded RNA virus[4]. HAV is classified into six genotypes, three infecting humans and three affecting simians, but there is only one known serotype[5].

Despite HAV being discovered more than 4 decades ago, it has been well characterized, and its detection and diagnosis have been widely implemented; changes in the socio-economic conditions and the control mechanisms of the virus have triggered new circulation and transmission scenarios that have generated new targets for its assessment. Some of them are the epidemiology and molecular surveillance of the virus, the different vaccination schemes and immune responses, the new susceptible populations (after the implementation of massive vaccination), and the study of the virus in environmental and food matrices.

Epidemiology and transmission: Old and new challenges

Although HAV epidemiology is complex, it is changing in those countries that are improving their public health and sanitation policies, considering that the most usual routes of HAV transmission are contaminated water ingestion and the contact with infected individuals[6]. Three circulation patterns have historically been described for

Table 1 Features of different types of hepatitis virus

	HAV	HBV	HCV	HDV	HEV
Family	*Picornaviridae*	*Hepadnaviridae*	*Flaviviridae*	Undefined[1]	*Hepeviridae*
Genus	*Hepatovirus*	*Orthohepadnavirus*	*Hepacivirus*	*Deltavirus*	*Orthohepevirus*
Genome	Positive single-stranded linear RNA	Double stranded gapped DNA	Positive single-stranded linear RNA	Negative single-stranded circular RNA	Positive single-stranded linear RNA
Genome length (kb)	7.5	3.2	9.6	1.7	7.2
Genotype	6 genotypes: I, II and III infect humans, and IV, V and VI infect non-human primates	10 genotypes (A to J)	8 (1 to 8)	8 (1 to 8)	8 (1 to 8)
Transmission	Fecal-oral	Parenteral, sexual, and perinatal	Exposure to infected blood	Exposure to infected blood and body fluids	Fecal-oral; zoonotic; blood transfusion
Treatment	None. In case of severe hepatitis, treatment of symptoms	Pegylated interferon-alpha and nucleoside/nucleotide analogues	DAA	Pegylated interferon-alpha	Ribavirin (in chronic HEV infection)
Prophylaxis	Yes (inactivated vaccine)	Yes (recombinant vaccine)	No	Yes (HBV vaccine)	No[2]
Clinical outcome of infection	Self-limited	Self-limited and chronic	Self-limited and chronic	Self-limited and chronic	Self-limited
Chronic infection rate	No	Depends on the age of acquisition of the infection. Birth or in infancy 90%, 1 yr and 5 yr of age 30%-50%, adulthood 5%. Hemodialysis patients 40%. Immune deficient patients 20%	80%	More frequent in HBV/HDV superinfection than coinfection	Acute infection in most of the cases. Chronic infection in immunosuppressed populations

[1]It is not defined yet in any of the established viral families.
[2]There is only one vaccine, approved and used only in China. HAV: Hepatitis A virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HEV: Hepatitis E virus; DAA: Direct antiviral agents.

Table 2 Hepatitis A virus highlights

	Hepatitis A virus
1	The risk of HAV infection is associated with the lack of safe water and poor and sanitation
2	Due to the vaccine introduction in childhood, young adults are becoming more susceptible to HAV infections
3	In countries where waterborne transmission is rare, outbreaks occur among men who have sex with men, injecting drug users and contaminated food
4	Since molecular detection is not routinely performed for diagnosis, surveillance programs, including viral amplification and sequencing, are needed to know the strains that circulate in a certain place
5	One of the greatest challenges for HAV is to increase vaccination coverage globally, still implementing the single-dose schedule, to decrease the new infections, and, in the long term, to achieve its eradication

HAV: Hepatitis A virus.

HAV: (1) In high endemicity areas from low- and middle-income countries, where the incidence varies from low to high over time and between different regions, there is a peak age of infection in early childhood that is frequently asymptomatic, the transmission pattern is person-to-person, and outbreaks are uncommon due to high rates of immunity from previous childhood infection; (2) In moderate endemicity areas, from middle-income countries (regions where sanitary conditions are variable), the incidence is high, the peak age of infection is in late childhood/adolescence or in young adults that is frequently symptomatic, the transmission pattern is also from person-to-person, related to food and water, and therefore outbreaks are common due to low rates of immunity from previous childhood infection; and (3) In low endemicity

Table 3 Hepatitis B virus highlights

	Hepatitis B virus
1	Several host factors, such as male gender, alcohol intake, and obesity have been associated to worse disease progression. Current challenge implies finding genetic markers to predict the course of HBV infection. In this line, different SNPs associated with the outcome of HBV infection have been recently identified
2	In the last years, new diagnostic assays have been developed in the framework of the diagnosis of HBV infection. The implementation of quantitative HBsAg, HBcAg, and HBV-RNA in routine clinical practice could probably improve the management of patients with CHB
3	Current antiviral treatments have some shortcomings, such as poor SVR or prolonged schedules. Direct antiviral agents against different HBV targets, including HBV cccDNA, are under evaluation. Moreover, immunemodulatory therapies to overcome host immune impairment observed in chronic infections are being investigated
4	Although a safe and cost-effective vaccine is available since the 1980s, an inadequate response is achieved in particular settings. New and more potent adjuvants, as well as formulations that include alternative viral antigens could improve the response rate vaccination
5	The development of new antiviral therapies that enables achieving functional cure as well as accurate diagnostic methods and more effective vaccines will contribute with the purpose of the WHO to eliminate by 2030 hepatitis as a global health problem

HBV: Hepatitis B virus; SNP: Single nucleotide polymorphisms; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core Antigen; CHB: Chronic hepatitis B; SVR: Sustained virological response; WHO: World Health Organization.

Table 4 Hepatitis C virus highlights

	Hepatitis C virus
1	WHO global hepatitis elimination strategy aims to reduce 90% of new HCV incidence, 65% of mortality and treat at least 80% of patients
2	DAA treatment leads to regression of clinical symptoms and liver disease complications even in those patients with other comorbidities, co-infections, or advanced liver disease
3	The immune response plays a central role in viral elimination. The understanding of the relationship between achieving protection and activation of immune responses is mandatory for the development of an effective prophylactic vaccine
4	Immune response restoration after DAA treatment is also under debate, certain immune features are reinvigorated, but many immune exhaustion signs may persist
5	SVR after DAA rates higher than 97% are usually attained, but still, a minor group of patients (4%-5%) fails to eradicate HCV due to resistance-associated variants, some of them arising after treatment but others naturally occurring in treatment naïve individuals
6	DAA efficacy impacts on transplantation from HCV-infected donors into infected or uninfected recipients; however, early outcome data are encouraging, experience is limited, and many issues remain under debate
7	HCC risk after DAA treatment has been extensively discussed; however, recent seminal reports support the notion of a reduced rate for occurrence or recurrence of HCC after DAA SVR
9	There are numerous HCV vaccine approaches including a few candidates who accomplished phase I trials, but a prophylactic HCV vaccine that can contribute to the eradication goal remains a pending issue

DAA: Direct antiviral agents; SVR: Sustained virological response; WHO: World Health Organization; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma.

areas from high income-countries, the incidence is low, the peak age of infection is in young adulthood, the transmission pattern is from person-to-person and also *via* food and water; and outbreaks are common due to low rates of immunity from previous childhood infection[3].

Nowadays, 34 countries have included vaccination against HAV in routine immunization programs among children[3]. Many countries use an inactivated HAV vaccine with a two-dose regimen, while other countries have successfully implemented it in their immunization programs in a single-dose[3,7]. However, long term results of the single-dose schedule have been only partially studied. The most recent investigation showed sustained immunologic protection for up to 9 years, with high levels of antibody titers, when children were vaccinated at 12 mo[8]. More studies that assess long-term seroprotection against HAV after single-dose vaccination scheme are necessary to monitor the effectiveness of this innovative strategy. In some territories vaccination is also recommended for people at risk of HAV infection, like those who travel to regions where HAV is endemic, drug users, men who have sex with men, and individuals with chronic liver disease[3].

The recent improvement of socio-economic, hygienic, and sanitation measures may translate into an increase in the number of adults who have never been infected in

Table 5 Hepatitis D virus highlights

	Hepatitis D virus
1	The natural course and outcome of acute hepatitis D differ according to HBV and HDV co-infection or superinfection
2	HDV and HBV genotypes in addition to host factors influence the course of chronic hepatitis
3	The implications on liver disease of HVD, HBV, and innate immunity interplay remain to be understood
4	Chronic setting leads to more severe hepatitis associated with higher rates of HCC and a faster progression to cirrhosis compared with HBV monoinfection. HDV pathologic changes are limited to the liver with histopathologic features that are not specific for it
5	HDV remains difficult to treat with the current available therapies, and although, several promising new therapies have been described treatment is still the greatest challenge in HDV infection

HDV: Hepatitis D virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma.

Table 6 Hepatitis E virus highlights

	Hepatitis E virus
1	HEV is transmitted by the fecal-oral route (involving contaminated waters) and also as a zoonosis
2	In the last years, many studies have focused on HEV detection in environmental and food matrices, and blood products as alternative sources of infection
3	A new etiological agent of human hepatitis E, Orthohepevirus C, previously known to infect rats, has been recently described
4	Although most cases of HEV infection produce acute hepatitis, chronic infections seem to be an increasing problem, particularly in Europe
5	Complications and extrahepatic manifestations are also increasingly recognized
6	Only one vaccine for HEV has been licensed in China, with little known data, which limits its use

HEV: Hepatitis E virus.

childhood and therefore lack immunity. Furthermore, despite pediatric immunization programs, many young adults may have been above the cut-off ages to be included when such social programs were introduced. Therefore, young adults are now becoming more susceptible to HAV infections, so in areas of low and middle-endemicity, the prevalence of symptomatic cases in this age group has increased[6]. In this sense, between the middle of 2016 and the beginning of 2018, several hepatitis A outbreaks were reported in Europe, the United States and South America, which disproportionately affected HAV unvaccinated young adult men, mainly men who have sex with men. This group presents an increased risk of infection same as persons, regardless sex orientation, who have oral-anal sexual contact[4]. Interestingly, through phylogenetic analysis accompanied by detailed questionnaires to capture the sexual history of the patients, it was possible to establish epidemiological links between cases, demonstrating that the viruses responsible for these outbreaks belonged to HAV genotype IA and grouped with one of these strains: VRD_521_2016, RIVM-HAV16-090, and V16-25801[6]. This highlights the importance of carrying out a more detailed epidemiological record of cases, including sexual history, which will help to establish the source and chain of infection.

Regarding travelers to endemic regions, although the WHO has recommended their vaccination, it does not always happen, increasing hepatitis A cases among this group. Furthermore, the movements of immigrants in some areas of the world (*e.g.*, in South America) led the virus to be transported from endemic areas (often without vaccination coverage) to non-endemic areas, introducing new viral strains[3,6]. Screening for immunoglobulin (Ig) G anti-HAV should be offered to this group; therefore, patients who test negative should be offered vaccination[4].

Detection and surveillance

Diagnosis of hepatitis A is performed by the detection of HAV IgM with serological assays. Specific antibodies are present in sera for at least 7 mo after infection, although in some individuals they remain for up to a year[4]. During acute infection, IgG anti-HAV appears, and it remains present in serum for life[3,4]. Serological surveillance is assumed as the main monitoring strategy for the infection. Since molecular detection is

not routinely performed for diagnosis, surveillance programs, including viral amplification and sequencing, are needed to understand the strains that circulate in a certain place or that are introduced by travelers; however, it is seldom carried out. Molecular surveillance includes the detection and study of HAV in environmental and food matrices, an area of study that has been carried out in recent years. For the purpose of molecular surveillance, the HAV Network (HAVNET) was created in 1999 [9]. This is an international HAV network of scientists who work in reference laboratories of hepatitis A and share molecular and epidemiological data on this virus, information that is useful for the scientific community. The HAVNET aims to increase the knowledge of HAV infections and map the worldwide distribution of HAV strains. As there is a strong geographical signal in the sequences, this can be used for source tracking.

The study of HAV in environmental and food matrices is a valuable tool for monitoring circulating HAV strains, to know the sources of infection and to take sanitation and prevention measures. After a large outbreak of foodborne hepatitis A in Europe in 2013-2014, the crucial role of sequence data analysis to investigate outbreaks and define transmission pathways was recognized, as well as the need of the agreement on a common genomic region for sequencing and a common protocol to perform HAV detection in food[10]. In this sense, with the aim of harmonizing the existing protocols for HAV detection in food, the European Committee for Standardization and the International Standards Organization developed and published a standard methodology for quantitative and qualitative determination of HAV (together with norovirus) in seven food matrices, using real-time (RT) polymerase chain reaction (PCR), which has allowed to obtain comparable results between laboratories[5]. Furthermore, the sequencing of a common consensus region was agreed to target the HAV VP1/2A junction and thus promote the protocol described in the HAVNET[10]. In this context, collaborations between the public health sector, the food sector, HAVNET, and other organizations, together with government dependencies, are highly recommended.

Although there is no legislation about the presence of HAV in environmental matrices at a global level, some countries have adopted measures for the surveillance of cases of food outbreaks due to HAV, which has led to strict controls of imported food, incorporating the mandatory control of this virus in some cases[11]. Foodborne HAV clinical cases and outbreaks are difficult to identify, track, and assess their magnitude due for many reasons: (1) The difficulty for patients to remember food consumption history before the onset of the disease; (2) The asymptomatic nature of many cases, which are not reported (in the case of outbreaks); (3) The long incubation period of HAV; (4) Viral contamination levels of a food item may be low and focal and, therefore, hard to detect; and (5) The scarce knowledge of health care teams about foodborne viral diseases[5].

The above issues highlight the new epidemiological scenarios of this virus, showing the targets to whom control and prevention actions should be directed. The main goal for the next years should be to increase vaccination coverage globally, implementing the single-dose schedule, so to decrease the new infections, and, in the long term, to achieve eradication.

HEPATITIS B VIRUS

The hepatitis B virus (HBV) was discovered by serendipity in the 1960s and subsequently several milestones were achieved such as the development of diagnostic tests in the early 1970s or the implementation, in the 1980s, of a safe and cost-effective vaccine with subsequent different therapies for the treatment of chronic hepatitis B (CHB) infection[12].

Despite these advances, the landscape is still far from satisfactory. Currently, an estimated 257 million people are living with CHB, and around 887000 deaths occur annually as a consequence of infection progression, mainly due to cirrhosis and hepatocellular carcinoma (HCC)[13]. Furthermore, it is expected in the coming decades that the problem of HBV infection might increase, particularly in developing countries, as a consequence of the limited access to diagnosis and treatment, in addition to the subclinical characteristics of the infection[14]. In fact, the WHO has proposed strategies to eliminate viral hepatitis as a Public Health problem by 2030. To achieve this goal, it will be necessary to implement prevention, diagnosis, and treatment measures, as well as to raise awareness among the population and primary care physicians from the infections caused by HBV[15].

Among the current challenges to overcome are the identification of host markers that would allow to predict accurately the evolution of infection and the implementation of a personalized medical approach, the development of anti-HBV therapies that enables achieving functional cure in chronically infected patients, as well as the restoration of the host's immune response, the implementation of new diagnostic methods, and the development of more effective vaccines that would lead to improving prevention policies in order to reduce the global burden of HBV disease.

Host factors

HBV infection has a wide range of clinical presentations, from subclinical to symptomatic in the acute stage, and from inactive carrier state to active chronic hepatitis with different degrees of severity[16]. Epidemiological data early established that the course of the infection is closely related to the age at which the infection is acquired, being the evolution to chronicity much more frequent in individuals infected at birth or in childhood[17]. Additionally, male gender, heavy alcohol consumption (more than 60 g/d), obesity, and comorbidities, such as co-infections with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis D virus (HDV), have also been reported to contribute to progression to end-stage liver disease[18-22]. Recently, genome-wide association studies have shown that the host genetic background may also affect the natural history of infection[23,24]. Several studies have identified single nucleotide polymorphisms (SNPs) in human leukocyte antigens (HLA) that have been associated with the outcome of HBV infection, either with clearance or progression of chronic infection, although some findings were not subsequently supported in other manuscripts. Among the more in depth characterized, it has been found that HLA-DP (rs3077 and rs9277535) and HLA-DQ (rs7453920 and rs2856718) SNPs were associated to HBV persistence[25-27] Notably, different studies have also identified several HLA polymorphisms associated with the response to the HBV vaccine[28,29]. Additionally, it was also reported that cytokine, chemokine, toll like receptor, sodium taurocholate cotransporting polypeptide, and vitamin D-related genes may influence the clinical outcomes of HBV infection[24,30,31]. Beyond the controversies observed among studies addressing the genetic polymorphisms involved in the outcome of HBV infection, mainly probably due to ethnic differences (haplotype structures and allele frequencies), these findings will undoubtedly help to individualize the risk of infection progression and to improve the effectiveness of HBV vaccination campaigns, contributing to the implementation of a personalized approach and a greater chance of accomplishing the achievement of eliminating HBV infection as a public health problem by 2030.

Diagnosis

In order to achieve global control of HBV infection, one of the main obstacles to overcome is the limited access to diagnostic resources. Since the 1980s, classical serological markers have been available, including detection of antigens s and e (HBsAg, HBeAg) and antibodies against antigen e and core (anti-HBe and anti-HBc), along with the later use of molecular markers to determine the viral load, for the diagnosis and management of HBV infection. The qualitative detection of HBsAg has been the hallmark of HBV infection. Its presence for more than 6 mo is pathognomonic of chronic infection, and HBsAg seroclearance is now considered the goal for functional cure, except for occult hepatitis B, in which HBsAg is not detected despite the persistence of the infection. In recent years, efforts have focused on the search for accurate tools for the monitoring of antiviral treatment in CHB. Complete cure of CHB infection implies elimination of the HBV from infected hepatocytes, which is hardly achievable because of the persistence of the covalently closed circular DNA (cccDNA) and integrated HBV-DNA. Since cccDNA detection is difficult to perform in routine diagnosis, surrogate markers have been developed, being the quantitative HBsAg (qHBsAg), the hepatitis B core-related antigen (HBcrAg), and serum HBV-RNA the most promising ones. In the last years, different assays to qHBsAg levels have been developed. In most studies carried out on HBeAg-positive patients, a positive correlation among HBsAg titers, serum HBV DNA, and liver cccDNA has been observed[32]. In contrast, this relationship was not verified in HBeAg-negative CHB cases[33]. The lack of correlation could be a consequence of S gene mutations associated with HBeAg seroconversion, affecting expression or secretion of HBsAg[34-36]. Nonetheless, several studies have shown that qHBsAg is a useful diagnostic tool, together with HBV-DNA levels, to discriminate inactive carriers from HBeAg-negative chronic hepatitis[37,38]. Furthermore, it has been described to be useful in predicting sustained HBsAg clearance and liver disease progression in inactive carriers[33]. Likewise, baseline and on-treatment qHBsAg levels have been shown to be a reliable

prognostic marker of sustained virological response (SVR) in treatment with pegylated interferon alpha (PEG-IFN-α). Consequently, current guidelines recommend its use for the management of HBV therapy[39-42]. The HBcrAg, another recently developed marker, detects the HBcAg, HBeAg and the 22 kDa precore protein. Different studies indicate that HBcrAg depicts a more accurate correlation with intrahepatic cccDNA transcriptional activity than qHBsAg, regardless of HBeAg status[43,44]. Furthermore, HBcrAg has been suggested as a prognostic factor for virological remission and HBsAg clearance in patients undergoing antiviral treatment[45], as well as a predictive marker for the development of HCC[46]. Nevertheless, its clinical use remains controversial. HBV-RNA detection has also raised interest as a possible surrogate marker of HBV transcriptional activity since serum HBV RNA levels significantly correlated with intrahepatic cccDNA concentrations among untreated patients[47,48]. Likewise, it has been suggested that HBV RNA has a predictive value as a diagnostic tool of HBeAg loss in patients under therapy, being proposed as a reliable marker for treatment discontinuation[49,50]. However, routine implementation still requires standardization of the methodology. Finally, several studies have identified other promising markers to monitor the management of CHB patients such as quantitative anti-HBcAg or cccDNA determination[51-53]. Further validation for their use in clinical practice is still required.

Therapy

Over the last 2 decades, notable progress has been achieved in the treatment of CHB infection. Currently available antiviral agents include PEG-IFN-α and nucleoside/nucleotide analogues (NAs) among which entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide are the first-line oral anti-HBV drugs due to the high genetic barrier to HBV resistance. Although suppression of HBV replication reduces the progression of liver disease and improves the outcomes in most patients, the actual obstacle to cure CHB is the persistence of cccDNA and integrated HBV DNA. Thus, the term 'functional cure' has been accepted as the ultimate goal to reach with HBV therapies[41,42]. However, PEG-IFN-α treatment has an unsatisfactory SVR rate in addition to several adverse effects, being therefore limited to a selected group of patients. On the other hand, although NAs have shown high efficacy in inhibiting viral replication, the HBsAg sustained clearance rate is poor, with a substantial risk of relapse when treatment is discontinued, the need for retreatment, and the risk of select drug resistant strains[54]. Therefore, the main challenge at present is the implementation of new strategies that increase the rate of loss of HBsAg or the sustained suppression of HBV replication compared to existing therapies by developing more efficient antiviral agents and immune-modulatory therapies to restore the functionality of the immune system. Direct antiviral agents targeting different HBV proteins or steps of the viral replication cycle are being evaluated. HBV entry inhibitors are molecules that target the NTCP receptor (NTCP: Sodium taurocholate cotransporting polypeptide is the host cell receptor required for HBV entry), preventing both the novo infection and reinfection cycles, being of great value to control CHB infection[55,56]. Also, HBsAg release inhibitors are promising drugs that combined with current antiviral treatments might help to induce HBsAg clearance [57]. Additionally, core protein assembly modulators and small interfering RNA targeting HBV transcripts are under evaluation in different clinical trials[58,59]. Another appealing strategy implies targeting the HBV cccDNA. Several molecules and clustered regularly interspaced short palindromic repeats technology have shown the ability to inhibit synthesis or eliminate the already formed cccDNA[60]. However, they are still under investigation due to delivery issues and unintended off-target effects [61]. Furthermore, antiviral agents against the protein X are also being addressed, both for their role in the epigenetic regulation of cccDNA and in the modulation of several host cell signaling pathways[62].

As mentioned above, the impairment of the host's immune system is another important factor for HBV persistence. Several approaches are being investigated for the pharmacological activation of the intrahepatic innate immune response, including the induction of IFN genes with antiviral properties[63] or the stimulation of toll-like receptors (TLR)[64], targeting adaptive cell effectors. In line with the latter strategy, some attractive methodologies include the use of checkpoint inhibitors that block the co-inhibitory receptors overexpressed in HBV-specific T cells to reverse immune dysfunction[65], the adoptive transfer of either genetically engineered T lymphocytes expressing chimeric antigen receptors or reinfusion of autologous restored T cells[66,67], as well as therapeutic vaccination that might boost host immune response[68]. However, the wide range of functional deficiencies observed in patients with CHB represents an important pitfall for the success of these therapies. Its use together with

antivirals agents is expected to lead to viral elimination as well as mounting strong immunological surveillance that limits viral reactivation.

Vaccine

Vaccination is the most powerful tool to control the spread of HBV. Since the 1980s, a recombinant HBV vaccine obtained by expressing the small envelope protein (HBsAgS) in yeast has been available. It is currently being implemented for infants in more than 189 countries, and in 109 of these a dose within the first 24 h of life has been introduced in vaccination schedules[69]. Following the global introduction of large-scale vaccination, a substantial decrease in the rate of HBsAg carriers was observed [70]. After three intramuscular doses, a protective response against HBV is achieved in more than 90% of healthy adults and more than 95% of infants, children, and adolescents. However, the response rate declines with age, particularly after the age of 40, as well as in people with obesity, smokers, comorbidities, genetic factors, or particular settings[71]. Failure to mount an adequate immune response is one of the main concerns regarding the HBV vaccine; therefore, to overcome this shortcoming, several attempts to enhance the immunogenicity have been addressed. On the one hand, new and more powerful adjuvants have been developed and evaluated, including liposome-based formulations, cytidine-phosphate-guanosine oligodeoxynucleotide (a TLR9 agonist) or virosomes[72]. These reformulations have shown a considerable improvement in seroconversion rates compared to the conventional vaccine, particularly in individuals with poor or no response[73]. Interestingly, the novel adjuvants may reduce the current schedule from three to two doses, contributing to a higher adherence rate to compliance with the vaccination scheme, which is another drawback[74]. Likewise, intradermal administration has shown to provide better responses than the intramuscular route[75].

On the other hand, recombinant vaccines derived from mammalian cells containing the medium and large envelope proteins, in addition to the already used small envelope protein, have been developed. This approach has the advantage of antigens displaying the same post-translational modifications and protein folding that occurs *in vivo*. This alternative approach showed a faster seroprotection rate as compared to the conventional vaccine, making it of particular interest for people with poor or no response. Furthermore, it could protect against HBV strains carrying vaccine induced or spontaneous HBsAgS mutants[76]. In fact, the emergence of vaccine escape mutants (VEMs) and the role of the HBV genetic variability both have been considered as possible shortcomings of the vaccine.

Shortly after the massive implementation of the HBV vaccine, the selection of variants with mutations in the wild type epitope has been reported. In many countries, where early large-scale vaccination was introduced, along with a decrease in the prevalence rate of infection, over time a significant increase in the frequency of VEMs [77] has been observed. Different studies have shown that VEMs can replicate, with the implicit risk of becoming the predominant strains in the coming decades. In addition to the selection pressure exerted by the implementation of large scale vaccination, due to the overlapping of the open reading frames in the HBV genome, mutations in the *Pol* gene can affect the *S* gene[78]. Consequently, the use of antiviral agents targeting the viral polymerase indirectly promotes the selection of mutants affecting the HBsAgS recognition by vaccine-induced antibodies. Beyond these assumptions, the transmission of VEM is a very unusual event and, although its strict vigilance is recommended, it does not pose a threat to the control of HBV infection. Therefore, the introduction of mutated antigens in the vaccine formulation is not currently being considered[79].

Finally, the genetic variability of HBV may represent a more significant problem than VEMs. Based on the genetic diversity, HBV is classified into 10 genotypes (A to J) and several subgenotypes. The HBV vaccine used today was developed decades ago, when the existence of the different HBV genotypes was unknown, using HBsAgS of genotype A2 as a prototype. Case reports of vaccinated people subsequently infected, mainly with the most divergent genotypes, have been described[80-82]. Although there is a paucity of data regarding cross-genotype preventive effect, greater protection against homologous genotype/sub-type than against heterologous strains of HBV have been reported[83]. However, empirical data from regions where the most divergent genotypes are prevalent suggests that cross protection is sufficient to prevent infection. Therefore, HBV diversity would not represent a major obstacle to the prophylaxis of infection.

HCV

In 2020 the Nobel Prize in Physiology or Medicine was awarded to the Americans Harvey J Alter (United States National Institutes of Health) and Charles M Rice (Rockefeller University) and to the British Michael Houghton (University of Alberta) for the discovery of the HCV. Alter demonstrated the existence of a non-A non-B hepatitis virus-associated with post-transfusion hepatitis in 1975, Houghton cloned and identified the viral genome and renamed it as HCV in 1989, and Rice established, from an edited version of the virus genome, a robust *in vitro* replication system in cell cultures in the 1990s and thus laid the foundation for future genetic and functional analysis[84-86].

Epidemiology and treatment

HCV is an enveloped single-stranded RNA virus of the *Flaviviridae* family. Due to a lack of proofreading activity of HCV RNA-dependent RNA polymerase (NS5B) and their high replication rate, a large number of viral variants are produced during infection[87]. Eight genotypes have been described, among them genotype 1 is prevalent worldwide, while the others were each characterized in different geographic regions, that is genotype 2 in West Africa, genotype 3 mostly in South Asia, genotype 4 in Central and North Africa, genotype 5 in South Africa, genotype 6 in South-East Asia, and genotype 7, which has been isolated from central African immigrants in Canada[88,89]. Recently, the novel genotype 8 was described as endemic in India[90].

HCV is estimated to infect more than 1% of the global population, and around 80% develop a slowly evolving, asymptomatic chronic liver disease characterized by cell damage, inflammation, and fibrosis that can progress, after a few decades, to cirrhosis in 30%-40% of cases or to HCC in 1%-3% of them[91]. Thus, HCV infection is strongly related to liver transplantation. In the absence of a vaccine, HCV treatments went through different stages, starting with prolonged regimens based on interferon as an immune system modulator with cure rates of less than 50% and high adverse effects, and going through successive generations of direct-action antivirals (DAA). However, the real improvement of the DAA regimen began in 2013, when an interferon-free treatment was available; since then, several DAA schemes targeted against the protease, the NS5A protein, or the polymerase became the standard of care. Currently, treatments are oral with almost no side effects and with SVR rates higher than 97% after 8 to 12 wk. Nowadays, successful treatment leads to regression of clinical symptoms and complications of liver disease even in those patients with other comorbidities, co-infections, or advanced liver disease[92-94]. In this new scenario, as mentioned above, in 2016 the World Health Assembly approved a global strategy to achieve viral hepatitis elimination (C and B), which concerning HCV aims to reduce 90% of new HCV infections (incidence), 65% of deaths (mortality), and treat at least 80% of patients who require treatment[95,96]. However, this objective is far from being reached. On the one hand, it is critical that each country implements systematic and organized programs of silent carrier detection to overcome the suboptimal rates of HCV screening. On the other hand, it is necessary to ensure access to treatment for all infected people, which is still difficult due to the high cost of it. Finally, it is essential to carry out primary prevention tasks to avoid the generation of new cases and the reinfection of patients already cured, especially in the groups at greatest risk[95,96].

Pathogenesis and immune response

Chronic hepatitis C pathogenic mechanisms as well as the immune response participation in the generation of liver damage are still topics of interest[91,97]. It has been thoroughly described that HCV alters liver homeostasis, leading to stress and inflammation[98]. The liver microenvironment is extremely complex with numerous immune cell populations that, along with the cytokines that they produce, play a central role in the viral elimination; thus the interplay between virus and host immune response may influence infection outcome[99-101]. Remarkably, in the chronic stage, the role of the immune cells becomes more complex since their altered functionality would contribute to liver damage[102]. Cellular immune surveillance of HCV infection induces interferon production and activates innate immune response, hence controlling the infection. As part of the innate immune response, natural killer, dendritic, and Kupffer cells present viral antigens from infected hepatocytes to the T and B lymphocytes, which in turn contribute to virus control. The immune system triggering is not enough to control HCV infection and in consequence, a persistent infection is established. However, the results behind this data are controversial, and the underlying mechanism by which various cell populations are involved is still

under discussion.

The understanding of the relationship between achieving protection and the activation of both innate and adaptive immune responses is mandatory for the development of an effective prophylactic vaccine that may control infection and transmission. Moreover, the restoration of the immune response after DAA treatment is also under debate, particularly because most reports have focused on immune cells in peripheral blood, and little is known related to the intrahepatic immune environment after rapid clearance of chronic HCV. Remarkably, a double scenario arises after DAA treatment, certain immune features are reinvigorated but many immune exhaustion signs may continue after viral elimination[103]. The majority of pro-inflammatory cytokines and chemokines reached normal values after long-term monitoring albeit IFN-α and tumor necrosis factor-related apoptosis-inducing ligand maintained high levels for months after treatment[104,105]. Regarding HCV-specific T cells, a partial recovery of the functionality, mainly proliferation capacity, was described. Nevertheless, it does not apply for every patient, and the restoration level was not homogeneous for all individuals. The suppression of HCV replication led to a decrease in expression of T lymphocyte exhaustion markers and an increase in HCV-specific IFN-γ responses after treatment[94,106,107]. However, the restoration of exhausted HCV-specific CD8+ T lymphocyte surface phenotype does not result, *per se*, in a complete functional restoration. Regarding CD4+ T cells, HCV antiviral treatment leads to a shift from a T helper 1 cell to a follicular helper T cell (Tfh) environment within HCV-specific cells. Likewise, HCV-specific CD8+ T lymphocytes, Tfh cells are likely to persist in an antigen-independent manner[108]. Furthermore, in chronic hepatitis C, regulatory T cells are usually elevated and display an activated phenotype in the course of infection that persists even after DAA therapy[109]. Natural killer (NK) cells have an important role in HCV infection control; however, phenotype and function of NK cells are altered in chronic HCV patients[110]. In recent years, several groups have investigated the recovery of the altered NK cell compartment upon successful antiviral treatment, but it is still a matter of research whether an active reinvigoration *via* certain signaling pathways or the resolution of inflammation after virus elimination are responsible for a seemingly restored NK cell compartment. Hence, such a persistent challenge of the immune system might trigger irreversible damage that in turn could affect the success of any therapeutic vaccine design or even any immunotherapy approach against HCC[94,103,111].

DAA resistance-associated variants

Current DAA therapy has demonstrated high efficacy, but still in a minor group of patients (4%-5%) it does not succeed in eradicating HCV, largely due to inadequate adherence but also due to relapse or viral fitness[112]. Since HCV is a rapidly evolving RNA virus, the exposure to DAAs triggers strong drug selection favoring mutants that offer partial resistance to them. Thus, the high SVR achieved with DAA still faces the challenge of resistance-associated variants (RAVs), some arising after treatment but others naturally occurring in treatment naïve individuals[113,114]. DAA treatment failure may be attributable to advanced liver disease, suboptimal therapy adherence, and the presence or generation of NS5A mutations[112]. The three HCV non-structural proteins have different RAV prevalence, which may be related to their distinct roles in the HCV life cycle that defines the resistance genetic barriers[115]. RAVs affecting each of the DAA classes have different properties and occur most commonly in the NS5A region, less commonly in the NS3 region, and uncommonly in the NS5B region. In all first-line DAA regimens, NS5A inhibitors are a crucial component because their RAVs have direct clinical impact[114,116]. Treatment-emergent RAVs that remain at high frequency after the end of therapy often have other fitness compensating mutations and may be more difficult to treat. Nowadays, there are several options for patients who have failed to respond to first line DAA therapy, and more than 90% of these patients are able to achieve SVR following retreatment, but the selection of the appropriate therapy depends on several factors and may require genotype and resistance testing. It should be noted that there are some notable genotype-specific differences with respect to retreatment, particularly in the case of prior exposure to NS5A inhibitors in patients with genotype 1 infection. Rescue treatment options with multiple targeted therapies, such as the pangenotypic combinations, sofosbuvir/velpatasvir/voxilaprevir (Vosevi), and glecaprevir/pibrentasvir (Mavyret), were effective in the majority of cases with DAA failure[112]. Interestingly, a recent European multicentric study showed that even Vosevi can fail in genotype 3 and genotype 1a infected individuals with cirrhosis, but this failure is not associated with a specific pattern of RAV. It is important to note that rescue treatment with multiple targeted therapies was effective in the majority of patients[117].

DAA treatment in the context of solid organ transplantation

The field of solid organ transplantation (SOT) has also been benefited with DAA development[118,119]. The efficacy of DAA has created a new opportunity to improve survival in end-organ failure patients through greater access to organ transplantation, since transplanting organs from HCV-infected donors into infected or uninfected recipients is now under consideration. Altogether, this has led to a better transplant outcome due to healthier patients receiving SOT and a significant reduction of waitlist mortality and healthcare costs. In the post-liver transplantation setting, early treatment is now recommended due to the high efficacy of DAAs, in association with a low side effect profile and easily mitigated drug-drug interactions[118,119]. The optimal treatment duration for each organ is not yet clear; in the years to come there will be increasing data and hopefully standardization of treatment. Some reports proposed 8-12 wk of DAA treatment for liver transplantation, but 2-4 wk seems to be enough for other organs[118,119]. However, it should be kept in mind that although it is expected that treatment eliminates the risk of infection, this is not a certainty; since the persistence of HCV RNA in peripheral blood mononuclear cells and/or the liver has been shown to occur post-SVR in liver transplantation recipients, with unclear clinical consequences. Although early outcome data are encouraging, the overall experience is limited, and many ethical issues and scientific questions remain, such as avoidance of selection bias, the optimal timing of DAA therapy, detailed evaluation of drug-drug interactions between DAAs and immunosuppressants, and long-term graft-patient outcomes. Moreover, there is no data on possible long-term hepatic and extrahepatic adverse effects associated to HCV exposure, even among those cured of the infection. As such, transplanting livers from HCV-infected donors into uninfected recipients requires special approval from governing bodies in the United States and in nearly all countries around the world[120].

HCC as a consequence of DAA treatment

Regarding the plausibility of HCC development in the context of DAA therapy, a risk reduction would be expected as viral clearance reduces morbidity and mortality rates. It should be considered, however, that HCV has a direct carcinogenic potential since some of the HCV-encoded proteins interact with cellular regulatory factors and produce oxidative stress, DNA damage, and deregulation of host cell checkpoints, thus promoting tumorigenesis[121,122]. Because HCV is an RNA virus and its genome does not undergo reverse transcription into DNA, its carcinogenic effects cannot be attributed to its integration into the hepatocyte genome. Lately, the risk of occurrence or recurrence of HCC in HCV patients who received DAA has been debated. IFN-based therapy reports demonstrated that achieving a SVR significantly diminished the risk for HCC[123]. Furthermore, these patients recover liver functionality with a positive impact on long-term disease-free survival[121,124]. On the other hand, initial reports on DAA therapy exposed a potential high risk of HCC occurrence and recurrence after treatment[121,125-127] and hypothesized that the occurrence of HCC would be the result of the emergence and spread of a pre-treatment "orphan" tumor clone that escapes immunological surveillance. The rapid virus elimination following DAA therapy may lead to an imbalance of the immunity that may rebound on immune control of the neoplastic clone[121]. However, the initial results were not conclusive or were even opposed due to the lack of homogeneity in the study design [125]. Recent seminal reports support the notion of a reduced rate for occurrence or recurrence of HCC after SVR obtained after DAA treatment, so the impact of DAAs on HCC risk is nowadays an old tale[93,128,129]. However, the time of DAA therapy initiation in HCC HCV positive patients is still controversial since it seems to condition treatment success[2,125,130,131]. In this sense, the best advice for physicians is to follow approved international or local guidelines and to keep updated to minimize risks or therapeutic failures[2,125,130,131].

Vaccine

The development of a prophylactic HCV vaccine that can contribute to the eradication goal still remains as a pending issue. The diversity of the virus, different behaviors of the virus in animal models or cell cultures, the limited models or individuals to test the vaccines, and the insufficient understanding of protective immunity against HCV are barriers to the development of an effective vaccine. It has been described, both in chimpanzees and humans, that immune system surveillance of primary infection is not necessarily efficient in controlling a recurrent one[132,133]. Therefore, spontaneous HCV immune control does not certainly generate protective immunity, hence diminishing confidence that prophylactic vaccination is possible. Furthermore,

compared to the initial HCV infection, a lower peak and duration of viremia characterized the reinfection in the same individual[113,134,135]. A faster and more effective viral replication control at second exposures indicates an adaptive immune response that may avoid chronic infection even though it cannot prevent reinfection. Therefore, a vaccine that induces T and B cell responses against multiple HCV genotypes and impedes the selection of virus escape mutants is needed[111,113]. While attenuated vaccines by the passage of the virus in non-human primate cell lines could be produced and suppress or inactivate genetic virulence factors, HCV does not replicate at high levels in non-human primate cell lines and no virulence factors have been defined for HCV yet. Therefore, concerns related to the production and the potential risk of attenuated vaccines could limit their utility[113]. In addition, HCV culture strains have adaptive mutations that enhance their ability of *in vitro* replication with an unknown impact on replication in humans. Inactivated whole HCV vaccines were also described; however, the lack of effective processes in the later phases for the purification of HCV represents an obstacle for the development of a complete virus vaccine[136]. On the other hand, there are numerous approaches involving viral antigens as immunogens, namely DNA-vaccines, adenovirus-based strategies, virus-like particles, HCV recombinant antigens conjugates to HBsAg, and HCV peptides in different delivery platforms[113,137-143]. Most of these candidate's vaccines have triggered humoral and cellular immune responses in rodents, and a small subset of them causes immunity in macaques, and fewer candidates in chimpanzees[113,144-148]. Likewise, only a few HCV vaccine developments accomplished the goal of phase I trials in volunteers not at risk for HCV infection[113,149-153]. Given that the partial results of the clinical trials are not completely encouraging, new strategies are required to improve and/or maintain antiviral immunity, and therefore there is a long way to go until a successful HCV vaccine could be used[137].

HCV infection is an example of the success of translational research, as a result, HCV infection is the only chronic viral infection that can be cured, and the hepatic or extrahepatic manifestations are mostly reversible[154]. Many countries are making significant progress in their fight against it, but HCV surveillance is at the base of any effort to control and eliminate the disease, since early diagnosis can prevent health problems that may result from infection and prevent transmission of the virus. The road is long, but with clear objectives the goal can be achieved.

HDV

In 1977, Rizzetto *et al*[155] identified a new antigen in the liver and serum of HBV infected patients who showed more severe hepatitis than their counterparts[155]. This observation led to the discovery of the HDV (also called a satellite virus), an unusual defective virus whose genome consists of a negative single-stranded circular RNA that encodes a single nucleocapsid protein, the delta antigen. The HDV virion, of 36 nm, consists of a ribonucleoprotein core complex and a lipoprotein envelope composed of the three HBV envelope proteins: Small (S-), medium (M-), and large (L-) HBsAg. HBV presence is mandatory for HDV replication, since HBsAg is required for HDV cell entry by NTCP, virion assembly, and export; however, its RNA replication is autonomous. HDV is maintained as episomes in the nucleus of the infected hepatocytes and transcribes the viral RNAs on behalf of the host cell machinery[156].

Clinical, epidemiological, and virological features

Two different scenarios may allow HDV infection: Either HBV and HDV simultaneously infect the host (co-infection) or HDV infection occurs in CHB patients (superinfection). In general, HDV is a highly pathogenic virus associated with more severe forms of acute hepatitis, including fulminant hepatitis. The natural course and outcome of acute hepatitis D differ according to the way infection takes place, whereas only 2% of coinfections evolve to chronicity, superinfection results in chronic infection in over 90% of the cases[157]. Irrespective of the type of infection, the chronic state leads to more severe hepatitis associated with higher rates of HCC and a faster progression to cirrhosis compared with HBV monoinfection, increasing this risk three times among HDV-HBV coinfected patients[157,158]. At least 5% of individuals with chronic HBV are co-infected with HDV, raising the HDV global burden of infection to an estimate of more than 62 million people, nearly 1% of the world's population[2,159]. Despite having a global distribution, HDV has a higher prevalence in Africa (Central and West Africa), Asia (Central and Northern Asia), Pacific Islands, Middle East, Eastern Europe, South America (Amazonian basin), and Greenland[2,160,161].

In addition to host factors, HDV and HBV genotypes influence the course of chronic hepatitis[162]. HDV genome analysis disclosed at least eight distinct HDV genotypes (HDV-1 to -8), with some displaying two to four sub-genotypes. Infection with

genotype 1, the most common one, has been associated with a wide spectrum of disease severity, while other genotypes appear to be more geographically restricted and to be linked with different degrees of disease severity. Infections with either genotype 2 and 4, the most commonly genotypes found in the Far East, generally develop milder forms of liver disease, whereas genotype 3 exclusively found in the Amazon region, has been documented as one of the most aggressive types, associated with severe and fulminant hepatitis outbreaks. HDV-5 is predominant in West Africa, whereas HDV 6, 7, and 8 were isolated in patients from central Africa[157]. Furthermore, HBV genotype could influence HDV infection and replication, being HDV viral loads are lower in patients co-infected with HBV genotype A, whereas co-infection is more frequently seen in genotype F CHB patients[156].

Transmission and diagnosis

HDV and HBV routes of transmission are alike, namely intravenous drug users or exposure to infected blood products and serous body fluids, but HDV mother to infant transmission is rare[162]. HIV infection, intravenous drug users, men who have sex with men, and individuals from areas of high HDV prevalence who are HBV-infected are at risk for co-infection with HDV[2,161]. The HDV antigen is only detectable transiently, therefore the diagnosis is made by measuring anti-HDV antibodies. HDV IgM appears in blood between the first and third weeks after infection and remains positive in the chronic phase with variable levels according to disease activity. HDV IgG is also detectable during active and resolved infection, so this test is useful for the screening of chronic or past HDV infection, while HDV RNA detection is applied to confirm active chronic hepatitis and to supervise therapy response. Anti-HDV IgM and HDV RNA assessment, together with HBV infection acute markers, should be tested to distinguish between acute co-infection HBV/HDV *vs* HDV superinfection [163].

Immune response and pathogenesis

Experimental and clinical studies suggested that HBV is a weak inducer of innate response and has developed strategies to evade innate immune sensing, whereas HDV has shown to activate the IFN pathway *via* melanoma differentiation antigen 5. It has been suggested that both HBV and HDV could inhibit the janus kinase/signal transducer and activator of transcription signaling pathway and hence the response to exogenous IFN. So, the constant activation of the IFN pathway may contribute to chronic viral pathogenesis; however, the implications on liver disease of HDV and HBV and innate immunity interplay remain to be understood. HDV activation of the type-I IFN pathway may promote an increase in the NK cell number, thereby inducing the killing of HBV-specific CD8 T cells by tumor necrosis factor-related apoptosis-inducing ligand-dependent mechanisms, hence worsening HBV pathogenesis in co-infected patients. Additionally, it has been described that HDV proteins affect autophagy by promoting HDV replication, cause oxidative stress, and modulate the transforming growth factor-β and nuclear transcription factor-kappa B signaling pathways. However, most of the studies have been performed in artificial systems that naturally tend to overexpression, so most of them need to be confirmed in actual infectious systems[156,164,165].

HDV pathologic changes are limited to the liver with histopathologic features that are not specific for it, but they tend to be more severe in HDV disease. The hepatocyte injury is typically focal, except in the most severe cases when confluent necrosis occurs, leading to submassive or massive necrosis accompanied by infiltration of inflammatory cells within the collapsed lobules and in the portal areas[157]. Liver biopsy is still of election to achieve an accurate inflammation grading and fibrosis staging since fibrosis noninvasive markers are not reliable in chronic HDV infection. The higher inflammation in HDV compared to HBV monoinfection alters elastography measurement, so the accuracy of transient elastography seems to be reasonable to detect cirrhosis but remains to be validated for grading lesser degrees of fibrosis[1,166-168].

Viral tropism

Several studies have proved the ability of HDV to replicate in a variety of tissues and cells after transfection; moreover, HDV-like viruses have been isolated from other species (birds, snakes). These findings question the hypothesis of an escaped human gene HDV origin and also alludes to the cooperation with other viruses to egress. Furthermore, it has been shown that HDV ribonucleoprotein can be assembled with envelope proteins that come from non-HBV related viruses, raising the question of HDV may also be harbored by other viruses[156].

Treatment

The ability to achieve SVR in the treatment of HDV remains uncertain given the high rates of late relapse. Therefore, HDV remains difficult to treat with the current available therapies. PEG-IFN is the election therapy but the absence of HDV treatment guidelines generate uncertainty concerning protocols. Nucleoside/NAs are ineffective because they do not reduce HBsAg levels, which is required for HDV propagation. However, despite the presence of HDV typically suppressing HBV replication, nucleoside/NA (entecavir or tenofovir) is generally recommended, particularly in patients with cirrhosis, regardless of HBV replication status[1]. Nevertheless, the Hep-Net International Delta Hepatitis Intervention Trial, a large multicenter program, treated patients with PEG-IFN-a-2a and/or adefovir for 48 wk. Six months after treatment completion, 28% of patients who were treated only with interferon continued to have undetectable HDV RNA with no additional benefit compared to those who also received adefovir and showed no response in individuals treated with adefovir alone. In a consecutive study in which patients were treated with PEG-IFN-α with or without tenofovir, only 23% of patients with interferon therapy presented levels of RNA under the detection limit 24 wk after stopping treatment with no extra benefit from the additional use of tenofovir[169]. Therefore, treatment is still the greatest challenge in HDV infection. So far, several promising new therapies have been described, some of which in combination with interferon, may result in sustained clearance of HDV. In this regard, myrcludex and lonafarnib are two promising treatments that are at the most advanced development stages. Myrcludex is an entry inhibitor while lonafarnib prevents HDV secretion, preventing both *de novo* and reinfection cycles. Other therapies are under evaluation in different clinical trials, such as hepcludex which has already been partially approved owing to its safe profile[170,171].

Despite these promising advances, we are in need of treatments achieving permanent HDV RNA suppression since high rates of relapse are associated with current IFN therapies in addition to increased transaminase levels after discontinuation. Interestingly, HDV coinfection prior to liver transplantation reduces the risk of graft reinfection and is associated with better patient survival than HBV-monoinfected patients. However, reinfection with HDV following liver transplantation may still occur, but tends to be aborted if HBV recurrence is also prevented[2,172].

The current knowledge on HDV highlights that the critical points to be addressed in future research must be directed to explain the virus and the immune system interaction linked to the pathogenesis that might allow treatment improvement against chronic liver disease produced by HDV.

HEPATITIS E VIRUS

The hepatitis E virus (HEV) is a causative agent of endemic and epidemic hepatitis worldwide, producing approximately 20 million infections every year, leading to an estimated 3.3 million symptomatic cases[173]. It is a spherical, non-enveloped virus that belongs to the family *Hepeviridae*, genus *Orthohepevirus*, a genus that is divided into four species (A-D)[174]. The strains of species A (Orthohepevirus A) are responsible for hepatitis E in humans. It comprises eight genotypes (HEV-1 to 8) displaying a geographical distribution and different epidemiological patterns. Genotypes that infect humans are 1-4 and 7[174,175].

HEV represents a significant public health challenge in resource-limited settings, mainly from Asia and Africa. In industrialized countries, it has historically been incorrectly regarded as having little clinical relevance[4]. However, in the last years, it has been recognized as an emerging and often undiagnosed disease in developed countries and some places of America, based on increasing reports of non-travel associated sporadic cases and chronic clinical presentations[176].

Epidemiology and transmission: Old and new challenges

Two epidemiological patterns have been observed for HEV. The first one is related to genotypes 1 (HEV-1) and 2 (HEV-2), which infect only humans and are transmitted mainly by the fecal-oral route, through water contaminated with the virus, resulting in frequent sporadic cases and occasional large outbreaks. These genotypes circulate in areas of high endemicity, generally in developing countries (due to poor sanitation) in Asia, the Middle East, North Africa, and some parts of America[175-177]. The second pattern, observed mainly in industrialized countries and some parts of America, is related to the zoonotic transmission of HEV genotypes 3 (HEV-3) and 4 (HEV-4), in

which pigs are considered a viral reservoir, although these viruses have also been detected in other animals, such as wild boar or deer[175,177]. Humans can become infected through direct contact (with many studies showing that farmers have higher levels of HEV antibodies)[178] or by ingestion of raw-undercooked animal meat or derived products, such as sausages or pates, that contain the virus[5]. Shellfish, fruits, and vegetables have also been implicated in viral transmission, probably due to pig slurry contaminating watercourses, which are used for irrigation, or being used as fertilizer[4,5]. HEV-3 has a worldwide distribution, while HEV-4 is restricted to Asia and Europe[177]. Interestingly, genotype 7 (HEV-7) has only been described in the Middle East and Dubai, from sporadic human cases and camels[174].

Since HEV is transmitted by the fecal-oral route (involving contaminated waters) and also as a zoonosis (having animal reservoirs), many studies in the last years have focused on HEV detection in environmental and food matrices as sources of HEV infection[177]. HEV has been detected in many environmental matrices, such as sewage, recreational waters (river, creek, dam), and tap waters, showing fecal contamination of the environment[179,180]. Viral presence in sewage represents an indicator of viral excretion of a given population, so it is useful for monitoring HEV circulation [181]. Water resources that are contaminated with wastewater are the main origin for the dissemination of enteric viruses and, in consequence, they could represent a viral reservoir with a dramatic impact on the population's health[179].

HEV food contamination that is not derived from pork is another route that is currently being studied, such as shellfish, fruits, vegetables, or milk, which have been postulated to be possible sources of infections, particularly in places where sporadic cases without an epidemiological link occur[5]. Many new lines of study are focused on the research of HEV in foods, methodologies for its detection in food matrices, and food outbreaks. The knowledge of these sources of infection will allow for improvements in the prevention of HEV infection.

Additionally, vertical transmission from mother to child[182] and transmission through blood transfusion[183] have also been described, but as less frequent routes. However, the transfusion route is currently becoming more relevant since an increasing number of cases are being reported in Europe and Asia[176]. This is particularly important for immunosuppressed populations since these patients could develop chronic infections and are commonly subjected to blood transfusions. Asymptomatic carriers of HEV could play a possible role as human viral reservoirs, and the virus can be transmitted during the donation, when the volunteer donates blood prior to the onset of the acute stage of hepatitis E[176]. In response to the threat posed by HEV to transfusion safety, many European countries have implemented screening for HEV-RNA in blood products, and many others are considering to do so [183]. However, in the rest of the world, there is still a lack of knowledge about this route of transmission.

In recent years, a few cases of acute and chronic human hepatitis E attributed, for the first time, to the HEV-C (*Orthohepevirus C*) species were reported in many parts of the world. Until now, this virus had only been detected in rats and ferrets (known as rat-HEV), and belongs to the genus *Orthohepevirus*, as well as the human-infecting HEV-A, although they are very divergent[4,184]. HEV-C genotype 1 was identified in both immunocompetent and immunocompromised patients who displayed acute and chronic infection as described in a recent large prospective study in Hong Kong, positioning this virus as a new etiological agent of hepatitis E. It is important to mention, as observed in one case, that the pre-existing HEV antibodies did not protect against HEV-C genotype 1. Also, routine hepatitis E diagnostic tests may overlook HEV-C infection[184]. Therefore, this is a new challenge in the field of viral hepatitis and specifically in understanding HEV epidemiology.

Clinical features

In most cases the infection produces an acute self-limited illness with a variety of clinical manifestations, ranging from asymptomatic course to acute liver failure, resulting in fatality rates of 0.2%-4%. The most common symptoms are abdominal pain, nausea, vomiting, anorexia, fever, and jaundice[182]. The course of the disease could be more severe in pregnant women infected with HEV-1, with high maternal, fetal, and neonatal morbidity and mortality rates, as high as 25%[182]. In turn, it has been described in individuals who have chronic liver disease that the mortality rate increases when infected by HEV[174]. Chronic HEV infections have been identified among immunocompromised persons infected with HEV-3 or HEV-4, including patients receiving cancer chemotherapy, recipients of organ transplant, and HIV-infected persons. In these cases, HEV-RNA had been detected in serum and/or stool samples for at least 6 mo[175,177]. Chronic hepatitis E seems to be an increasing

problem, particularly in Europe, where areas with high chronicity rates have been identified.

Hepatitis E also shows a spectrum of serious complications and extrahepatic manifestations, which are being increasingly recognized[174]. Some of them include acute or chronic liver failure, neurological disorders, pancreatitis, renal injury, cryoglobulinemia, hematological disorders, and thyroiditis[174,175,177]. The mechanisms of HEV-associated extrahepatic injuries are not fully understood yet and represent a challenge for the study of hepatitis E and its management.

Diagnosis

Diagnosis of hepatitis E infection can be carried out using direct techniques, which allow for the detection of the viral antigens and nucleic acid, as well as by the detection of IgG and IgM HEV-specific antibodies, although it may require a combination of both, molecular and serological assays, to confirm infection and for monitoring the treatment in chronically infected patients[185]. Laboratory diagnostic techniques for HEV detection vary in their specificity and sensitivity, something important to consider when using any of them, and to make comparisons. Currently, the gold standard test is the PCR for HEV-RNA amplification[185]. In the case of acute hepatitis E, a differential diagnosis should be performed to exclude other viral hepatitis and other causes (autoimmune, toxic, *etc.*) of liver disease. HEV-RNA detection can be carried out in serum samples (although the viremic period is short) as well as in stool samples, in which virions are shed for a longer period of time[4]. HEV antigen can also be performed, using double-antibody sandwich enzyme immunoassay techniques, which can be detected in serum, feces, or urine[185], although it is not extensively used. Acute hepatitis can also be diagnosed by IgM anti-HEV detection[173]. It is worth mentioning that the time of diagnosis and sample extraction is crucial. HEV is not generally taken into account in an initial assessment of a sick individual, due to still being regarded as an "emerging" disease, and many clinicians have limited knowledge of the disease[4]. This delay in sampling could lead to false negative results for viral RNA detection. In these cases, specific IgM testing is useful. For chronic infections, diagnosis is performed by detecting the presence of HEV-RNA by RT-PCR (and/or its variants Nested-PCR and RT-PCR) in blood for more than 6 mo[174]. The titer of antibodies against HEV may be lower in these patients, as well as in those immunosuppressed, so detection of HEV IgM and IgG should be interpreted with caution[185]. Although viral genotyping is not routinely performed, its determination is important in order to understand the clinical and epidemiological pattern (especially in risk patients, as immunosuppressed individuals, pregnant women, *etc.*), as well as for viral surveillance and to monitor the introduction of new genotypes/strains in a given region.

Antiviral treatment and vaccine

Antiviral therapy is not usually required in acute HEV infection since the virus is spontaneously cleared. However, treatment with ribavirin may be considered in cases of severe acute hepatitis E or acute-on-chronic liver failure[174].

In the case of chronic infections, ribavirin monotherapy for 3 mo is recommended. Decreasing levels of immunosuppression at diagnosis of chronic HEV infection is also advisable in solid organ transplant recipients. After 3 mo, HEV-RNA should be assessed in stool and serum samples. If RNA is undetectable, European Association for the Study of the Liver suggests stopping ribavirin therapy. If RNA replication persists, therapy with ribavirin should be continued for an additional 3 mo (6 mo course of ribavirin monotherapy in total). In the case of liver transplant recipients with lack of response to ribavirin, PEG-IFN therapy for 3 mo could be considered[174,186].

Even though many HEV vaccines have been developed worldwide, only one has been licensed in China (Helicon®). This vaccine is based on a recombinant HEV peptide derived from genotype 1, corresponding to a fragment of the open reading frame 2, which encodes the capsid protein of HEV. It is recommended to be used in individuals aged > 16 years and at high risk of HEV infection (food handlers, animal husbandry, soldiers, women of childbearing age, travelers to endemic areas, *etc.*). However, very little is known about many aspects of this vaccine, which limits its use, such as the efficacy (it has only been proved to prevent symptomatic hepatitis E due to genotype 4), immunogenicity and safety, especially in specific populations, like pregnant women, transplant patients and subjects with chronic liver disease[187].

The foregoing highlights new challenges regarding hepatitis E worldwide, showing that further research about epidemiological, clinical, and virological aspects are needed to understand better the different HEV scenarios and implications around the world.

CONCLUSION

In this review, we summarized the most relevant topics that are being analyzed or that have recently arisen in the setting of viral hepatitis. Although in recent years significant progress has been made in the knowledge of viral hepatitis, there are still many aspects to be resolved. It is necessary to continue working on improving diagnosis to maintain a constant and continuous epidemiological follow-up of infected populations, expand knowledge on the mechanisms of pathogenesis of each virus, improve treatment, and develop or improve the efficiency of vaccines. It is important to understand that strategies must be both local and global, as this represents that most successful path for viral hepatitis to cease being a major public health problem.

ACKNOWLEDGEMENTS

The authors thank Dr Jose Debes (Associate Professor, Department of Medicine, Faculty, School of Public Health University of Minnesota, Minneapolis, MN, United States) for reading and correcting the English language used in the manuscript.

REFERENCES

1 **Cheung A**, Kwo P. Viral Hepatitis Other than A, B, and C: Evaluation and Management. *Clin Liver Dis* 2020; **24**: 405-419 [PMID: 32620280 DOI: 10.1016/j.cld.2020.04.008]
2 **Te H**, Doucette K. Viral hepatitis: Guidelines by the American Society of Transplantation Infectious Disease Community of Practice. *Clin Transplant* 2019; **33**: e13514 [PMID: 30817047 DOI: 10.1111/ctr.13514]
3 **World Health Organization**. Hepatitis A fact sheet. [Internet] [accessed 17 August 2020]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-a
4 **Webb GW**, Kelly S, Dalton HR. Hepatitis A and Hepatitis E: Clinical and Epidemiological Features, Diagnosis, Treatment, and Prevention. *Clin Microbiol Newsl* 2020; **42**: 171-179 [PMID: 33110280 DOI: 10.1016/j.clinmicnews.2020.10.001]
5 **Di Cola G**, Fantilli AC, Pisano MB, Ré VE. Foodborne transmission of hepatitis A and hepatitis E viruses: A literature review. *Int J Food Microbiol* 2021; **338**: 108986 [PMID: 33257099 DOI: 10.1016/j.ijfoodmicro.2020.108986]
6 **Mariojoules J**, Castro G, Pisano MB, Barbero P, Fantilli A, Borda M, Canna F, Barbás G, Ré V. Hepatitis A outbreak affecting men who have sex with men (MSM) in central Argentina, occurred in July 2017-April 2018, later than the European outbreak. *J Clin Virol* 2019; **117**: 49-53 [PMID: 31202104 DOI: 10.1016/j.jcv.2019.05.014]
7 **Espul C**, Benedetti L, Linares M, Cuello H, Lo Castro I, Thollot Y, Rasuli A. Seven-year follow-up of the immune response after one or 2 doses of inactivated hepatitis A vaccine given at 1 year of age in the Mendoza Province of Argentina. *Hum Vaccin Immunother* 2017; **13**: 2707-2712 [PMID: 28933624 DOI: 10.1080/21645515.2017.1358326]
8 **Urueña A**, González JE, Rearte A, Pérez Carrega ME, Calli R, Pagani MF, Uboldi A, Vicentín R, Caglio P, Cañero-Velasco MC, Gentile A, Ramonet M, Vizzotti C. Single-dose Universal Hepatitis A Immunization in One-year-old Children in Argentina: High Prevalence of Protective Antibodies up to 9 Years After Vaccination. *Pediatr Infect Dis J* 2016; **35**: 1339-1342 [PMID: 27636725 DOI: 10.1097/INF.0000000000001322]
9 **Hepatitis A Network (HAVNET)**. [Internet] [accessed 16 December 2019]. Available from: https://www.rivm.nl/en/havnet
10 **Enkirch T**, Severi E, Vennema H, Thornton L, Dean J, Borg ML, Ciccaglione AR, Bruni R, Christova I, Ngui SL, Balogun K, Němeček V, Kontio M, Takács M, Hettmann A, Korotinska R, Löve A, Avellón A, Muñoz-Chimeno M, de Sousa R, Janta D, Epštein J, Klamer S, Suin V, Aberle SW, Holzmann H, Mellou K, Ederth JL, Sundqvist L, Roque-Afonso AM, Filipović SK, Poljak M, Vold L, Stene-Johansen K, Midgley S, Fischer TK, Faber M, Wenzel JJ, Takkinen J, Leitmeyer K. Improving preparedness to respond to cross-border hepatitis A outbreaks in the European Union/European Economic Area: towards comparable sequencing of hepatitis A virus. *Euro Surveill* 2019; **24** [PMID: 31311618 DOI: 10.2807/1560-7917.ES.2019.24.28.1800397]
11 **Mbayed V**, Mozgovoj M, Oteiza JM, Stupka J, Red de Seguridad Alimentaria de CONICET. In: Informe del grupo "Ad Hoc" "Virus transmitidos por alimentos". 2019. [Internet] [accessed 17 August 2020]. Available from: https://rsa.conicet.gov.ar/a dhoc/virus-transmitidos-por-alimentos/
12 **Nguyen MH**, Wong G, Gane E, Kao JH, Dusheiko G. Hepatitis B Virus: Advances in Prevention, Diagnosis, and Therapy. *Clin Microbiol Rev* 2020; **33** [PMID: 32102898 DOI: 10.1128/CMR.00046-19]
13 **World Health Organization**. Global Health Sector Strategy on Viral Hepatitis 2016-2021. Towards Ending Viral Hepatitis 2016. [Internet] [accessed 17 August 2020]. Available from: http://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/

14 **Zampino R**, Boemio A, Sagnelli C, Alessio L, Adinolfi LE, Sagnelli E, Coppola N. Hepatitis B virus burden in developing countries. *World J Gastroenterol* 2015; **21**: 11941-11953 [PMID: 26576083 DOI: 10.3748/wjg.v21.i42.11941]

15 **Hatzakis A**, Lazarus JV, Cholongitas E, Baptista-Leite R, Boucher C, Busoi CS, Deuffic-Burban S, Chhatwal J, Esmat G, Hutchinson S, Malliori MM, Maticic M, Mozalevskis A, Negro F, Papandreou GA, Papatheodoridis GV, Peck-Radosavljevic M, Razavi H, Reic T, Schatz E, Tozun N, Younossi Z, Manns MP. Securing sustainable funding for viral hepatitis elimination plans. *Liver Int* 2020; **40**: 260-270 [PMID: 31808281 DOI: 10.1111/liv.14282]

16 **Chu CM**, Liau Y. Natural History of Hepatitis B Virus Infection. In: Liaw YF., Zoulim F. (eds) Hepatitis B Virus in Human Diseases. Molecular and Translational Medicine. Humana Press, Cham, 2016: 217-247

17 **Li Z**, Hou X, Cao G. Is mother-to-infant transmission the most important factor for persistent HBV infection? *Emerg Microbes Infect* 2015; **4**: e30 [PMID: 26060603 DOI: 10.1038/emi.2015.30]

18 **Sagnelli E**, Stroffolini T, Mele A, Imparato M, Sagnelli C, Coppola N, Almasio PL. Impact of comorbidities on the severity of chronic hepatitis B at presentation. *World J Gastroenterol* 2012; **18**: 1616-1621 [PMID: 22529690 DOI: 10.3748/wjg.v18.i14.1616]

19 **Ruggieri A**, Gagliardi MC, Anticoli S. Sex-Dependent Outcome of Hepatitis B and C Viruses Infections: Synergy of Sex Hormones and Immune Responses? *Front Immunol* 2018; **9**: 2302 [PMID: 30349537 DOI: 10.3389/fimmu.2018.02302]

20 **Loomba R**, Yang HI, Su J, Brenner D, Iloeje U, Chen CJ. Obesity and alcohol synergize to increase the risk of incident hepatocellular carcinoma in men. *Clin Gastroenterol Hepatol* 2010; **8**: 891-898, 898.e1-898. e2 [PMID: 20621202 DOI: 10.1016/j.cgh.2010.06.027]

21 **Iida-Ueno A**, Enomoto M, Tamori A, Kawada N. Hepatitis B virus infection and alcohol consumption. *World J Gastroenterol* 2017; **23**: 2651-2659 [PMID: 28487602 DOI: 10.3748/wjg.v23.i15.2651]

22 **Ganesan M**, Eikenberry A, Poluektova LY, Kharbanda KK, Osna NA. Role of alcohol in pathogenesis of hepatitis B virus infection. *World J Gastroenterol* 2020; **26**: 883-903 [PMID: 32206001 DOI: 10.3748/wjg.v26.i9.883]

23 **Li Y**, Si L, Zhai Y, Hu Y, Hu Z, Bei JX, Xie B, Ren Q, Cao P, Yang F, Song Q, Bao Z, Zhang H, Han Y, Wang Z, Chen X, Xia X, Yan H, Wang R, Zhang Y, Gao C, Meng J, Tu X, Liang X, Cui Y, Liu Y, Wu X, Li Z, Wang H, Hu B, He M, Gao Z, Xu X, Ji H, Yu C, Sun Y, Xing B, Yang X, Tan A, Wu C, Jia W, Li S, Zeng YX, Shen H, He F, Mo Z, Zhou G. Genome-wide association study identifies 8p21.3 associated with persistent hepatitis B virus infection among Chinese. *Nat Commun* 2016; **7**: 11664 [PMID: 27244555 DOI: 10.1038/ncomms11664]

24 **Zhang Z**, Wang C, Liu Z, Zou G, Li J, Lu M. Host Genetic Determinants of Hepatitis B Virus Infection. *Front Genet* 2019; **10**: 696 [PMID: 31475028 DOI: 10.3389/fgene.2019.00696]

25 **Qiu B**, Jiang W, Olyaee M, Shimura K, Miyakawa A, Hu H, Zhu Y, Tang L. Advances in the genome-wide association study of chronic hepatitis B susceptibility in Asian population. *Eur J Med Res* 2017; **22**: 55 [PMID: 29282121 DOI: 10.1186/s40001-017-0288-3]

26 **Trinks J**, Nishida N, Hulaniuk ML, Caputo M, Tsuchiura T, Marciano S, Haddad L, Blejer J, Bartoli S, Ameigeiras B, Frías SE, Vistarini C, Heinrich F, Remondegui C, Ceballos S, Echenique G, Charre Samman M, D'Amico C, Rojas A, Martínez A, Ridruejo E, Fernández RJ, Burgos Pratx L, Salamone H, Nuñez F, Galdame O, Gadano A, Corach D, Sugiyama M, Flichman D, Tokunaga K, Mizokami M. Role of HLA-DP and HLA-DQ on the clearance of hepatitis B virus and the risk of chronic infection in a multiethnic population. *Liver Int* 2017; **37**: 1476-1487 [PMID: 28267888 DOI: 10.1111/liv.13405]

27 **Akcay IM**, Katrinli S, Ozdil K, Doganay GD, Doganay L. Host genetic factors affecting hepatitis B infection outcomes: Insights from genome-wide association studies. *World J Gastroenterol* 2018; **24**: 3347-3360 [PMID: 30122875 DOI: 10.3748/wjg.v24.i30.3347]

28 **Kennedy RB**, Ovsyannikova IG, Palese P, Poland GA. Current Challenges in Vaccinology. *Front Immunol* 2020; **11**: 1181 [PMID: 32670279 DOI: 10.3389/fimmu.2020.01181]

29 **Wang WT**, Zhao XQ, Li GP, Chen YZ, Wang L, Han MF, Li WN, Chen T, Chen G, Xu D, Ning Q, Zhao XP. Immune response pattern varies with the natural history of chronic hepatitis B. *World J Gastroenterol* 2019; **25**: 1950-1963 [PMID: 31086463 DOI: 10.3748/wjg.v25.i16.1950]

30 **Moudi B**, Heidari Z, Mahmoudzadeh-Sagheb H. Impact of host gene polymorphisms on susceptibility to chronic hepatitis B virus infection. *Infect Genet Evol* 2016; **44**: 94-105 [PMID: 27346643 DOI: 10.1016/j.meegid.2016.06.043]

31 **Hu HH**, Liu J, Lin YL, Luo WS, Chu YJ, Chang CL, Jen CL, Lee MH, Lu SN, Wang LY, You SL, Yang HI, Chen CJ; REVEAL-HBV Study Group. The rs2296651 (S267F) variant on NTCP (SLC10A1) is inversely associated with chronic hepatitis B and progression to cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis B. *Gut* 2016; **65**: 1514-1521 [PMID: 26642861 DOI: 10.1136/gutjnl-2015-310686]

32 **Thompson AJ**, Nguyen T, Iser D, Ayres A, Jackson K, Littlejohn M, Slavin J, Bowden S, Gane EJ, Abbott W, Lau GK, Lewin SR, Visvanathan K, Desmond PV, Locarnini SA. Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology* 2010; **51**: 1933-1944 [PMID: 20512987 DOI: 10.1002/hep.23571]

33 **Manesis EK**, Papatheodoridis GV, Tiniakos DG, Hadziyannis ES, Agelopoulou OP, Syminelaki T, Papaioannou C, Nastos T, Karayiannis P. Hepatitis B surface antigen: relation to hepatitis B

replication parameters in HBeAg-negative chronic hepatitis B. *J Hepatol* 2011; **55**: 61-68 [PMID: 21145875 DOI: 10.1016/j.jhep.2010.10.027]

34 **Pollicino T**, Amaddeo G, Restuccia A, Raffa G, Alibrandi A, Cutroneo G, Favaloro A, Maimone S, Squadrito G, Raimondo G. Impact of hepatitis B virus (HBV) preS/S genomic variability on HBV surface antigen and HBV DNA serum levels. *Hepatology* 2012; **56**: 434-443 [PMID: 22271491 DOI: 10.1002/hep.25592]

35 **Mirabelli C**, Surdo M, Van Hemert F, Lian Z, Salpini R, Cento V, Cortese MF, Aragri M, Pollicita M, Alteri C, Bertoli A, Berkhout B, Micheli V, Gubertini G, Santoro MM, Romano S, Visca M, Bernassola M, Longo R, De Sanctis GM, Trimoulet P, Fleury H, Marino N, Mazzotta F, Cappiello G, Spanò A, Sarrecchia C, Zhang JM, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Specific mutations in the C-terminus domain of HBV surface antigen significantly correlate with low level of serum HBV-DNA in patients with chronic HBV infection. *J Infect* 2015; **70**: 288-298 [PMID: 25452041 DOI: 10.1016/j.jinf.2014.10.015]

36 **Kuhnhenn L**, Jiang B, Kubesch A, Vermehren J, Knop V, Susser S, Dietz J, Carra G, Finkelmeier F, Grammatikos G, Zeuzem S, Sarrazin C, Hildt E, Peiffer KH. Impact of HBV genotype and mutations on HBV DNA and qHBsAg levels in patients with HBeAg-negative chronic HBV infection. *Aliment Pharmacol Ther* 2018; **47**: 1523-1535 [PMID: 29637585 DOI: 10.1111/apt.14636]

37 **Liaw YF**. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative chronic HBV infection. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 631-641 [PMID: 31477873 DOI: 10.1038/s41575-019-0197-8]

38 **Cornberg M**, Wong VW, Locarnini S, Brunetto M, Janssen HLA, Chan HL. The role of quantitative hepatitis B surface antigen revisited. *J Hepatol* 2017; **66**: 398-411 [PMID: 27575311 DOI: 10.1016/j.jhep.2016.08.009]

39 **Martinot-Peignoux M**, Asselah T, Marcellin P. HBsAg quantification to optimize treatment monitoring in chronic hepatitis B patients. *Liver Int* 2015; **35** Suppl 1: 82-90 [PMID: 25529092 DOI: 10.1111/liv.12735]

40 **Chuaypen N**, Sriprapun M, Praianantathavorn K, Payungporn S, Wisedopas N, Poovorawan Y, Tangkijvanich P. Kinetics of serum HBsAg and intrahepatic cccDNA during pegylated interferon therapy in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B. *J Med Virol* 2017; **89**: 130-138 [PMID: 27307409 DOI: 10.1002/jmv.24601]

41 **European Association for the Study of the Liver**. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]

42 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

43 **Wong DK**, Seto WK, Cheung KS, Chong CK, Huang FY, Fung J, Lai CL, Yuen MF. Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA. *Liver Int* 2017; **37**: 995-1001 [PMID: 27992681 DOI: 10.1111/liv.13346]

44 **Testoni B**, Lebossé F, Scholtes C, Berby F, Miaglia C, Subic M, Loglio A, Facchetti F, Lampertico P, Levrero M, Zoulim F. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. *J Hepatol* 2019; **70**: 615-625 [PMID: 30529504 DOI: 10.1016/j.jhep.2018.11.030]

45 **Jung KS**, Park JY, Chon YE, Kim HS, Kang W, Kim BK, Kim SU, Kim do Y, Han KH, Ahn SH. Clinical outcomes and predictors for relapse after cessation of oral antiviral treatment in chronic hepatitis B patients. *J Gastroenterol* 2016; **51**: 830-839 [PMID: 26687058 DOI: 10.1007/s00535-015-1153-1]

46 **Tada T**, Kumada T, Toyoda H, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Kitabatake S, Yama T, Tanaka J. HBcrAg predicts hepatocellular carcinoma development: An analysis using time-dependent receiver operating characteristics. *J Hepatol* 2016; **65**: 48-56 [PMID: 27034253 DOI: 10.1016/j.jhep.2016.03.013]

47 **Butler EK**, Gersch J, McNamara A, Luk KC, Holzmayer V, de Medina M, Schiff E, Kuhns M, Cloherty GA. Hepatitis B Virus Serum DNA andRNA Levels in Nucleos(t)ide Analog-Treated or Untreated Patients During Chronic and Acute Infection. *Hepatology* 2018; **68**: 2106-2117 [PMID: 29734472 DOI: 10.1002/hep.30082]

48 **Huang H**, Wang J, Li W, Chen R, Chen X, Zhang F, Xu D, Lu F. Serum HBV DNA plus RNA shows superiority in reflecting the activity of intrahepatic cccDNA in treatment-naïve HBV-infected individuals. *J Clin Virol* 2018; **99-100**: 71-78 [PMID: 29353073 DOI: 10.1016/j.jcv.2017.12.016]

49 **Wang J**, Yu Y, Li G, Shen C, Meng Z, Zheng J, Jia Y, Chen S, Zhang X, Zhu M, Song Z, Wu J, Shao L, Qian P, Mao X, Wang X, Huang Y, Zhao C, Zhang J, Qiu C, Zhang W. Relationship between serum HBV-RNA levels and intrahepatic viral as well as histologic activity markers in entecavir-treated patients. *J Hepatol* 2017 [PMID: 28870671 DOI: 10.1016/j.jhep.2017.08.021]

50 **van Bömmel F**, van Bömmel A, Krauel A, Wat C, Pavlovic V, Yang L, Deichsel D, Berg T, Böhm S. Serum HBV RNA as a Predictor of Peginterferon Alfa-2a Response in Patients With HBeAg-Positive Chronic Hepatitis B. *J Infect Dis* 2018; **218**: 1066-1074 [PMID: 29741634 DOI: 10.1093/infdis/jiy270]

51 **Caviglia GP**, Abate ML, Tandoi F, Ciancio A, Amoroso A, Salizzoni M, Saracco GM, Rizzetto M, Romagnoli R, Smedile A. Quantitation of HBV cccDNA in anti-HBc-positive liver donors by

droplet digital PCR: A new tool to detect occult infection. *J Hepatol* 2018; **69**: 301-307 [PMID: 29621551 DOI: 10.1016/j.jhep.2018.03.021]

52 **Mu D**, Yuan FC, Chen Y, Jiang XY, Yan L, Jiang LY, Gong JP, Zhang DZ, Ren H, Liao Y. Baseline value of intrahepatic HBV DNA over cccDNA predicts patient's response to interferon therapy. *Sci Rep* 2017; **7**: 5937 [PMID: 28725013 DOI: 10.1038/s41598-017-05242-y]

53 **Huang JT**, Yang Y, Hu YM, Liu XH, Liao MY, Morgan R, Yuan EF, Li X, Liu SM. A Highly Sensitive and Robust Method for Hepatitis B Virus Covalently Closed Circular DNA Detection in Single Cells and Serum. *J Mol Diagn* 2018; **20**: 334-343 [PMID: 29656833 DOI: 10.1016/j.jmoldx.2018.01.010]

54 **Cornberg M**, Lok AS, Terrault NA, Zoulim F; 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference‡. *J Hepatol* 2020; **72**: 539-557 [PMID: 31730789 DOI: 10.1016/j.jhep.2019.11.003]

55 **Shimura S**, Watashi K, Fukano K, Peel M, Sluder A, Kawai F, Iwamoto M, Tsukuda S, Takeuchi JS, Miyake T, Sugiyama M, Ogasawara Y, Park SY, Tanaka Y, Kusuhara H, Mizokami M, Sureau C, Wakita T. Cyclosporin derivatives inhibit hepatitis B virus entry without interfering with NTCP transporter activity. *J Hepatol* 2017; **66**: 685-692 [PMID: 27890789 DOI: 10.1016/j.jhep.2016.11.009]

56 **Donkers JM**, Appelman MD, van de Graaf SFJ. Mechanistic insights into the inhibition of NTCP by myrcludex B. *JHEP Rep* 2019; **1**: 278-285 [PMID: 32039379 DOI: 10.1016/j.jhepr.2019.07.006]

57 **Bazinet M**, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, Jimbei P, Iarovoi L, Smesnoi V, Musteata T, Jucov A, Dittmer U, Krawczyk A, Vaillant A. Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy. *Gastroenterology* 2020; **158**: 2180-2194 [PMID: 32147484 DOI: 10.1053/j.gastro.2020.02.058]

58 **Han K**, Cremer J, Elston R, Oliver S, Baptiste-Brown S, Chen S, Gardiner D, Davies M, Saunders J, Hamatake R, Losos J, Leivers M, Hood S, van der Berg F, Paff M, Ritter JM, Theodore D. A Randomized, Double-Blind, Placebo-Controlled, First-Time-in-Human Study to Assess the Safety, Tolerability, and Pharmacokinetics of Single and Multiple Ascending Doses of GSK3389404 in Healthy Subjects. *Clin Pharmacol Drug Dev* 2019; **8**: 790-801 [PMID: 30861337 DOI: 10.1002/cpdd.670]

59 **Yuen MF**, Gane EJ, Kim DJ, Weilert F, Yuen Chan HL, Lalezari J, Hwang SG, Nguyen T, Flores O, Hartman G, Liaw S, Lenz O, Kakuda TN, Talloen W, Schwabe C, Klumpp K, Brown N. Antiviral Activity, Safety, and Pharmacokinetics of Capsid Assembly Modulator NVR 3-778 in Patients with Chronic HBV Infection. *Gastroenterology* 2019; **156**: 1392-1403. e7 [PMID: 30625297 DOI: 10.1053/j.gastro.2018.12.023]

60 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durantel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]

61 **Yang HC**, Chen PJ. The potential and challenges of CRISPR-Cas in eradication of hepatitis B virus covalently closed circular DNA. *Virus Res* 2018; **244**: 304-310 [PMID: 28627393 DOI: 10.1016/j.virusres.2017.06.010]

62 **Lucifora J**, Arzberger S, Durantel D, Belloni L, Strubin M, Levrero M, Zoulim F, Hantz O, Protzer U. Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. *J Hepatol* 2011; **55**: 996-1003 [PMID: 21376091 DOI: 10.1016/j.jhep.2011.02.015]

63 **Imam H**, Kim GW, Mir SA, Khan M, Siddiqui A. Interferon-stimulated gene 20 (ISG20) selectively degrades N6-methyladenosine modified Hepatitis B Virus transcripts. *PLoS Pathog* 2020; **16**: e1008338 [PMID: 32059034 DOI: 10.1371/journal.ppat.1008338]

64 **Niu C**, Li L, Daffis S, Lucifora J, Bonnin M, Maadadi S, Salas E, Chu R, Ramos H, Livingston CM, Beran RK, Garg AV, Balsitis S, Durantel D, Zoulim F, Delaney WE 4th, Fletcher SP. Toll-like receptor 7 agonist GS-9620 induces prolonged inhibition of HBV *via* a type I interferon-dependent mechanism. *J Hepatol* 2018; **68**: 922-931 [PMID: 29247725 DOI: 10.1016/j.jhep.2017.12.007]

65 **Gane E**, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, Schwabe C, Dunbar PR. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study. *J Hepatol* 2019; **71**: 900-907 [PMID: 31306680 DOI: 10.1016/j.jhep.2019.06.028]

66 **Bertoletti A**. ImmTAV, a New Immunotherapy Targeting the Source of HBV Infection. *Hepatology* 2020; **72**: 1514-1517 [PMID: 32865247 DOI: 10.1002/hep.31527]

67 **Kah J**, Koh S, Volz T, Ceccarello E, Allweiss L, Lütgehetmann M, Bertoletti A, Dandri M. Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection. *J Clin Invest* 2017; **127**: 3177-3188 [PMID: 28737510 DOI: 10.1172/JCI93024]

68 **Boni C**, Janssen HLA, Rossi M, Yoon SK, Vecchi A, Barili V, Yoshida EM, Trinh H, Rodell TC, Laccabue D, Alfieri A, Brillo F, Fisicaro P, Acerbi G, Pedrazzi G, Andreone P, Cursaro C, Margotti M, Santoro R, Piazzolla V, Brunetto MR, Coco B, Cavallone D, Zhao Y, Joshi A, Woo J, Lau AH, Gaggar A, Subramanian GM, Massetto B, Fung S, Ahn SH, Ma X, Mangia A, Ferrari C. Combined GS-4774 and Tenofovir Therapy Can Improve HBV-Specific T-Cell Responses in Patients With Chronic Hepatitis. *Gastroenterology* 2019; **157**: 227-241. e7 [PMID: 30930022 DOI:

10.1053/j.gastro.2019.03.044]
69 **World Health Organization**. Immunization coverage. [Internet] [accessed 17 August 2020]. Available from: https://www.who.int/news-room/fact-sheets/detail/immunization-coverage
70 **Ott JJ**, Horn J, Krause G, Mikolajczyk RT. Time trends of chronic HBV infection over prior decades - A global analysis. *J Hepatol* 2017; **66**: 48-54 [PMID: 27592304 DOI: 10.1016/j.jhep.2016.08.013]
71 **Yang S**, Tian G, Cui Y, Ding C, Deng M, Yu C, Xu K, Ren J, Yao J, Li Y, Cao Q, Chen P, Xie T, Wang C, Wang B, Mao C, Ruan B, Jiang T, Li L. Factors influencing immunologic response to hepatitis B vaccine in adults. *Sci Rep* 2016; **6**: 27251 [PMID: 27324884 DOI: 10.1038/srep27251]
72 **Schillie S**, Harris A, Link-Gelles R, Romero J, Ward J, Nelson N. Recommendations of the Advisory Committee on Immunization Practices for Use of a Hepatitis B Vaccine with a Novel Adjuvant. *MMWR Morb Mortal Wkly Rep* 2018; **67**: 455-458 [PMID: 29672472 DOI: 10.15585/mmwr.mm6715a5]
73 **Walayat S**, Ahmed Z, Martin D, Puli S, Cashman M, Dhillon S. Recent advances in vaccination of non-responders to standard dose hepatitis B virus vaccine. *World J Hepatol* 2015; **7**: 2503-2509 [PMID: 26523203 DOI: 10.4254/wjh.v7.i24.2503]
74 **Trantham L**, Kurosky SK, Zhang D, Johnson KD. Adherence with and completion of recommended hepatitis vaccination schedules among adults in the United States. *Vaccine* 2018; **36**: 5333-5339 [PMID: 29909136 DOI: 10.1016/j.vaccine.2018.05.111]
75 **Schnyder JL**, De Pijper CA, Garcia Garrido HM, Daams JG, Goorhuis A, Stijnis C, Schaumburg F, Grobusch MP. Fractional dose of intradermal compared to intramuscular and subcutaneous vaccination - A systematic review and meta-analysis. *Travel Med Infect Dis* 2020; **37**: 101868 [PMID: 32898704 DOI: 10.1016/j.tmaid.2020.101868]
76 **Elhanan E**, Boaz M, Schwartz I, Schwartz D, Chernin G, Soetendorp H, Gal Oz A, Agbaria A, Weinstein T. A randomized, controlled clinical trial to evaluate the immunogenicity of a PreS/S hepatitis B vaccine Sci-B-Vac™, as compared to Engerix B®, among vaccine naïve and vaccine non-responder dialysis patients. *Clin Exp Nephrol* 2018; **22**: 151-158 [PMID: 28456864 DOI: 10.1007/s10157-017-1416-7]
77 **Ye H**, Teng J, Lin Z, Wang Y, Fu X. Analysis of HBsAg mutations in the 25 years after the implementation of the hepatitis B vaccination plan in China. *Virus Genes* 2020; **56**: 546-556 [PMID: 32542478 DOI: 10.1007/s11262-020-01773-1]
78 **Di Lello FA**, Ridruejo E, Martínez AP, Pérez PS, Campos RH, Flichman DM. Molecular epidemiology of hepatitis B virus mutants associated with vaccine escape, drug resistance and diagnosis failure. *J Viral Hepat* 2019; **26**: 552-560 [PMID: 30576055 DOI: 10.1111/jvh.13052]
79 **Gerlich WH**. Prophylactic vaccination against hepatitis B: achievements, challenges and perspectives. *Med Microbiol Immunol* 2015; **204**: 39-55 [PMID: 25523195 DOI: 10.1007/s00430-014-0373-y]
80 **Schlabe S**, van Bremen K, Aldabbagh S, Glebe D, Bremer CM, Marsen T, Mellin W, Cristanziano VD, Eis-Hübinger AM, Spengler U. Hepatitis B virus subgenotype F3 reactivation with vaccine escape mutations: A case report and review of the literature. *World J Hepatol* 2018; **10**: 509-516 [PMID: 30079137 DOI: 10.4254/wjh.v10.i7.509]
81 **O'Halloran JA**, De Gascun CF, Dunford L, Carr MJ, Connell J, Howard R, Hall WW, Lambert JS. Hepatitis B virus vaccine failure resulting in chronic hepatitis B infection. *J Clin Virol* 2011; **52**: 151-154 [PMID: 21802353 DOI: 10.1016/j.jcv.2011.06.020]
82 **Stramer SL**, Wend U, Candotti D, Foster GA, Hollinger FB, Dodd RY, Allain JP, Gerlich W. Nucleic acid testing to detect HBV infection in blood donors. *N Engl J Med* 2011; **364**: 236-247 [PMID: 21247314 DOI: 10.1056/NEJMoa1007644]
83 **Inoue T**, Tanaka Y. Cross-Protection of Hepatitis B Vaccination among Different Genotypes. *Vaccines (Basel)* 2020; **8** [PMID: 32824318 DOI: 10.3390/vaccines8030456]
84 **Alter HJ**. THE GORDON WILSON LECTURE: THE HEPATITIS C VIRUS: FROM HIPPOCRATES TO CURE. *Trans Am Clin Climatol Assoc* 2019; **130**: 104-118 [PMID: 31516174]
85 **Houghton M**. The long and winding road leading to the identification of the hepatitis C virus. *J Hepatol* 2009; **51**: 939-948 [PMID: 19781804 DOI: 10.1016/j.jhep.2009.08.004]
86 **Luna JM**, Saeed M, Rice CM. Taming a beast: lessons from the domestication of hepatitis C virus. *Curr Opin Virol* 2019; **35**: 27-34 [PMID: 30875640 DOI: 10.1016/j.coviro.2019.02.008]
87 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562 DOI: 10.1126/science.2523562]
88 **Murphy DG**, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C virus genotype 7, a new genotype originating from central Africa. *J Clin Microbiol* 2015; **53**: 967-972 [PMID: 25520447 DOI: 10.1128/JCM.02831-14]
89 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]
90 **Borgia SM**, Hedskog C, Parhy B, Hyland RH, Stamm LM, Brainard DM, Subramanian MG, McHutchison JG, Mo H, Svarovskaia E, Shafran SD. Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes. *J Infect Dis* 2018; **218**: 1722-1729 [PMID: 29982508 DOI: 10.1093/infdis/jiy401]
91 **Manns MP**, Buti M, Gane E, Pawlotsky JM, Razavi H, Terrault N, Younossi Z. Hepatitis C virus

infection. *Nat Rev Dis Primers* 2017; **3**: 17006 [PMID: 28252637 DOI: 10.1038/nrdp.2017.6]
92 **Herzer K**, Gerken G, Kroy D, Tacke F, Plewe J, Eurich D, Spengler U, Strassburg CP, Welker MW, Pischke S, Sterneck M, Mehrabi A, Weiss KH, Herber A, Berg T, Zimmermann T, Galle PR, Heinzow H, Schmidt H, Markova A, Serfert Y, Manns MP, Zeuzem S, Wedemeyer H. Impact of direct-acting antiviral therapy on the need for liver transplantation related to hepatitis C in Germany. *J Hepatol* 2018; **69**: 982-984 [PMID: 30089577 DOI: 10.1016/j.jhep.2018.07.001]
93 **Waziry R**, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017; **67**: 1204-1212 [PMID: 28802876 DOI: 10.1016/j.jhep.2017.07.025]
94 **Wedemeyer H**, Khera T, Strunz B, Björkström NK. Reversal of Immunity After Clearance of Chronic HCV Infection-All Reset? *Front Immunol* 2020; **11**: 571166 [PMID: 33133084 DOI: 10.3389/fimmu.2020.571166]
95 **Hollande C**, Parlati L, Pol S. Micro-elimination of hepatitis C virus. *Liver Int* 2020; **40** Suppl 1: 67-71 [PMID: 32077601 DOI: 10.1111/liv.14363]
96 **Matičič M**, Lombardi A, Mondelli MU, Colombo M; ESCMID Study Group for Viral Hepatitis (ESGVH). Elimination of hepatitis C in Europe: can WHO targets be achieved? *Clin Microbiol Infect* 2020; **26**: 818-823 [PMID: 31978546 DOI: 10.1016/j.cmi.2020.01.014]
97 **Tang L**, Marcell L, Kottilil S. Systemic manifestations of hepatitis C infection. *Infect Agent Cancer* 2016; **11**: 29 [PMID: 27222662 DOI: 10.1186/s13027-016-0076-7]
98 **Goto K**, Roca Suarez AA, Wrensch F, Baumert TF, Lupberger J. Hepatitis C Virus and Hepatocellular Carcinoma: When the Host Loses Its Grip. *Int J Mol Sci* 2020; **21** [PMID: 32357520 DOI: 10.3390/ijms21093057]
99 **Rios DA**, Valva P, Casciato PC, Frias S, Soledad Caldirola M, Gaillard MI, Bezrodnik L, Bandi J, Galdame O, Ameigeiras B, Krasniansky D, Brodersen C, Mullen E, Matteo EN, Preciado MV. Chronic hepatitis C liver microenvironment: role of the Th17/Treg interplay related to fibrogenesis. *Sci Rep* 2017; **7**: 13283 [PMID: 29038590 DOI: 10.1038/s41598-017-13777-3]
100 **Valva P**, Gismondi MI, Casciato PC, Galoppo M, Lezama C, Galdame O, Gadano A, Galoppo MC, Mullen E, De Matteo EN, Preciado MV. Distinctive intrahepatic characteristics of paediatric and adult pathogenesis of chronic hepatitis C infection. *Clin Microbiol Infect* 2014; **20**: O998-1009 [PMID: 24942073 DOI: 10.1111/1469-0691.12728]
101 **Mengshol JA**, Golden-Mason L, Rosen HR. Mechanisms of Disease: HCV-induced liver injury. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 622-634 [PMID: 17978819 DOI: 10.1038/ncpgasthep0961]
102 **Jenne CN**, Kubes P. Immune surveillance by the liver. *Nat Immunol* 2013; **14**: 996-1006 [PMID: 24048121 DOI: 10.1038/ni.2691]
103 **Ghosh A**, Romani S, Kottilil S, Poonia B. Lymphocyte Landscape after Chronic *Hepatitis C Virus* (HCV) Cure: The New Normal. *Int J Mol Sci* 2020; **21** [PMID: 33050486 DOI: 10.3390/ijms21207473]
104 **Gorin JB**, Malone DFG, Strunz B, Carlsson T, Aleman S, Björkström NK, Falconer K, Sandberg JK. Plasma FABP4 is associated with liver disease recovery during treatment-induced clearance of chronic HCV infection. *Sci Rep* 2020; **10**: 2081 [PMID: 32034167 DOI: 10.1038/s41598-020-58768-z]
105 **Debes JD**, van Tilborg M, Groothuismink ZMA, Hansen BE, Schulze Zur Wiesch J, von Felden J, de Knegt RJ, Boonstra A. Levels of Cytokines in Serum Associate With Development of Hepatocellular Carcinoma in Patients With HCV Infection Treated With Direct-Acting Antivirals. *Gastroenterology* 2018; **154**: 515-517. e3 [PMID: 29102620 DOI: 10.1053/j.gastro.2017.10.035]
106 **Shrivastava S**, Bhatta M, Ward H, Romani S, Lee R, Rosenthal E, Osinusi A, Kohli A, Masur H, Kottilil S, Wilson E. Multitarget Direct-Acting Antiviral Therapy Is Associated With Superior Immunologic Recovery in Patients Coinfected With Human Immunodeficiency Virus and Hepatitis C Virus. *Hepatol Commun* 2018; **2**: 1451-1466 [PMID: 30556035 DOI: 10.1002/hep4.1258]
107 **Barili V**, Fisicaro P, Montanini B, Acerbi G, Filippi A, Forleo G, Romualdi C, Ferracin M, Guerrieri F, Pedrazzi G, Boni C, Rossi M, Vecchi A, Penna A, Zecca A, Mori C, Orlandini A, Negri E, Pesci M, Massari M, Missale G, Levrero M, Ottonello S, Ferrari C. Targeting p53 and histone methyltransferases restores exhausted CD8+ T cells in HCV infection. *Nat Commun* 2020; **11**: 604 [PMID: 32001678 DOI: 10.1038/s41467-019-14137-7]
108 **Smits M**, Zoldan K, Ishaque N, Gu Z, Jechow K, Wieland D, Conrad C, Eils R, Fauvelle C, Baumert TF, Emmerich F, Bengsch B, Neumann-Haefelin C, Hofmann M, Thimme R, Boettler T. Follicular T helper cells shape the HCV-specific CD4+ T cell repertoire after virus elimination. *J Clin Invest* 2020; **130**: 998-1009 [PMID: 31697649 DOI: 10.1172/JCI129642]
109 **Langhans B**, Nischalke HD, Krämer B, Hausen A, Dold L, van Heteren P, Hüneburg R, Nattermann J, Strassburg CP, Spengler U. Increased peripheral $CD4^+$ regulatory T cells persist after successful direct-acting antiviral treatment of chronic hepatitis C. *J Hepatol* 2017; **66**: 888-896 [PMID: 28040549 DOI: 10.1016/j.jhep.2016.12.019]
110 **Njiomegnie GF**, Read SA, Fewings N, George J, McKay F, Ahlenstiel G. Immunomodulation of the Natural Killer Cell Phenotype and Response during HCV Infection. *J Clin Med* 2020; **9** [PMID: 32268490 DOI: 10.3390/jcm9041030]
111 **Stuart JD**, Salinas E, Grakoui A. Immune system control of hepatitis C virus infection. *Curr Opin Virol* 2021; **46**: 36-44 [PMID: 33137689 DOI: 10.1016/j.coviro.2020.10.002]

112 **Parlati L**, Pol S. Direct acting antivirals failure: cause and retreatment options. *Expert Rev Gastroenterol Hepatol* 2018; **12**: 1245-1250 [PMID: 30791789 DOI: 10.1080/17474124.2018.1541237]

113 **Bailey JR**, Barnes E, Cox AL. Approaches, Progress, and Challenges to Hepatitis C Vaccine Development. *Gastroenterology* 2019; **156**: 418-430 [PMID: 30268785 DOI: 10.1053/j.gastro.2018.08.060]

114 **Hayes CN**, Imamura M, Chayama K. Management of HCV patients in cases of direct-acting antiviral failure. *Expert Rev Gastroenterol Hepatol* 2019; **13**: 839-848 [PMID: 31392907 DOI: 10.1080/17474124.2019.1651642]

115 **Zeng H**, Li L, Hou Z, Zhang Y, Tang Z, Liu S. Direct-acting Antiviral in the Treatment of Chronic Hepatitis C: Bonuses and Challenges. *Int J Med Sci* 2020; **17**: 892-902 [PMID: 32308542 DOI: 10.7150/ijms.43079]

116 **Paolucci S**, Premoli M, Novati S, Gulminetti R, Maserati R, Barbarini G, Sacchi P, Piralla A, Sassera D, De Marco L, Girello A, Mondelli MU, Baldanti F. Baseline and Breakthrough Resistance Mutations in HCV Patients Failing DAAs. *Sci Rep* 2017; **7**: 16017 [PMID: 29167469 DOI: 10.1038/s41598-017-15987-1]

117 **Dietz J**, Di Maio VC, de Salazar A, Merino D, Vermehren J, Paolucci S, Kremer AE, Lara M, Pardo MR, Zoller H, Degasperi E, Peiffer KH, Sighinolfi L, Téllez F, Graf C, Ghisetti V, Schreiber J, Fernández-Fuertes E, Boglione L, Muñoz-Medina L, Stauber R, Gennari W, Figueruela B, Santos J, Lampertico P, Zeuzem S, Ceccherini-Silberstein F, García F, Sarrazin C; HCV Virology Italian Resistance Network (VIRONET-C) collaborators; Spanish GEHEP-004 Collaborators; Members of the German HCV resistance study group. Failure on voxilaprevir, velpatasvir, sofosbuvir and efficacy of rescue therapy. *J Hepatol* 2021; **74**: 801-810 [PMID: 33220331 DOI: 10.1016/j.jhep.2020.11.017]

118 **Kahn JA**. The use of organs from hepatitis C virus-viremic donors into uninfected recipients. *Curr Opin Organ Transplant* 2020; **25**: 620-625 [PMID: 33105203 DOI: 10.1097/MOT.0000000000000826]

119 **Kappus MR**, Wolfe CR, Muir AJ. Direct-Acting Antivirals and Organ Transplantation: Is There Anything We Can't Do? *J Infect Dis* 2020; **222**: S794-S801 [PMID: 33245347 DOI: 10.1093/infdis/jiaa420]

120 **Ghany MG**, Morgan TR; AASLD-IDSA Hepatitis C Guidance Panel. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology* 2020; **71**: 686-721 [PMID: 31816111 DOI: 10.1002/hep.31060]

121 **Muzica CM**, Stanciu C, Huiban L, Singeap AM, Sfarti C, Zenovia S, Cojocariu C, Trifan A. Hepatocellular carcinoma after direct-acting antiviral hepatitis C virus therapy: A debate near the end. *World J Gastroenterol* 2020; **26**: 6770-6781 [PMID: 33268960 DOI: 10.3748/wjg.v26.i43.6770]

122 **Lemon SM**, McGivern DR. Is hepatitis C virus carcinogenic? *Gastroenterology* 2012; **142**: 1274-1278 [PMID: 22537433 DOI: 10.1053/j.gastro.2012.01.045]

123 **Morgan RL**, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* 2013; **158**: 329-337 [PMID: 23460056 DOI: 10.7326/0003-4819-158-5-201303050-00005]

124 **Cabibbo G**, Celsa C, Calvaruso V, Petta S, Cacciola I, Cannavò MR, Madonia S, Rossi M, Magro B, Rini F, Distefano M, Larocca L, Prestileo T, Malizia G, Bertino G, Benanti F, Licata A, Scalisi I, Mazzola G, Di Rosolini MA, Alaimo G, Averna A, Cartabellotta F, Alessi N, Guastella S, Russello M, Scifo G, Squadrito G, Raimondo G, Trevisani F, Craxì A, Di Marco V, Cammà C; Rete Sicilia Selezione Terapia – HCV (RESIST-HCV) and Italian Liver Cancer (ITA. LI.CA.) Group. Direct-acting antivirals after successful treatment of early hepatocellular carcinoma improve survival in HCV-cirrhotic patients. *J Hepatol* 2019; **71**: 265-273 [PMID: 30959157 DOI: 10.1016/j.jhep.2019.03.027]

125 **Gao X**, Zhan M, Wang L, Ding Y, Niu J. Timing of DAA Initiation After Curative Treatment and Its Relationship with the Recurrence of HCV-Related HCC. *J Hepatocell Carcinoma* 2020; **7**: 347-360 [PMID: 33299823 DOI: 10.2147/JHC.S279657]

126 **Conti F**, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, Foschi FG, Lenzi M, Mazzella G, Verucchi G, Andreone P, Brillanti S. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol* 2016; **65**: 727-733 [PMID: 27349488 DOI: 10.1016/j.jhep.2016.06.015]

127 **Reig M**, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X, Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol* 2016; **65**: 719-726 [PMID: 27084592 DOI: 10.1016/j.jhep.2016.04.008]

128 **Saraiya N**, Yopp AC, Rich NE, Odewole M, Parikh ND, Singal AG. Systematic review with meta-analysis: recurrence of hepatocellular carcinoma following direct-acting antiviral therapy. *Aliment Pharmacol Ther* 2018; **48**: 127-137 [PMID: 29851093 DOI: 10.1111/apt.14823]

129 **Ioannou GN**, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017 [PMID: 28887168 DOI: 10.1016/j.jhep.2017.08.030]

130 **Wörns MA**, Galle PR, Zeuzem S, Schirmacher P, Manns M, Vogel A. Drug Treatment for Chronic

Hepatitis C Infection and Cancer Risk. *Dtsch Arztebl Int* 2017; **114**: 597-602 [PMID: 28927498 DOI: 10.3238/arztebl.2017.0597]

131 **Cabibbo G**, Petta S, Barbàra M, Missale G, Virdone R, Caturelli E, Piscaglia F, Morisco F, Colecchia A, Farinati F, Giannini E, Trevisani F, Craxì A, Colombo M, Cammà C; ITA. LI.CA study group. A meta-analysis of single HCV-untreated arm of studies evaluating outcomes after curative treatments of HCV-related hepatocellular carcinoma. *Liver Int* 2017; **37**: 1157-1166 [PMID: 28061016 DOI: 10.1111/liv.13357]

132 **Bassett SE**, Guerra B, Brasky K, Miskovsky E, Houghton M, Klimpel GR, Lanford RE. Protective immune response to hepatitis C virus in chimpanzees rechallenged following clearance of primary infection. *Hepatology* 2001; **33**: 1479-1487 [PMID: 11391537 DOI: 10.1053/jhep.2001.24371]

133 **Prince AM**. Immunity in hepatitis C virus infection. *Vox Sang* 1994; **67** Suppl 3: 227-228 [PMID: 7526553 DOI: 10.1111/j.1423-0410.1994.tb04581.x]

134 **Sacks-Davis R**, Grebely J, Dore GJ, Osburn W, Cox AL, Rice TM, Spelman T, Bruneau J, Prins M, Kim AY, McGovern BH, Shoukry NH, Schinkel J, Allen TM, Morris M, Hajarizadeh B, Maher L, Lloyd AR, Page K, Hellard M; InC3 study group. Hepatitis C Virus Reinfection and Spontaneous Clearance of Reinfection--the InC3 Study. *J Infect Dis* 2015; **212**: 1407-1419 [PMID: 25883387 DOI: 10.1093/infdis/jiv220]

135 **Osburn WO**, Fisher BE, Dowd KA, Urban G, Liu L, Ray SC, Thomas DL, Cox AL. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. *Gastroenterology* 2010; **138**: 315-324 [PMID: 19782080 DOI: 10.1053/j.gastro.2009.09.017]

136 **Lothert K**, Offersgaard AF, Pihl AF, Mathiesen CK, Jensen TB, Alzua GP, Fahnøe U, Bukh J, Gottwein JM, Wolff MW. Development of a downstream process for the production of an inactivated whole hepatitis C virus vaccine. *Sci Rep* 2020; **10**: 16261 [PMID: 33004836 DOI: 10.1038/s41598-020-72328-5]

137 **Capone S**, Brown A, Hartnell F, Sorbo MD, Traboni C, Vassilev V, Colloca S, Nicosia A, Cortese R, Folgori A, Klenerman P, Barnes E, Swadling L. Optimising T cell (re)boosting strategies for adenoviral and modified vaccinia Ankara vaccine regimens in humans. *NPJ Vaccines* 2020; **5**: 94 [PMID: 33083029 DOI: 10.1038/s41541-020-00240-0]

138 **Fauvelle C**, Colpitts CC, Keck ZY, Pierce BG, Foung SK, Baumert TF. Hepatitis C virus vaccine candidates inducing protective neutralizing antibodies. *Expert Rev Vaccines* 2016; **15**: 1535-1544 [PMID: 27267297 DOI: 10.1080/14760584.2016.1194759]

139 **Cox AL**, Thomas DL. Hepatitis C virus vaccines among people who inject drugs. *Clin Infect Dis* 2013; **57** Suppl 2: S46-S50 [PMID: 23884065 DOI: 10.1093/cid/cit329]

140 **Youn JW**, Hu YW, Tricoche N, Pfahler W, Shata MT, Dreux M, Cosset FL, Folgori A, Lee DH, Brotman B, Prince AM. Evidence for protection against chronic hepatitis C virus infection in chimpanzees by immunization with replicating recombinant vaccinia virus. *J Virol* 2008; **82**: 10896-10905 [PMID: 18753204 DOI: 10.1128/JVI.01179-08]

141 **Elmowalid GA**, Qiao M, Jeong SH, Borg BB, Baumert TF, Sapp RK, Hu Z, Murthy K, Liang TJ. Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. *Proc Natl Acad Sci USA* 2007; **104**: 8427-8432 [PMID: 17485666 DOI: 10.1073/pnas.0702162104]

142 **Christiansen D**, Earnest-Silveira L, Chua B, Meuleman P, Boo I, Grubor-Bauk B, Jackson DC, Keck ZY, Foung SKH, Drummer HE, Gowans EJ, Torresi J. Immunological responses following administration of a genotype 1a/1b/2/3a quadrivalent HCV VLP vaccine. *Sci Rep* 2018; **8**: 6483 [PMID: 29691437 DOI: 10.1038/s41598-018-24762-9]

143 **von Delft A**, Donnison TA, Lourenço J, Hutchings C, Mullarkey CE, Brown A, Pybus OG, Klenerman P, Chinnakannan S, Barnes E. The generation of a simian adenoviral vectored HCV vaccine encoding genetically conserved gene segments to target multiple HCV genotypes. *Vaccine* 2018; **36**: 313-321 [PMID: 29203182 DOI: 10.1016/j.vaccine.2017.10.079]

144 **Lang Kuhs KA**, Ginsberg AA, Yan J, Wiseman RW, Khan AS, Sardesai NY, O'Connor DH, Weiner DB. Hepatitis C virus NS3/NS4A DNA vaccine induces multiepitope T cell responses in rhesus macaques mimicking human immune responses [corrected]. *Mol Ther* 2012; **20**: 669-678 [PMID: 21952169 DOI: 10.1038/mt.2011.188]

145 **Fattori E**, Zampaglione I, Arcuri M, Meola A, Ercole BB, Cirillo A, Folgori A, Bett A, Cappelletti M, Sporeno E, Cortese R, Nicosia A, Colloca S. Efficient immunization of rhesus macaques with an HCV candidate vaccine by heterologous priming-boosting with novel adenoviral vectors based on different serotypes. *Gene Ther* 2006; **13**: 1088-1096 [PMID: 16554842 DOI: 10.1038/sj.gt.3302754]

146 **Jeong SH**, Qiao M, Nascimbeni M, Hu Z, Rehermann B, Murthy K, Liang TJ. Immunization with hepatitis C virus-like particles induces humoral and cellular immune responses in nonhuman primates. *J Virol* 2004; **78**: 6995-7003 [PMID: 15194776 DOI: 10.1128/JVI.78.13.6995-7003.2004]

147 **Colloca S**, Barnes E, Folgori A, Ammendola V, Capone S, Cirillo A, Siani L, Naddeo M, Grazioli F, Esposito ML, Ambrosio M, Sparacino A, Bartiromo M, Meola A, Smith K, Kurioka A, O'Hara GA, Ewer KJ, Anagnostou N, Bliss C, Hill AV, Traboni C, Klenerman P, Cortese R, Nicosia A. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. *Sci Transl Med* 2012; **4**: 115ra2 [PMID: 22218691 DOI: 10.1126/scitranslmed.3002925]

148 **Garrone P**, Fluckiger AC, Mangeot PE, Gauthier E, Dupeyrot-Lacas P, Mancip J, Cangialosi A, Du Chéné I, LeGrand R, Mangeot I, Lavillette D, Bellier B, Cosset FL, Tangy F, Klatzmann D, Dalba C. A prime-boost strategy using virus-like particles pseudotyped for HCV proteins triggers broadly

neutralizing antibodies in macaques. *Sci Transl Med* 2011; **3**: 94ra71 [PMID: 21813755 DOI: 10.1126/scitranslmed.3002330]

149 **Swadling L**, Halliday J, Kelly C, Brown A, Capone S, Ansari MA, Bonsall D, Richardson R, Hartnell F, Collier J, Ammendola V, Del Sorbo M, Von Delft A, Traboni C, Hill AV, Colloca S, Nicosia A, Cortese R, Klenerman P, Folgori A, Barnes E. Highly-Immunogenic Virally-Vectored T-cell Vaccines Cannot Overcome Subversion of the T-cell Response by HCV during Chronic Infection. *Vaccines (Basel)* 2016; **4** [PMID: 27490575 DOI: 10.3390/vaccines4030027]

150 **Swadling L**, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, Halliday J, Kelly C, Bowen D, Fergusson J, Kurioka A, Ammendola V, Del Sorbo M, Grazioli F, Esposito ML, Siani L, Traboni C, Hill A, Colloca S, Davis M, Nicosia A, Cortese R, Folgori A, Klenerman P, Barnes E. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Sci Transl Med* 2014; **6**: 261ra153 [PMID: 25378645 DOI: 10.1126/scitranslmed.3009185]

151 **Frey SE**, Houghton M, Coates S, Abrignani S, Chien D, Rosa D, Pileri P, Ray R, Di Bisceglie AM, Rinella P, Hill H, Wolff MC, Schultze V, Han JH, Scharschmidt B, Belshe RB. Safety and immunogenicity of HCV E1E2 vaccine adjuvanted with MF59 administered to healthy adults. *Vaccine* 2010; **28**: 6367-6373 [PMID: 20619382 DOI: 10.1016/j.vaccine.2010.06.084]

152 **Law JL**, Chen C, Wong J, Hockman D, Santer DM, Frey SE, Belshe RB, Wakita T, Bukh J, Jones CT, Rice CM, Abrignani S, Tyrrell DL, Houghton M. A hepatitis C virus (HCV) vaccine comprising envelope glycoproteins gpE1/gpE2 derived from a single isolate elicits broad cross-genotype neutralizing antibodies in humans. *PLoS One* 2013; **8**: e59776 [PMID: 23527266 DOI: 10.1371/journal.pone.0059776]

153 **Drane D**, Maraskovsky E, Gibson R, Mitchell S, Barnden M, Moskwa A, Shaw D, Gervase B, Coates S, Houghton M, Basser R. Priming of CD4+ and CD8+ T cell responses using a HCV core ISCOMATRIX vaccine: a phase I study in healthy volunteers. *Hum Vaccin* 2009; **5**: 151-157 [PMID: 19246990 DOI: 10.4161/hv.5.3.6614]

154 **Parlati L**, Hollande C, Pol S. Treatment of hepatitis C virus infection. *Clin Res Hepatol Gastroenterol* 2020; 101578 [PMID: 33272891 DOI: 10.1016/j.clinre.2020.11.008]

155 **Rizzetto M**, Canese MG, Aricò S, Crivelli O, Trepo C, Bonino F, Verme G. Immunofluorescence detection of new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. *Gut* 1977; **18**: 997-1003 [PMID: 75123 DOI: 10.1136/gut.18.12.997]

156 **Lucifora J**, Delphin M. Current knowledge on Hepatitis Delta Virus replication. *Antiviral Res* 2020; **179**: 104812 [PMID: 32360949 DOI: 10.1016/j.antiviral.2020.104812]

157 **Farci P**, Niro GA. Clinical features of hepatitis D. *Semin Liver Dis* 2012; **32**: 228-236 [PMID: 22932971 DOI: 10.1055/s-0032-1323628]

158 **Mahale P**, Aka P, Chen X, Pfeiffer RM, Liu P, Groover S, Mendy M, Njie R, Goedert JJ, Kirk GD, Glenn JS, O'Brien TR. Hepatitis D virus infection, cirrhosis and hepatocellular carcinoma in The Gambia. *J Viral Hepat* 2019; **26**: 738-749 [PMID: 30661282 DOI: 10.1111/jvh.13065]

159 **Chen HY**, Shen DT, Ji DZ, Han PC, Zhang WM, Ma JF, Chen WS, Goyal H, Pan S, Xu HG. Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. *Gut* 2019; **68**: 512-521 [PMID: 30228220 DOI: 10.1136/gutjnl-2018-316601]

160 **Wedemeyer H**, Manns MP. Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 31-40 [PMID: 20051970 DOI: 10.1038/nrgastro.2009.205]

161 **World Health Organization**. Hepatitis D. [Internet] [accessed 17 August 2020]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-d

162 **Hardikar W**. Viral hepatitis. *J Paediatr Child Health* 2019; **55**: 1038-1043 [PMID: 31317618 DOI: 10.1111/jpc.14562]

163 **Noureddin M**, Gish R. Hepatitis delta: epidemiology, diagnosis and management 36 years after discovery. *Curr Gastroenterol Rep* 2014; **16**: 365 [PMID: 24293018 DOI: 10.1007/s11894-013-0365-x]

164 **Khabir M**, Aliche AZ, Sureau C, Blanchet M, Labonté P. Hepatitis Delta Virus Alters the Autophagy Process To Promote Its Genome Replication. *J Virol* 2020; **94** [PMID: 31748400 DOI: 10.1128/JVI.01936-19]

165 **Zhang Z**, Urban S. Interplay between Hepatitis D Virus and the Interferon Response. *Viruses* 2020; **12** [DOI: 10.3390/v12111334]

166 **Alves Vasconcelos MP**, DallÁcqua DV, Wedemeyer H, Witkin SS, Mendes-Corrêa MC, Villalobos-Salcedo JM. Noninvasive Models for Predicting Liver Fibrosis in Individuals with Hepatitis D Virus/Hepatitis B Virus Coinfection in the Brazilian Amazon Region. *Am J Trop Med Hyg* 2020; **103**: 169-174 [PMID: 32431268 DOI: 10.4269/ajtmh.19-0688]

167 **Da BL**, Surana P, Takyar V, Kleiner DE, Heller T, Koh C. Vibration-controlled transient elastography for the detection of cirrhosis in chronic hepatitis D infection. *J Viral Hepat* 2020; **27**: 428-436 [PMID: 31742822 DOI: 10.1111/jvh.13235]

168 **Takyar V**, Surana P, Kleiner DE, Wilkins K, Hoofnagle JH, Liang TJ, Heller T, Koh C. Noninvasive markers for staging fibrosis in chronic delta hepatitis. *Aliment Pharmacol Ther* 2017; **45**: 127-138 [PMID: 27813124 DOI: 10.1111/apt.13834]

169 **Wedemeyer H**, Yurdaydin C, Hardtke S, Caruntu FA, Curescu MG, Yalcin K, Akarca US, Gürel S, Zeuzem S, Erhardt A, Lüth S, Papatheodoridis GV, Keskin O, Port K, Radu M, Celen MK, Idilman

R, Weber K, Stift J, Wittkop U, Heidrich B, Mederacke I, von der Leyen H, Dienes HP, Cornberg M, Koch A, Manns MP; HIDIT-II study team. Peginterferon alfa-2a plus tenofovir disoproxil fumarate for hepatitis D (HIDIT-II): a randomised, placebo controlled, phase 2 trial. *Lancet Infect Dis* 2019; **19**: 275-286 [PMID: 30833068 DOI: 10.1016/S1473-3099(18)30663-7]

170 **Rizzetto M**. Targeting Hepatitis D. *Semin Liver Dis* 2018; **38**: 66-72 [PMID: 29471567 DOI: 10.1055/s-0037-1621711]

171 **Asselah T**, Loureiro D, Tout I, Castelnau C, Boyer N, Marcellin P, Mansouri A. Future treatments for hepatitis delta virus infection. *Liver Int* 2020; **40** Suppl 1: 54-60 [PMID: 32077603 DOI: 10.1111/liv.14356]

172 **Baskiran A**, Akbulut S, Sahin TT, Koc C, Karakas S, Ince V, Yurdaydin C, Yilmaz S. Effect of HBV-HDV co-infection on HBV-HCC co-recurrence in patients undergoing living donor liver transplantation. *Hepatol Int* 2020; **14**: 869-880 [PMID: 32895876 DOI: 10.1007/s12072-020-10085-3]

173 **World Health Organization**. Fact sheet Hepatitis E. [Internet] [accessed 17 August 2020]. Available from: https://www.who.int/es/news-room/fact-sheets/detail/hepatitis-e

174 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines on hepatitis E virus infection. *J Hepatol* 2018; **68**: 1256-1271 [PMID: 29609832 DOI: 10.1016/j.jhep.2018.03.005]

175 **Hoofnagle JH**, Nelson KE, Purcell RH. Hepatitis E. *N Engl J Med* 2012; **367**: 1237-1244 [PMID: 23013075 DOI: 10.1056/NEJMra1204512]

176 **Wen GP**, Chen CR, Song XY, Tang ZM, Ji WF, Wang SL, Zhang K, Zhang J, Ou SH, Zheng ZZ, Xia NS. Long-term HEV carriers without antibody seroconversion among eligible immunocompetent blood donors. *Emerg Microbes Infect* 2018; **7**: 125 [PMID: 29977038 DOI: 10.1038/s41426-018-0125-y]

177 **Pisano MB**, Martinez-Wassaf MG, Mirazo S, Fantilli A, Arbiza J, Debes JD, Ré VE. Hepatitis E virus in South America: The current scenario. *Liver Int* 2018; **38**: 1536-1546 [PMID: 29788538 DOI: 10.1111/liv.13881]

178 **Lee JT**, Shao PL, Chang LY, Xia NS, Chen PJ, Lu CY, Huang LM. Seroprevalence of Hepatitis E Virus Infection among Swine Farmers and the General Population in Rural Taiwan. *PLoS One* 2013; **8**: e67180 [PMID: 23840620 DOI: 10.1371/journal.pone.0067180]

179 **Masachessi G**, Pisano MB, Prez VE, Martínez LC, Michelena JF, Martínez-Wassaf M, Giordano MO, Isa MB, Pavan JV, Welter A, Nates SV, Ré V. Enteric Viruses in Surface Waters from Argentina: Molecular and Viable-Virus Detection. *Appl Environ Microbiol* 2018; **84** [PMID: 29269500 DOI: 10.1128/AEM.02327-17]

180 **Chen YJ**, Cao NX, Xie RH, Ding CX, Chen EF, Zhu HP, Sun JM, Shang XP, Wang XX, Miao ZP. Epidemiological investigation of a tap water-mediated hepatitis E virus genotype 4 outbreak in Zhejiang Province, China. *Epidemiol Infect* 2016; **144**: 3387-3399 [PMID: 27546066 DOI: 10.1017/S0950268816001898]

181 **Martínez Wassaf MG**, Pisano MB, Barril PA, Elbarcha OC, Pinto MA, Mendes de Oliveira J, DiGiusto P, Nates SV, Ré VE. First detection of hepatitis E virus in Central Argentina: environmental and serological survey. *J Clin Virol* 2014; **61**: 334-339 [PMID: 25213209 DOI: 10.1016/j.jcv.2014.08.016]

182 **Debing Y**, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis E virology: Implications for clinical practice. *J Hepatol* 2016; **65**: 200-212 [PMID: 26966047 DOI: 10.1016/j.jhep.2016.02.045]

183 **Domanović D**, Tedder R, Blümel J, Zaaijer H, Gallian P, Niederhauser C, Sauleda Oliveras S, O'Riordan J, Boland F, Harritshøj L, Nascimento MSJ, Ciccaglione AR, Politis C, Adlhoch C, Flan B, Oualikene-Gonin W, Rautmann G, Strengers P, Hewitt P. Hepatitis E and blood donation safety in selected European countries: a shift to screening? *Euro Surveill* 2017; **22** [PMID: 28449730 DOI: 10.2807/1560-7917.ES.2017.22.16.30514]

184 **Sridhar S**, Yip CCY, Wu S, Cai J, Zhang AJ, Leung KH, Chung TWH, Chan JFW, Chan WM, Teng JLL, Au-Yeung RKH, Cheng VCC, Chen H, Lau SKP, Woo PCY, Xia NS, Lo CM, Yuen KY. Rat Hepatitis E Virus as Cause of Persistent Hepatitis after Liver Transplant. *Emerg Infect Dis* 2018; **24**: 2241-2250 [PMID: 30457530 DOI: 10.3201/eid2412.180937]

185 **Al-Sadeq DW**, Majdalawieh AF, Mesleh AG, Abdalla OM, Nasrallah GK. Laboratory challenges in the diagnosis of hepatitis E virus. *J Med Microbiol* 2018; **67**: 466-480 [PMID: 29485390 DOI: 10.1099/jmm.0.000706]

186 **Shrestha A**, P Gupta B, K Lama T. Current Treatment of Acute and Chronic Hepatitis E Virus Infection: Role of Antivirals. *Euroasian J Hepatogastroenterol* 2017; **7**: 73-77 [PMID: 29201777 DOI: 10.5005/jp-journals-10018-1216]

187 **WHO**. Hepatitis E vaccine: WHO position paper, May 2015--Recommendations. *Vaccine* 2016; **34**: 304-305 [PMID: 26232546 DOI: 10.1016/j.vaccine.2015.07.056]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4045-4087

DOI: 10.3748/wjg.v27.i26.4045

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Biomarkers in the diagnosis of pancreatic cancer: Are we closer to finding the golden ticket?

Robert S O'Neill, Alina Stoita

ORCID number: Robert S O'Neill 0000-0002-9576-2248; Alina Stoita 0000-0001-9460-2149.

Author contributions: O'Neill RS researched the paper and wrote the initial draft of the manuscript; Stoita A designed the paper, researched studied and reviewed the manuscript.

Conflict-of-interest statement: The authors have no conflicts of interests relevant to this manuscript.

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Robert S O'Neill, Alina Stoita, Department of Gastroenterology, St Vincent's Hospital Sydney, Sydney 2010, Australia

Robert S O'Neill, St George and Sutherland Clinical School, Faculty of Medicine, University of New South Wales, Sydney 2010, Australia

Alina Stoita, St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Sydney 2010, Australia

Corresponding author: Alina Stoita, FRACP, MBBS, Doctor, Lecturer, Department of Gastroenterology, St Vincent's Hospital Sydney, 390 Victoria Street, Sydney 2010, Australia. alina.stoita@svha.org.au

Abstract

Pancreatic cancer (PC) is a leading cause of cancer related mortality on a global scale. The disease itself is associated with a dismal prognosis, partly due to its silent nature resulting in patients presenting with advanced disease at the time of diagnosis. To combat this, there has been an explosion in the last decade of potential candidate biomarkers in the research setting in the hope that a diagnostic biomarker may provide a glimmer of hope in what is otherwise quite a substantial clinical dilemma. Currently, serum carbohydrate antigen 19-9 is utilized in the diagnostic work-up of patients diagnosed with PC however this biomarker lacks the sensitivity and specificity associated with a gold-standard marker. In the search for a biomarker that is both sensitive and specific for the diagnosis of PC, there has been a paradigm shift towards a focus on liquid biopsy and the use of diagnostic panels which has subsequently proved to have efficacy in the diagnosis of PC. Currently, promising developments in the field of early detection on PC using diagnostic biomarkers include the detection of microRNA (miRNA) in serum and circulating tumour cells. Both these modalities, although in their infancy and yet to be widely accepted into routine clinical practice, possess merit in the early detection of PC. We reviewed over 300 biomarkers with the aim to provide an in-depth summary of the current state-of-play regarding diagnostic biomarkers in PC (serum, urinary, salivary, faecal, pancreatic juice and biliary fluid).

Key Words: Pancreatic cancer; Cancer; Biomarkers; Diagnostic; Review

Country/Territory of origin: Australia

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

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: February 27, 2021
Revised: March 24, 2021
Accepted: June 15, 2021
Article in press: June 15, 2021
Published online: July 14, 2021

P-Reviewer: Ruess DA
S-Editor: Gao CC
L-Editor: A
P-Editor: Liu JH

Core Tip: Circulating biomarkers are an attractive method for pancreatic cancer (PC) diagnosis. Over 300 biomarkers are presented in this review, however no gold standard biomarker exists. While carbohydrate antigen 19-9 possesses modest sensitivity in PC diagnosis, a lack of specificity is a limitation for its use. More recent studies have shifted towards the concept of a liquid biopsy along with measuring expression of RNA based markers in different mediums. Panels comprising multiple candidate biomarkers have emerged, demonstrating modest diagnostic value. Further studies are required to validate these findings, along with assessment in an asymptomatic population to determine their value in screening.

Citation: O'Neill RS, Stoita A. Biomarkers in the diagnosis of pancreatic cancer: Are we closer to finding the golden ticket? *World J Gastroenterol* 2021; 27(26): 4045-4087
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4045.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4045

INTRODUCTION

Pancreatic cancer (PC), most recently declared as a medical emergency by the United European Gastroenterology in a position paper, is a leading cause of cancer related mortality on a global scale, being the 12th most common cancer diagnosis, and the seventh leading cause of cancer related death[1-3]. The mortality associated with PC is significant compared to its solid organ tumor counterparts, accounting for approximately 4% of cancer related deaths with a Mortality/Incidence ratio of 98%, and has a dismal 5-year survival rate of approximately 9% which has only incrementally improved over the past forty years due to improvements in neoadjuvant and adjuvant therapeutic options[3-5]. This poor prognosis is attributed to patients being diagnosed with advanced disease at the time of presentation and the relatively silent nature of the disease[6]. It is estimated that, at the time of diagnosis 80%-90% of patients have unresectable disease[7]. It is postulated that diagnosis at an earlier stage would increase the 5-year survival rate as this would allow for curative resection along with adjuvant chemotherapy[8,9].

Due to the overwhelming number of patients having unresectable disease at the time of diagnosis there has been an emphasis on the identification of novel diagnostic modalities or biomarkers that can assist clinicians in detecting PC at an early stage. Currently there is no defined PC screening strategy for the general population that is comparable to screening colonoscopies for colorectal cancer (CRC) and the programs that exist are only limited to high risk patients (familial PC and hereditary PC syndromes) which represent only 5%-10% of all PC patients[10-12].

The goal of early detection of PC in otherwise asymptomatic patients is optimistic however so far impractical due to low incidence of PC in the general population, where even with a screening assay with a high specificity, implementing a screening program might result in increased levels of anxiety in the screened population with the potential for false positive results[13]. Further to this, the vast majority of studies have assessed the utility of diagnostic biomarkers in patients with symptomatic disease, rather than as a surveillance or screening biomarker in the general population.

A biomarker is defined as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease'. Currently carbohydrate antigen 19-9 (CA19-9) is regarded as the best serological biomarker available so far in the diagnosis of PC, however the majority of studies endorsing the use of CA19-9 as a complementary test in the diagnosis of PC acknowledge it is not specific or sensitive enough to be used for screening[14,15]. A number of other biomarkers have been proposed and these will be reviewed here[16]. Variation exists in the biomarker domain, with studies utilizing serum, biliary fluid, pancreatic juice, urine, faeces and pancreatic cystic fluid for analysis of potential agents to determine their worth as a malignancy biomarker, however these methods of assessment vary in their invasiveness, sensitivity and specificity[17-20].

Due to the currently rapidly evolving landscape of potential biomarkers for early diagnosis of PC and the apparent lack of a gold standard diagnostic assay in the general population, the aim of this review is to provide a comprehensive update on the current diagnostic biomarkers implicated in PC with over 300 biomarkers

reviewed here.

SEROLOGICAL BIOMARKERS OF PC

Serum has been the most utilized modality for specimen collection for biomarker analysis, and it is the preferred specimen for analysis due to simplicity of collection and low risk, however it has limitations, particularly the potential for dilution of candidate tumour markers and the potential for these markers to be obscured by other serum proteins that exist within samples[21].

Glycolipids and proteins

CA19-9: CA19-9 is a tetrasaccharide expressed on the surface of cancer cells. It is the most well-known serological biomarker used in PC diagnosis, and was initially described in 1979 as a tumor antigen recognised by the monoclonal antibody NS19-9 in the case of CRC[22,23]. CA19-9 is not specific for PC alone, and has been implicated in colon, gastric and biliary tract cancer[24-26]. CA19-9 has only been reported to be elevated in only 80% of all PC patients, and has been used in monitoring disease progress or responsiveness to treatment[27,28]. CA19-9 has also been demonstrated to be elevated in benign conditions such as chronic pancreatitis (CP), biliary obstruction and cholangitis highlighting a lack of specificity[29,30]. In addition to this, CA19-9 is related to the Lewis blood group antigens and only those patients who belong to the Le (α-β+) or Le (α + β-) blood groups will express the antigen, its sensitivity in the diagnosis of PC is questionable as 10% of the population have a Le (α-β-) phenotype which lacks the enzyme 1,4-fucosyl transferase that is essential for the production of CA19-9[31,32].

Only a scarce number of studies have evaluated serum CA19-9 Levels in the general, asymptomatic population as a screening modality for PC. These studies were conducted in Japanese, Korean and Taiwanese populations and reported a low positive predictive value (PPV) of serum CA19-9 in the diagnosis of PC in a screening setting[33-35].

A recent meta-analysis assessing the diagnostic value of CA19-9 in PC compared to carcinoembryonic antigen (CEA) reported a summary sensitivity of 0.80 in the diagnosis of PC, along with a summary specificity of 0.75 and area under the curve (AUC) of 0.84[36].

To improve the diagnostic performance of CA19-9, it has been combined with a number of other biomarkers in the research setting[37,38]. This has translated to improved diagnostic value. Of note, sialylated tumor-related antigen, including sialyl-Lewis A glycan isomers, has recently been demonstrated to be superior to CA19-9 when used in isolation, as well as improving the sensitivity and specificity when used in combination with CA19-9[39-41] (Table 1).

CEA: CEA is a foetal glycoprotein that is not usually produced in large quantities after birth. Aside from its role in the surveillance and prognosis of CRC, CEA has also been implicated in ovarian, cervical, lung and breast cancer[42]. A number of studies have investigated the diagnostic value of CEA for PC, however the results reported are inconsistent throughout the literature.

The predictive value of CEA in the diagnosis and prognosis of PC has been recently evaluated in a relatively small systematic review and meta-analysis published by Meng *et al*[43] in 2017. Through the analysis of 19 studies including 3650 participants, a CEA-based panel was deemed to have greater diagnostic accuracy compared to CEA or CA19-9 alone with an AUC and *Q* value of 0.90 and 0.84 respectively, however the sensitivity of the panels demonstrated no advantage over CA19-9 or CEA when utilized in isolation[43]. A meta-analysis conducted in 2018 comparing CA19-9 to CEA included 13 studies with 4537 participants and 1277 patients diagnosed with PC[36]. This study demonstrated a superior sensitivity of CA19-9 compared to CEA (ratio of sensitivity = 1.54), along with a superior AUC (ratio of AUC = 1.24). A recommendation was made that both markers should be utilized for early diagnosis of PC due to their convenient, efficient and non-invasive properties.

CA125: CA125 is a high-molecular-weight mucin-like glycoprotein that has been associated with ovarian cancer, CRC and cholangiocarcinoma[44-46]. The role of CA125 in PC has only been established in the past decade with small studies demonstrating its superiority to CA19-9 in predicting resectability of PC, along with correlating with metastasis-associated disease burden[47,48]. There is unique clinical utility for CA125 given that serum levels do not correlate with serum bilirubin levels

Table 1 Serum protein biomarkers implicated in the diagnosis of pancreatic cancer

Class	Candidate marker
Glycolipids and proteins	CA19-9[27,28,33-38,144,160,182,187,213,221], sTRA[39-41], CEA[43], CA125[47,48,50], CA242[55,53], Osteonectin[57], Osteopontin[58-61], DUPAN-2[65-70], LAMC2[73-75], ULBP2[78-80], sCD40L[82], LRG1[84], C4BPA[86], Cofilin-1[88], sgC1qR[91], Trypsinogen-2[92,93], DKK1[96], THBS-2[99-102], THBS-1[103], AGR2[108], REG1A[108], REGIII[108], REG1β[111], REG4[114-117], SYCN[108], LOXL2[108], PARK7/DJ-1[126], TTR[129,130], TTF1[134], TTF2[134], TTF3[134], GPNMB[138], PRX-1[139], TFPI[141], TIMP-1[144], MMP-9[144], IGFBP-1[146], IGFBP-2[147-149], IGFBP-3[147,149], MSLN[148,154], C5[152], MMP-7[155-157], cathepsin-D[156], MMP-12[157], OPG[160], Kisspeptin[165], Galectin[171], MUC16[48,182], MUC5AC[37,182], PAM4[187], HSP27[190,191], CAM17.1[192,193], Fuc-Hpt[194], SAA [196], APN/CD13[200], M2-PK[203,204], APOA2[206-208], APOC1[209], APOC2[210], APOE[211-212], ITIH[213], APOA1[213], APOL1 [213]
Growth factors	TGF-B[215], VEGF[217], FGF-10/KGF-2[138], PDGF[220], TSGF[221]
Cytokines and chemokines	IP-10[220], IL-6[220,230-232], MIC-1/GDF15[227,228], IL-11[229], YKL-40[232,233], IL-8[230,234,235,237,241], IL-10[214], IL-1β[214], OSM [138], TNF-α[240-244], M-CSF[214], CXCL11[138], SCF[138,247-248], Eotaxin[250], HGF[250], MCP-1[250], CXCL10[250]
Adhesion molecules	CEACAM1[253,254], ICAM-1[160,262-263]

CEA: Carcinoembryonic antigen; TTF: Thyroid transcription factor; sTRA: Sialylated tumor-related antigen; IL: Interleukin.

and it is not significantly altered in the case of patients who are jaundiced[49].

A recent meta-analysis comprising eight studies with 1235 participants demonstrated a pooled sensitivity of 59% and specificity of 78% for CA125 in the diagnosis of PC, while the AUC and Q-value of the CA125-based diagnostic panel were 0.89 and 0.82 respectively[50]. This panel was deemed to be superior to CA125 or CA19-9 when used in isolation. Although this demonstrated a favourable result for the use of a CA125-based diagnostic panel going forward, the meta-analysis was limited by its size and heterogeneity between studies.

CA242: CA242 is a sialic acid-containing carbohydrate antigen which has been reported to have a high correlation with CA19-9 in patients diagnosed with PC[51-53]. Serum CA242 has also been demonstrated to be highest in patients diagnosed with PC compared to other solid organ malignancies, such as cervical cancer or oesophageal cancer[54].

In a 2015 meta-analysis comprising 21 studies and 3497 participants, CA242 was evaluated in conjunction with CA19-9 and CEA in diagnosing PC[55]. CA242 pooled sensitivity for detection of PC was 67.8%, with a subsequent pooled specificity of 83.0%. When combined with CA19-9, a sensitivity of 90.0% was achieved. More recently, a biomarker panel of CA19-9, serum periostin (POSTN) and CA242 was able to discriminate early stage PC from controls with an AUC of 0.98, along with benign conditions (AUC = 0.90)[53]. When utilized in isolation however, receiver operating characteristic (ROC) curve analysis returned an inferior result for CA242 in comparison to CA19-9 in distinguishing early stage PC from healthy controls.

Osteonectin: Osteonectin is a glycoprotein that has been previously demonstrated to have a key function in PC through promoting invasion and metastasis[56]. There is limited data on the use of Osteonectin in the diagnosis of PC, with a small prospective study reporting significantly elevated serum levels in those diagnosed with PC compared to controls, and a plasma level of > 100.18 ng/mL on ROC curve analysis resulting in an AUC of 86% for predicting PC[57].

Osteopontin: Osteopontin (OPN), a protein associated with the extracellular matrix (ECM), has been previously reported to be upregulated in PC preoperative serum, where when elevated it was found to have a sensitivity and specificity of 80% and 97% [58]. More recently serum levels of OPN and tissue inhibitor of metalloproteinase 1 (TIMP-1) were able to distinguish PC from CP and healthy controls. Additionally, when combined with CA19-9, diagnostic accuracy improved than compared to when used in isolation[59].

A meta-analysis published in 2014 demonstrated that the serum OPN levels in patients with PC was significantly greater compared to controls[60]. More recently, a pilot study published in 2016 identified that levels of OPN were higher in patients with PC compared to those with CP and control subjects, further affirming its potential role as a diagnostic biomarker in PC[61].

Duke pancreatic monoclonal antigen type 2: Duke pancreatic monoclonal antigen type 2 (DUPAN-2) is the precursor for CA19-9 has been reported to be elevated in patients with PC who are negative for the Lewis blood group phenotype highlighting an advantage over the conventional biomarker CA19-9[62-64]. There is minimal literature evaluating serum DUPAN-2 in the diagnosis of PC and the sensitivity of the biomarker in diagnosing PC is less than desirable, with its use shifting from diagnosis to prognosis more recently[65-70].

Laminin γ2: Laminin γ2 (LAMC2), an ECM glycoprotein, has been previously demonstrated to be inversely related to overall patient survival in patients with PC and over-expression has been proposed as a poor prognostic factor in patients diagnosed with PC[71,72]. Its value as a diagnostic biomarker has been assessed in a number of studies where when used in isolation and in conjunction with CA125 and CA19-9 in a panel, LAMC2 has demonstrated efficacy in PC diagnosis[73-75].

UL16 binding protein 2: UL16 binding protein 2 (ULBP2) is an NKG2D ligand present on NK cells that has been implicated in tumorigenesis[76,77]. Initially identified in 2011, ULBP2 was found to be elevated in PC patients compared to healthy controls [78]. ULBP2 has been utilized in combination with MIC-1, where it was reported to be significantly elevated in the serum of patients with PC compared to controls[79]. This elevation of ULBP2 in the sera of patients with PC was further validated in 2017 where in a small single centre study, serum levels of ULBP2, dickkopf-1 (DKK1) and CA19-9 were all significantly elevated in those diagnosed with PC compared to those with benign pancreatic disease and controls[80]. There is very little published with regard to the role of ULBP2 in the diagnosis of PC, with more recent data highlighting a potential role as a predictor of poor prognosis[81].

Soluble CD40 ligand: Soluble CD40 ligand (sCD40L) was first evaluated as a diagnostic and prognostic marker for PC in a study in 2014, where serum levels were significantly elevated in PC patients compared to controls[82]. Considering a lack of validation and small sample size, its routine clinical use is not recommended.

Leucine-rich a2-glycoprotein-1: Leucine-rich a2-glycoprotein-1 (LRG1) is an inflammatory protein present in human sera[83]. Although it was able to distinguish between patients with PC, CP or healthy controls, however the authors were not able to demonstrate effectiveness for LRG-1 as an early diagnostic marker[84].

C4b-binding protein a-chain: C4b-binding protein a-chain (C4BPA) is a serum protein implicated in B cell proliferation and CD40 activation which can reverse immune suppression and stimulate anti-tumour T cell responses[85]. It was demonstrated in a single study to be significantly elevated in patients with PC compared to healthy controls, with a subsequent AUC of 0.860 which was superior to CA19-9[86].

Cofilin-1: Cofilin-1 belongs to a family of proteins known as the actin depolymerizing factor/cofilin family, and has been implicated in chemotaxis, cell migration and tumor cell invasion[87]. There is minimal literature describing the role of cofilin-1 as a diagnostic biomarker of PC, with a single study in 2017 measuring the immune complex levels of cofilin-1 in sera and reporting that levels were significantly elevated in those diagnosed with PC compared to healthy controls and those with CP[88].

Soluble gC1qR: Soluble gC1qR (sgC1qR) is a multifunctional cellular protein which has previously been implicated in inflammation and malignancy[89,90]. With regard to PC, only a single small study has assessed its role as a circulating diagnostic biomarker, where it was demonstrated to be significantly increased in those diagnosed with metastatic PC compared to controls[91].

Serum trypsinogen-2: Serum trypsinogen-2 evaluation as a diagnostic biomarker is limited in the literature. A small study performed in 1996 demonstrated that high levels of serum trypsinogen-2 were present in those with BTC and PC, while also being elevated in benign obstructive disease highlighting a lack of sensitivity associated with the marker[92]. Another small single centre study showed the levels in those with PC and CP were significantly elevated compared to controls[93].

DKK1: DKK1 is a soluble inhibitor of Wnt/B-catenin signalling and has been demonstrated to be over-expressed in a number of solid organ malignancies[94,95]. DKK1 has been previously reported to be superior to CA19-9 on ROC curve analysis in differentiating patients with PC compared to controls with an AUC of 0.919 compared to 0.853 [96], while a more recent review highlights its potential as a target for cancer immuno-

therapy rather than diagnosis[97].

Thrombospondin-2 and thrombospondin-1: Thrombospondin-2 (THBS2) is a glycoprotein that mediates cell-to-cell and cell-to-matrix interactions which has previously been implicated in malignancy, particularly CRC[98]. When utilized with CA19-9, it can boost detection of PC in high-risk populations which has been more recently affirmed[99-101]. Le Large *et al*[102] reported an AUC of 0.952 for THBS2 and CA19-9 in discriminating patients with cancer compared to healthy donors, however there was no difference in plasma THBS2 expression between patients with PC and distal cholangiocarcinoma highlighting a potential diagnostic dilemma and a lack of specificity associated with the assay[102].

Serum THBS1 has been demonstrated to significantly decrease up to 24 mo prior to the diagnosis of PC and when used in combination with CA19-9, an AUC of 0.86 was achieved significantly outperforming both markers utilized in isolation[103].

Anterior gradient homolog 2 protein: Anterior gradient homolog 2 protein (AGR2) is a protein that has been previously identified as having a crucial role in embryogenesis. It is found in the endoplasmic reticulum and on the cell surface, and is expressed by multiple solid organ malignancies[104,105]. It has been previously implicated in the initiation of PC and is expressed in premalignant lesions of the pancreas[106,107]. As a diagnostic biomarker in PC, only a handful of studies exist reporting its elevation in PC compared to controls, with utilisation in a diagnostic assay with CA19-9 and REG1β resulting in modest diagnostic accuracy[108].

Regenerating protein family: REG1β, a member of the regenerating (REG) islet-derived family of proteins, which is present in pancreatic acinar cells, and subsequently is implicated in the regeneration of pancreatic islets[109]. REG family members have also been implicated in PC[110]. REG islet-derived 1 alpha (REG1A) and REGIII were initially demonstrated to be elevated in plasma in murine PC models, while REG1β was first studied in 2013 and was demonstrated to be significantly elevated in PC serum compared to healthy participants and those with benign disease [108,111].

REG4 is also over-expressed in a number of solid organ malignancies, including those of the gastrointestinal tract[112,113]. It acts an antiapoptotic factor through the Akt signalling pathway and has been demonstrated to be elevated in the serum of patient with PC compared to controls[114,115]. Serum REG4 has been reported to be superior to CA19-9 on AUC analysis, however there is inconsistencies in both sensitivity and specificity between studies[116,117].

Syncollin: Usually expressed in pancreatic acinar granules on the luminal side of the granular membrane, syncollin (SYCN) acts to concentrate and mature zymogens, while also regulating exocytosis and has previously been identified in the pancreatic juice of patients diagnosed with PC[118-120]. Initially evaluated in humans in 2013, SYCN was found to be significantly elevated in the serum of patients with PC compared to health controls and those with benign disease. In addition to this, it was also able to identify patients with PC in which serum CA19-9 was normal suggesting superior sensitivity. When combined with the serum biomarker REG1β and CA19-9, it was demonstrated to have an average AUC of 0.895 when discriminating patients with PC compared to healthy controls[108]. Although there is a lack of data to determine whether the findings of the aforementioned studies are generalisable, SYCN does display merit in terms of its sensitivity in patients diagnosed with PC compared to CA19-9.

Lysyl oxidase-like 2: Lysyl oxidase-like 2 (LOXL2) is a member of the lysyl oxidase (LOX) family of secreted, copper-dependent amine oxidases which have been implicated in malignancy due to their ability to promote epithelial-mesenchymal transition[121,122]. Additionally, its expression presents poorer overall survival and worse clinicopathological parameters irrespective of malignancy[123]. LOXL2 has been reported to be elevated in serum of patients with PC compared to controls, however was inferior to CA19-9 and its general ability to distinguish PC from controls was not deemed to be significant[108].

PARK7/DJ-1: DJ-1 is a multifunctional protein which has been implicated in Parkinson's disease, however is also an oncogene that has been demonstrated to be over-expressed in a number of solid organ malignancies[124,125]. DJ-1 was first evaluated in 47 patients with PC in 2011 and shown to be elevated in patients with PC compared to those with CP and controls, with an AUC superior to CA19-9 (0.6647)

[126]. Further studies are warranted to determine whether the results of this study can be replicated.

Transthyretin: Transthyretin (TTR) is the major carrier for the hormones thyroxin and tri-iodothyronine, and has been previously demonstrated to be elevated in patients with endocrine tumours but decreased in solid organ malignancies including epithelial ovarian carcinoma[127,128]. Studies are heterogenous, one study showing serum TTR level decreased by at least 2-fold when compared to control participants and other showing TTR is elevated in patients diagnosed with PC[129,130].

Trefoil factors: Trefoil factors (TFFs) are small, secretory mucin-associated proteins which are involved in the protection of epithelial cells, however an oncogenic role has been noted particularly in the case of gastric cancer[131-133]. In 2019 a small study demonstrated significant elevation of TFF1 and TFF2 in early PC compared to benign controls and CP patients. In addition to this, when combined with CA19-9, the panel of TFF (TFF1, TFF2 and TFF3) resulted in an AUC of 0.93 in discriminating early PC from benign controls[134].

Osteoactivin/glycoprotein nonmetastatic melanoma protein B: Glycoprotein nonmetastatic melanoma protein B (GPNMB) is a type 1 transmembrane protein which has been described as a promoter of metastasis and cellular invasion in malignancy[135-137]. A single study analyzed pre-treatment sera of patients with PC compared to controls and demonstrated modest diagnostic accuracy for PC[138].

Peroxiredoxin-1: Described as an important protector against redox damage, peroxiredoxin-1 (PRX-1) has also been implicated in PC where in the serum of patients it was significantly elevated compared to healthy controls and correlated with aggressive clinicopathological parameters. When combined with CA19-9, the AUC was significantly higher that PRX-1 when utilized in isolation[139].

Tissue factor pathway inhibitor: Tissue factor pathway inhibitor (TFPI) is a plasma Kunitz-type serine proteinase inhibitor which controls coagulation initiation, while also being implicated in malignancy[140]. An isolated study has assessed the role of TFPI in PC, where when utilized in combination with tenascin C and CA19-9 in a biomarker panel, it was demonstrated to improve the diagnostic performance of CA19-9 in discriminating early-stage cancer from healthy controls[141].

TIMP-1: TIMP-1 possesses an inhibitory effect on most MMPs along with playing a role in the regulation of cell proliferation and apoptosis[142,143]. TIMP-1 has a sensitivity of 47.1%, specificity of 69.2% and AUC of 0.64 which, in conjunction with matrix metalloproteinase-9 (MMP-9), were both deemed inferior to CA19-9 as a marker for detecting PC[144].

Insulin-like growth factor binding protein: Insulin-like growth factor binding protein 1 (IGFBP-1) is a downstream target of insulin and inhibits IGF-1 activity[145]. Wolpin *et al*[146] demonstrated that low plasma levels of IGFBP-1 predicted an increased risk of PC in a nested case-control study. In a pilot 2016 study IGFBP-2 and IGFBP-3 were shown to be able to discriminate PC patients with early stage disease from healthy controls, along with being superior to CA19-9 when utilized in combination[147]. Kendrick *et al*[148] showed that IGFBP2 and mesothelin (MSLN) were weak diagnostic classifiers individually but their utilization in a diagnostic biomarker panel was recommended. Additionally, in the case of premalignant lesions, Kim *et al*[149] reported that a biomarker panel of six candidate proteins including IGFBP-2 and IGFBP-3 had high discriminatory power in distinguishing intraductal papillary mucinous neoplasm (IPMN) and controls.

Complement component 5: Component 5 (C5) is a complement protein, which when cleaved into two fragments, C5a and C5b, is implicated in the formation of the membrane attack complex (MAC), a structure that is vital in the innate immune system[150,151]. Wingren *et al*[152] reported that C5 was differentially overexpressed, along with a number of inflammatory and growth factors in the serum of patients with PC compared to normal controls subjects.

MSLN: Initially evaluated in 2009, circulating MSLN was described as a useful biomarker for PC where it was detected in 73 of the 74 patients with PC[153]. However more recently, serum MSLN was found to be a weak diagnostic classifier of PC[148]. This supports the findings of Sharon *et al*[154] who identified that serum MSLN and megakaryocyte potentiating factor did not differ significantly between cohorts

diagnosed with PC, biliary carcinoma, benign pancreatic conditions, healthy controls and benign non-pancreatic conditions, and as such was concluded that it was not useful as a biomarker for the assessment of malignancy.

MMP: In a small study Kuhlmann *et al*[155] reported a 100% positive predictive value when MMP-7 was combined with CA19-9 in patients with periampullary carcinoma. MMP-7 has also been utilized in a panel comprising CA19-9, cathepsin D with an impressive AUC of 0.900 for discriminating patients with PC from normal healthy controls[156]. Kahlert *et al*[157] also reported that serum MMP-7 and MMP-12 were strong classifiers for the diagnosis of patients with PC compared to healthy controls.

Osteoprotegerin: Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily and is mainly associated with regulation of bone turnover that has also been implicated in malignancy[158,159]. It has been previously combined in a biomarker panel with intercellular adhesion molecule 1 (ICAM-1) and CA19-9 and was able to discriminate PC patients from healthy controls with a sensitivity and specificity of 78% and 94% respectively[160]. This study contrasts with the findings of Nolen *et al*[161] where when combined in a panel with CA19-9 and OPN, OPG was not effective in predicting PC in prospectively collected serum samples in a large screening cohort.

Kisspeptin: Kisspeptin, initially implicated in melanoma, has been demonstrated to be expressed physiologically in a number of different tissues, suggesting it possesses antitumoral properties[162-164]. Recently, in a cohort of 128 patients with PC, serum levels of Kisspeptin were elevated in those with PC compared to healthy controls and ROC curve analysis demonstrated an AUC of 0.797 in discriminating PC from healthy controls, however it was deemed inferior to CA19-9[165].

Galectin-3: Galectin-3 is a member of the β-galactoside-binding protein family which has been previously demonstrated to be associated with a number of solid organ malignancies, including those of the gastrointestinal tract[166-169]. It has been reported to be over-expressed in PC tissue specimens and elevated in the serum of patients with PC[170]. Yi *et al*[171] further built upon this finding in a prospective screening study, where in 1850 healthy participants a single case of PC was diagnosed in a patient with elevated serum levels, a lack of specificity cited as a barrier to implementation.

Mucins: Mucins are a family of glycoproteins that serve a number of functions, and line the surface of epithelial cells in the gastrointestinal tract[172,173]. In normal pancreatic tissue, a number of mucins are expressed, these being MUC1, MUC5B, MUC6, MUC11, MUC12, MUC17, MUC20 and MUC21, while other members of the mucin family are usually undetectable[173-179]. Mucins have previously been demonstrated to have a role in PC in promoting metastasis, chemoresistance and tumorigenicity, while a recent meta-analysis identified MUC1, MUC4, MUC5AC and MUC16 as key biomarkers in the diagnosis of PC[180,181]. On peripheral blood sampling, MUC16/CA125 Levels have been previously demonstrated to be strongly associated with metastatic disease[48]. Serum MUC5AC has also been reported to have efficacy in differentiating resectable early-stage PC from healthy controls, along with median circulating levels being significantly elevated compared to benign controls and CP. Furthermore, when utilized in combination with CA19-9, diagnostic accuracy was improved significantly for resectable PC cases compared to healthy controls[37]. When combining measurements of CA19-9 assay with detection of CA19-9 on MUC5AC and MUC16, the sensitivity of PC detection improved, with greater sensitivity and near 100% specificity achieved[182].

PAM4: PAM4 antibody is a monoclonal antibody which binds to large-size mucin, and it has been previously been reported that expression of the PAM4-reactive antigen on immunohistology may provide a method for early detection of PC[183-185]. The PAM4 antigen is absent from normal pancreatic tissue or pancreatic tissue associated with benign disease[186]. A 2012 study conducted by Gold *et al*[187] reported the overall sensitivity of PAM4 detection of PC at 75%, with associated high discriminatory power with respect to benign disease, however this has yet to be replicated.

Heat shock protein 27: Heat shock protein 27 (HSP27) is a molecular chaperone which acts to prevent aggregation of misfolded proteins, along with playing a role in the degradation of these proteins[188]. Additionally, it also plays a role in promoting tumour metastasis[189]. In patients diagnosed with PC, HSP27 detection in serum has

been demonstrated to have a sensitivity of 100% and specificity of 84%, however a lack of specificity is highlighted by elevated levels also being reported in CP and cannot be recommended as a diagnostic biomarker in PC[190,191].

CAM17.1: CAM17.1 monoclonal antibody is a monoclonal antibody which detects a mucous glycoprotein that is specific for intestinal mucous, also known as CAM17.1. CAM17.1 is overexpressed in PC but has a low sensitivity and specificity of 78% and 76% respectively in diagnosing PC[192,193].

Fucosylated haptoglobin: Recently fucosylated haptoglobin (Fuc-Hpt) has emerged as a novel biomarker in PC, where it has been demonstrated to be almost equivocal to CA19-9 on ROC curve analysis and also correlates with disease stage[194]. Although this does demonstrate promise as a diagnostic biomarker, it is postulated that Fuc-Hpt is produced by metastatic deposits in the liver, and as such lacks utility in the diagnosis of early stage disease, but rather is able to identify liver metastasis that may not be detected on radiological assessment[195].

Serum amyloid A: Serum amyloid A (SAA) is an acute phase protein which has previously been implicated in a number of disease processes, however with regard to malignancy Yokoi *et al*[196] reported levels of SAA to be elevated in patients with PC compared to controls, although a sensitivity of 96.5% was observed for the detection of PC, and a specificity of 31.9% highlights a shortcoming in its use as a potential diagnostic biomarker.

Aminopeptidase N: Aminopeptidase N (APN/CD13) is a membrane bound metalloproteinase which is expressed in a number of different tumour types and cells, and has been suggested to play a role in tumor progression, proliferation, invasion and angiogenesis[197-199]. APN/CD13 was first evaluated in 2016 by Pang *et al*[200] where an AUC of 0.904 was reported in differentiating PC from benign pancreatic tumours, CP and healthy controls, however this study was limited in its size.

M2-pyruvate kinase: M2-pyruvate kinase (M2-PK) is a glycolytic enzyme that has been demonstrated to have a role in cancer metabolism[201,202]. Initially evaluated in 2004, serum M2-PK was reported to be elevated in patients with PC with a sensitivity and specificity of 85% and 41% respectively, which was subsequently validated in 2008 however elevation was also seen in patients with CP thus highlighting a lack of specificity associated with its implementation as a diagnostic biomarker[203,204].

Apolipoprotein isoforms: Apolipoproteins (APOs), which are produced in the liver and intestine, act as lipid carriers, and in doing so, act as ligands for cell membrane receptors, enzyme cofactors and structural components of lipoproteins (after binding to lipids)[205]. A large number of APOs have been reported to have a role in malignancy with serum APOA2, APOC1, APOC2 and APOE being implicated in PC diagnosis and prognosis.

APOA2, specifically APOA2-ATQ/AT has been demonstrated to be able to distinguish patients with early stage PC compared to healthy controls as well as identifying patients at high risk of pancreatic malignancy. The AUC value for APOA2-ATQ/AT was superior compared to CA19-9 in detecting early stage PC[206]. APOA2 was prospectively evaluated in 2019 where it was identified to be useful when utilized in combination with CA19-9 to improve detection of PC up to 18 mo prior to diagnosis and was suggested to be a useful first measure of PC detection prior to imaging[207]. This was built upon in 2020, where APOA2-ATQ/AT was implemented in a screening cohort in which an elevated level resulted in a PPV of 33.3% for the diagnosis of PC [208].

APOC1 has been implicated in PC where in pre-operative serum, higher levels were reported to correlate with poor prognosis highlighting the potential role of APOC1 as contributing to aggressiveness in PC[209]. Similarly, APOC2 was investigated by Xue *et al*[210] who reported that serum levels independently predicted survival in patients diagnosed with PC.

Serum APOE has been demonstrated to have a sensitivity and specificity of 76.2% and 71.4% respectively for distinguishing patients with PC compared to controls[211, 212]. This study published a superior sensitivity of APOE in diagnosing PC to CA19-9, however it lacked specificity in the diagnosis and was proposed that utilization in combination with CA19-9 could prove beneficial in the future[211]. More recently, when combined in a biomarker panel with inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3), APOA1, APOL1 and CA19-9, a sensitivity and specificity of 95% and 94.1% respectively was reported for the diagnosis of PC[213].

Serum growth factors

Transforming growth factor-beta: According to the findings of Yako *et al*[214] there is a lack of a definitive consensus on the role of transforming growth factor-beta (TGF-β) as a diagnostic biomarker in PC, with serum levels varying in those diagnosed with the malignancy. In addition to this TGF-β has also been implicated in the diagnosis of PC where it has been demonstrated to be elevated in serum samples compared to benign controls, while high levels in serum also significantly correlated with reduced patient survival[215].

Vascular endothelial growth factor: Vascular endothelial growth factor (VEGF) has been reported to have an important role in PC development, while VEGF-A expression has been reported be an important predictor for both distant metastasis and poor prognosis in PC[216]. There is a lack of data affirming the role of serum VEGF as a diagnostic biomarker for PC, with biliary VEGF considered a more accurate diagnostic modality[217].

Fibroblast growth factor 10/keratinocyte growth factor-2: Fibroblast growth factor 10/keratinocyte growth factor-2 (FGF-10/KGF-2) is a regulator of the pancreatic epithelial progenitor cell proliferation and has been implicated in pancreatic morphogenesis along with epithelial mesenchymal transition[218,219]. FGF-10/KGF-2 has been demonstrated to be significantly overexpressed in the sera of patients diagnosed with PC pre-treatment compared to controls, in conjunction with a number of other novel cytokine candidate markers[138].

Platelet-derived growth factor: There is limited data pertaining to the use of platelet-derived growth factor (PDGF) in the diagnosis of PC, however it has been proposed in a panel including IP-10, interleukin (IL)-6 and CA19-9 which demonstrated diagnostic superiority in the discrimination of PC patients from patients with benign disease both in a training and independent test set[220].

Tumour specific growth factor: There is limited data pertaining to the role of tumour specific growth factor (TSGF) in the diagnosis of PC, with a single centre study reporting an increase in specificity for PC when TSGF is used in combination with CA242 and CA19-9 while another study assessed the utility of TSGF as a monitor of response to treatment[221,222].

Serum cytokines and chemokines

Macrophage inhibitory cytokine-1/Growth Differentiation Factor-15: Macrophage inhibitory cytokine-1/Growth Differentiation Factor-15 (MIC-1/GDF15) is a distant member of the TGF-β superfamily of cytokines that has been implicated with inflammation and carcinogenesis, along with serum elevation being detected in a number of pathologies including heart failure and renal failure[223-226].

A meta-analysis published in 2018 aimed to compare MIC-1/GDF15 to CA19-9 as a diagnostic biomarker in PC, identifying fourteen studies with a total of 2826 participants. MIC-1/GDF15 was reported to have a sensitivity of 80% and specificity of 88%, and a diagnostic odds ratio (DOR) of 24.57 which was superior to CA19.9 (DOR = 17.76). In addition to this the AUC of MIC-1/GDF15 in diagnosing PC was 0.8945, which was moderately superior to CA19.9. The conclusion from this study was that MIC-1/GDF15 had comparable diagnostic accuracy to CA19-9, however it was noted that there was marked heterogeneity between studies and that the results should be interpreted with caution[227].

With regard to PC, the authors of this study have recently demonstrated that in a prospective PC screening cohort deemed to be high risk for developing PC based on familial and genetic factors, MIC-1/GDF15 had moderate predictive capacity for patients who subsequently were diagnosed with PC on endoscopic ultrasound (EUS) and biopsy. However, the participants enrolled were considered high risk for developing PC, highlighting a potential issue with generalising the results of this study[228].

ILs: ILs are cytokines that constitute a substantial proportion of those cytokines present in the tumor microenvironment. With regards to their role as diagnostic biomarkers in PC, a considerable number of cytokines have been evaluated in patients diagnosed with PC with variable results (Table 1). There is heterogeneity between studies with insufficient evidence to support their use in routine clinical practice as diagnostic biomarkers, with previous studies demonstrating a lack of diagnostic capacity for PC compared to CRC or benign disease[235].

Oncostatin M (OSM) forms part of the IL-6 cytokine family and has been implicated in promoting epithelial mesenchymal transition, along with being linked to a number of solid organ malignancies[236-238]. Serum levels of OSM have been found to be significantly elevated in patients with PC compared to controls in a single centre study limiting generalisability[138]. There is limited data on the utility of CXC motif ligand 8 (CXCL8)/IL-8 as a diagnostic biomarker in PC. In a relatively small cohort study CXCL8 seems to be superior to CA19-9 and CEA[239].

TNF-α: There is variability in the data pertaining to TNF-α as a diagnostic biomarker in PC. Although the majority of studies report elevated levels of TNF-α in serum compared to healthy controls, a lack of specificity is highlighted as a pitfall in its routine use as a diagnostic biomarker[240-243].

Macrophage colony-stimulating factor: Serum macrophage colony-stimulating factor (M-CSF) has been demonstrated to be elevated in patients with PC compared to controls, along with correlating with advanced stage disease and with non-resectable tumors. Aside from those studies included in the 2016 systematic review published by Yako *et al*[214] there is limited published literature assessing the value of M-CSF as a serological biomarker in the diagnosis of PC.

CXCL11/interferon inducible T cell alpha chemokine: CXCL11 is a CXC chemokine which stimulates the phosphorylation of mitogen-activated protein kinase kinases pathways, resulting in cellular proliferation and prevention of apoptosis[244]. Initially evaluated in 2014, serum CXCL11 was found to be over-expressed in patients with PC compared to controls highlighting a potential role as a diagnostic biomarker, in addition to having a predictive role for gemcitabine and erlotinib treatment response in patients with PC[138].

Stem cell factor: Stem cell factor (SCF) is a ligand that is involved in cell proliferation, differentiation and cell survival, and aside from normal cellular physiology, SCF has been implicated in PC and CRC, with serum levels being noted to be elevated in PC compared to healthy controls, however studies are limited[138,245-248].

Eotaxin: Eotaxin is a protein which is implicated in the recruitment of eosinophils into inflammatory sites which has also been implicated in malignancy[249]. Serum eotaxin was assessed by Zeh *et al*[250] in a single centre study in 2005 in conjunction with hepatocyte growth factor, monocyte chemoattractant protein-1 and CXCL10, were it was able to distinguish PC from healthy controls with a sensitivity of 85.7% and specificity of 92.3%, which was superior to CA19-9.

Serum adhesion molecules

CEA-related cell adhesion molecules: CEA-related cell adhesion molecules (CEACAM) proteins belong to the immunoglobulin supergene family comprised of a variable-like domain as well constant C2-like Ig domains which are required for functionality as well as adhesion. The most well-known CEACAMs related to malignancy are CEACAM1, CEACAM5 (more commonly known as CEA), and CEACAM6. Both CEACAM5 and CEACAM6 are associated with the membrane through a glycosylphosphatidylinositol linkage, while CEACAM1 is anchored to the cellular membrane by transmembrane domains. CEACAM1 have been previously demonstrated to be elevated in a number of tumor entities including PC, however a lack of sensitivity and specificity has been cited as a barrier to its use[217,251-254]. More recently, the role of CEACAMs, including CEACAM1 has shifted from diagnosis to treatment, with CEACAM1 being implicated in cancer immunotherapy[255].

CEACAM6 is a cell surface adhesion receptor that has been previously reported to modulate the ECM in PC[256]. Expression of CEACAM6 was noted in 92% of PC specimens assessed in a 2005 study[257]. Although relatively specific for PC on serum analysis, there is scant evidence to suggest the CEACAM6 as a serological biomarker is useful in the detection of PC with a shift in focus to disruption of CAECAM6 as a therapeutic option in PC[258]. CEACAM5, or CEA, has been demonstrated to have limited efficacy in the diagnosis of PC as described previously, due to it being overexpressed in a number of solid organ malignancies[259,260].

ICAM-1: ICAM-1 is a glycoprotein that functions in cell-cell and cell-ECM adhesion, along with acting as a macrophage chemoattractant[261]. Serum ICAM-1 has been previously evaluated in a number of studies, where it has been demonstrated to be superior to CA19-9 in PC diagnosis. Although preliminary studies have demonstrated promise, its inability to distinguish between early and late-stage PC have been

identified as a potential dilemma limiting its implementation as a screening and diagnostic biomarker[262,263].

Serum non-coding RNAs

Long non-coding RNAs: Long non-coding RNAs (LncRNAs) belong to a group of RNAs that are longer than 200 nucleotides and are not translated into proteins. These RNAs are abundant in cells, and were previously thought to be of minimal value with minimal influence on biological behaviour[264]. This belief has however changed over the past 10 years, with more recent data suggesting that lncRNAs have a diverse range of function, including chromatin modification, gene transcription, post-translational modification and regulation of intracellular signalling pathways[265]. In addition to this, they play a role in either the promotion or suppression of tumor growth, through involvement in intracellular signalling pathways[266] (Table 2).

LncRNA in PC have the potential to modulate both intrinsic and acquired chemo-resistance. Additionally, lncRNA also possess the capacity to act as a miRNA sponge, to perform chromatin remodelling, and promote gene transcription in candidate tumour suppressor genes by binding to gene promotors[267-270]. In terms of the role of lncRNAs as a diagnostic marker in PC a number of candidates have been evaluated with mixed results, and studies are limited to single cohort studies yet to be validated [271]. Perhaps the most promising study to date in search for a lncRNA biomarker was published in 2020, which utilized analysis of the extracellular vesicle lncRNA profile by extracellular vesicle lncRNA sequencing in patients diagnosed with PC and CP. This was performed utilizing a support vector machine algorithm to detect a d-signature for eight different extracellular vesicular long RNA. This study demonstrated that through utilisation of the d-signature, an AUC of 0.949 was able to be achieved in identifying resectable stage I/II PC, while also demonstrating superiority when compared to CA19-9 when distinguishing PC from CP[272].

MiRNAs: MiRNAs are noncoding 20-25 nucleotide endogenous RNA sequences who regulate gene expression and are able to regulate the biological function of many tumors[273]. MiRNAs have become prominent in the field of oncology in the diagnosis, prognosis and monitoring of therapy of cancer. In addition to their presence in serum, miRNAs have also been detected in cerebrospinal fluid, breast milk, saliva and urine[274,275]. Although the method through which miRNA are released into the peripheral circulation from active malignancies is still being determined, their ability to withstand severe conditions along with extended storage highlights an exciting potential diagnostic biomarker. Due to the lack of a gold-standard diagnostic biomarker for PC, research into the efficacy of miRNA as a diagnostic biomarker in PC has progressed rapidly in the past decade with a large number of candidate miRNA biomarkers utilized in serum for the detection of PC as demonstrated in Table 2. Perhaps the most comprehensive analysis to date reviewing candidate miRNAs utilized in PC comes from a large meta-analysis published in 2018 encompassing 80 studies which detected miRNA in blood (including whole blood, serum and plasma samples that concluded that candidate miRNA biomarkers are useful in PC, particularly when used in combination, however no standing panel was reported to exist at this stage[276].

The rapid expansion of miRNA utilization in serum in the diagnosis of PC highlights its potential value as a future diagnostic biomarker modality which could be implemented into routine clinical practice, however determination of which miRNA possesses the greatest diagnostic accuracy is required. Panel based assays represent a very attractive methodology for miRNA detection which have been identified as having superior diagnostic accuracy, however further validation of specific candidate miRNAs is required.

Serum liquid biopsy

Exosomes: Exosomes are membrane-bound nano-capsules that transfer molecules between cells[308]. Their role in the diagnosis of PC is limited to only a handful of studies which were recently included in a relatively small systematic review meta-analysis which also assessed circulating tumor cells (CTCs) and cell-free DNA (cfDNA). In six papers included, exosomes were found to have strong diagnostic value with an AUC of 0.9819[309]. It was postulated that they possessed value in the field of PC detection due to pancreatic cells possessing a strong exocrine function, along with the high activity of PC cells. A number of different types of exosomes were analyzed as demonstrated in Table 3.

Table 2 Serum based non-coding RNA biomarkers implicated in the diagnosis of pancreatic cancer

Type	Candidate marker
LncRNA	LINC-PINT[277], SNHG15[278,279], LINC01238[280], ABHD11-AS1[281], HULC[282,283], UFC1[284]
MiRNA	miR-21[285-289], miR-25[288,290,297], miR-210-3p[289], miR-29a[290], miR-19a[290], miR-210[285,291], miR-155[285,292], miR-499a-5p[293], miR-125a-3p[294], miR-6893-5p[294], miR-125b-1-3p[294], miR-6075[294], miR-6836-3p[294], miR-1469[294], miR-6729-5p[294], miR-575[294], miR-204-3p[294], miR-6820-5p[294], miR-4294[294], miR-4476[294], miR-4792[294], miR-196a[285,295], miR-18a[296,297], miR-10b[292-298], miR-106b[292], miR-642-3p[299], miR-885-5p[299], miR-22-3p[299], miR-34a[286], miR-191[297], miR-451a[300], miR-121-5p[298], miR-30c[298], miR-483-5p[290,297], miR-1290[301,302], miR-24[290,297,301], miR-134[301], miR-146a[301], miR-378[301], miR-484[301], miR-628-4p[301], miR-1825[301], miR-1246[302], miR-482-3p[287], miR-16[295], miR-27a-3p[303], miR-192[304], miR-885-5p[299], miR-22-3p[299], miR-642b-3p[299], miR-492[305], miR-663a[305], miR-194[304], miR-223[306], miR-774-5p[307], miR-409-3p[307], miR-128-3p[307], miR-20a[290,297], miR-27a[297], miR-29c[297], miR-30a.5p[297], miR-323.3p[297], miR-345[297]

MiRNA: MicroRNA; LINC-PINT: Long intergenic non-protein coding RNA, P53 induced transcript; SNHG15: Small nucleolar RNA host gene 15; ABHD11-AS1: ABHD11 antisense RNA 1; HULC: Highly up-regulated in liver cancer.

Table 3 Serum based 'liquid biopsy' biomarkers implicated in the diagnosis of pancreatic cancer

	Biomarkers
Exosomes	Exosomes: GPC1[310,313], miR-10b[310], miR-30c[310], miR-181-a[310], miR-let7a[310], miR-17-5p[311], miR-21[311], miR-1246[312], miR-4644[312], miR-3976[312], miR-4306[312]
ctDNA	*KRAS*[314-317], *ADAMTS1*[318], *BNC1*[318]
CTC	CAPI+/CD45-[319], CK+[319], CEA+[319], CD45-/DAPI+/CEP8[320], CD45[321], CCK19[321], Pdx-1[321], Kras mutation[322], CEP8[323], CK[323], CD45[323], DAPI[323], chromosome 8[324], Folate-receptor positive CTCs[326]

Tspan8: Tetraspanin 8; EpCAM: Epithelial cell adhesion molecule; MET: mesenchymal-epithelial transition factor; CD104: Integrin 4-beta; GPC1: Glypican 1; GNAS: Guanine Nucleotide binding protein; KRAS: KRAS Proto-Oncogene, GTPase; ADAMTS1: A disintegrin and metalloproteinase with thrombospondin motifs 1; BNC1: Basonuclin 1; CD45: Leukocyte common antigen; CK19: Cytokeratin 19; Pdx-1: Pancreatic and duodenal homeobox 1; ADAMTS1: A disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13; BNC1: Zinc finger protein basonuclin-1.

CTCs: Initially identified in 1896 in metastatic breast cancer, CTCs are cells that are shed from primary tumor or metastatic deposits which enter the bloodstream directly and can be detected forming what is known as a real-time "liquid biopsy"[325]. In a recently published systematic review and meta-analysis, seven articles were identified which utilized CTCs in the diagnosis of PC, of which multiple methods of detection were used highlighting heterogeneity between study methodology. The pooled sensitivity and specificity of CTCs were 74% and 83% respectively, with and AUC of 0.8166. The authors' conclusion was that CTCs had moderate diagnostic value in PC [309].

CTCs demonstrated inferiority when compared to exosomes in the systematic review due to their inferior sensitivity and specificity, however their AUC was still deemed acceptable from a diagnostic capacity for PC. Folate receptor positive CTCs have also been implicated as a novel diagnostic biomarker in those patients diagnosed with periampullary malignancy on ligand-targeted polymerase chain reaction demonstrating a significant elevation compared to those with benign pancreatic disease[326]. In addition to this, when utilized in combination with CA19-9, it was reported to have a superior sensitivity and specificity of 97.8% and 83.3% respectively, compared to when used in isolation. CTCs have yet to be utilized in a prospective screening population. Decreased blood flow to malignant pancreatic tissue along with increased CTC accumulation in the liver due to the portal circulation are posed as challenges in the detection of PC related CTCs[327].

Circulating tumor DNA: cfDNA, initially identified in 1948, is fragmented DNA identified in the circulation. It has been applied to many areas of medicine, ranging from prenatal assessment, renal failure, and stroke where it has had mixed results[328-330]. In the case of medical oncology, the detection and utilisation of cfDNA secreted from tumours, referred to as circulating tumor DNA (ctDNA) has been met with a number of challenges, namely the ability to discriminate ctDNA from normal cfDNA, and low levels of ctDNA hampering detection[331].

The diagnostic value of ctDNA in PC has been deemed to be promising with a recent meta-analysis being able to identify seven articles assessing ctDNA in the

diagnosis of PC showed a pooled sensitivity and specificity were 64% and 92% respectively, with an AUC of 0.9478[309]. In this review, ctDNA was deemed inferior to CTCs from a sensitivity perspective, however the AUC was superior in diagnosing PC. This was attributable to the inability to detect low levels of circulating ctDNA in early stages of cancer when overall tumor burden was low, highlighting a dilemma in utilizing this form of diagnostic biomarker in early stages of disease and as a screening modality. A summary of the included ctDNA biomarkers can be viewed in Table 3.

Plasma ctDNA quantification of hot-spot mutations in KRAS and GNAS has also been reported to be useful in predicting tumor burden in patients diagnosed with PC. In addition to this, digital PCR (dPCR) provided accurate tumor-derived mutant KRAS detection in plasma in resectable PC and improved post-resection recurrence prediction compared to CA19-9[332].

URINARY BIOMARKERS

Urine protein biomarkers

Urine proteins have also been established as a means through which PC can be detected, with previous proof-of-concept studies demonstrating that protein signatures associated with PC can be detected in the urine[333]. Radon *et al*[334] were able to build upon this, where they reported that three proteins, lymphatic vessel endothelial hyaluronan receptor 1, REG1A and thyroid transcription factor 1, when combined in a biomarker panel, were able to detect patients with PC with an AUC of 0.89 and 0.92 in training and validation datasets respectively, compared to healthy controls. Although further validation is required, this presents an inexpensive and non-invasive option for screening in patients for PC, and was suggested to be added to the current screening modalities utilized in high-risk patients to determine its efficacy prospectively[334]. Aside from this there is relatively little published with regard to the urinary proteome in the detection of PC and other proteins implicated are limited to single centre cohort studies (Table 4).

Urine non-coding RNA

MiRNA: Urinary miRNA has previously been utilized in the detection of bladder cancer, however, there is scant literature to support the use of urinary miRNA in the detection of PC[339]. In a small British study, Debernardi *et al*[340] were able to demonstrate that miR-143, miR-223 and miR-30e were significantly over-expressed in patients with stage I PC compared to age-matched healthy individuals. MiR-1246 has also been assessed as a urinary biomarker, where significantly higher levels of expression were noted in patients with PC compared to controls, with an AUC of 0.90 which was superior to serum miR-1246 (AUC = 0.87)[18]. Considering the non-invasive capacity of urine sampling, coupled with the rapid expansion and interest in use of miRNA in the detection of malignancy, further studies should aim to determine whether experimental studies can translate into larger prospective clinical studies.

Urine liquid biopsy

Urinary cfDNA: Considering the rapid expansion of the concept of a 'liquid biopsy', the hypothesis that tumour DNA could be detected through the urine with urinary cfDNA originating from the shedding of cells directly from the genitourinary tract or *via* the circulation passing through the kidney and filtering through the glomerulus also known as transrenal DNA has emerged as a method of biomarker detection. Terasawa *et al*[341] were able to detect urine KRAS mutations in 48% of participants diagnosed with PC, which was equivocal with the serum detection rate. This method of detection however is influenced by the patient's underlying kidney function.

Exosomes: More recently, the ratio of miR-3940-5p/miR-8069 in urine exosomes has been implicated in PC. This ratio was noted to be elevated in patients diagnosed with early stage PC, with a sensitivity of 93.0% and PPV of 78.4%[342].

Other urinary markers

Detection of volatile organic compounds (VOCs) is a relatively novel area in malignancy diagnosis, which utilized odors that emanate from urine, breath and faeces. These compounds are produced by bacterial dysbiosis which is secondary to malignancy. Recently Nissinen *et al*[343] were able to demonstrate through using field asymmetric waveform ion mobility spectrometry that patients diagnosed with PC could be distinguished from healthy controls with a sensitivity and specificity of 79%

Table 4 Urinary biomarkers implicated in the detection of pancreatic cancer

Type	Candidate marker
Protein	LYVE1[334], REG1A[334], TTF1[334], TIMP1[335], MMP-2[335], NGAL[336], PGE2 metabolites[337], CD59 glycoprotein (CD59)[338], ANXA2[338], 21 kDA gelsolin fragment[338], S100A9[338]
Liquid biopsy	UcfDNA: KRAS mutation[341]; Exosomal miRNA: miR-3940-5p[342], miR-8069[342]
RNA	MiRNA: miR-143[340], miR-223[340], miR30e[340], miR-1246[18]
Metallomics	Calcium[344], magnesium[344]
Other	VOCs[343]

MiRNA: MicroRNA; ANXA2: Annexin A2; S200A9: Protein S100-A9.

and 79% respectively through the detection of VOCs in the urine. Additionally, the analysis of the metallomic signature of urine is also a relatively uncharted area in the field of PC, with a study published by Schilling *et al*[344] recently demonstrating that in those diagnosed with PC, urine calcium and magnesium were significantly lower compared to healthy controls. They were able to demonstrate through combined analysis that these metals were accurate indicators for metal dyshomeostasis in PC with a sensitivity of 99.5%.

PANCREATIC JUICE BIOMARKERS

Pancreatic juice is usually obtained during the ERCP which is an invasive procedure with potential morbidity and mortality and is not used routinely as a screening procedure. Alternatively, pancreatic juice can be collected during the endoscopy from the duodenum after secretin administration which has the risk of secretin induced pancreatitis and contamination of the sample with duodenal and gastric juice. While attractive, pancreatic juice biomarkers are unlikely to be used in large populational studies but it might be useful in selected cases in which endoscopy or ERCP is indicated (Table 5).

Protein based biomarkers

Protein biomarkers are the most well explored candidate biomarkers in the medium of pancreatic juice. Conventional markers utilized in serum, such as CA19-9 and CEA, have been implicated in pancreatic juice where the sensitivity of CA19-9 is questionable, while CEA demonstrated merit in predicting malignant transformation of IPMNs along with the diagnosis of PC[345-352]. Aside from these biomarkers, a large number of proteins have been assessed in the pancreatic juice of patients with variable results, however considering that evidence supporting these biomarkers is limited to only a handful of small cohort studies, their implementation as a diagnostic tool is not recommended.

Although mucins have been extensively investigated in the diagnosis of PC, with regard to pancreatic juice there is limited literature published on its value. Levels have been demonstrated to be elevated in the case of MUC1, and KL-6 mucin, a type of MUC1, was investigated by Matsumoto *et al*[354] and reported to be significantly elevated in the pancreatic juice of patients with PC and IPMC compared to inflammatory lesions and IPMNs however its specificity was less than desirable.

Non-coding RNA

When compared to serum and saliva, pancreatic juice has proved to be less fruitful with regard to candidate miRNA biomarkers in PC diagnosis. Both miR-21 and miR-155 have been demonstrated to be elevated in the pancreatic juice of patients diagnosed with PC compared to CP[362], while Wang *et al*[363] was also able to report a specificity of 88% and sensitivity of 87% when four circulating miRNAs in pancreatic juice (miR-205, miR-210, miR-492 and miR-1427) were used in combination for detecting PC. In addition to miRNA assessed in pancreatic juice, MSLN mRNA has also been implicated in the diagnosis of PC on pancreatic juice[364].

Table 5 Pancreatic Juice biomarkers implicated in the detection of pancreatic cancer

Type	Candidate marker
Protein	CA19-9[345-347,349], MIC-1[349], NGAL[349], CEA[347,348,350-352], AMYP[353], PRSS1[353], glycoprotein GP2-1[353], CCDC132[353], REG1A[353], REG1B[353], REG3A[353], LIPRP2[353], KL-6/MUC1[354], CPA5[355], inactive LIPRP1[355], KLK1[355], HBD[355], TTR [355], S100P[356], MMP-9[357], MMP-7[155], DJ-1[357] A1BG[357], PAP-1[358], AGR2[359], IL-8[360], Cathepsin E[361]
RNA	MiRNA: miR-21[362], miR-155[362] , miR-205[363], miR-210[363], miR-492[363], miR-1427[363]; mRNA: mesothelin[364]; Other: hTERT [365,366], telomerase activity[367-369]
Liquid biopsy	Exosomes: CEACAM1[371], CEACAM 5[371], tenascin C[371], MMP7[371], LAMB3[371], LAMC2[371], MUC1[372], MUC4[372], MUC5AC[372], MUC6[372], MUC16[372], CFTR[372], MDR1[372], ex-miR-21[373], ex-miR-155[373]; Methylated DNA: KRAS[374,377], ppENK[375,376], p16[375,376], Cyclin D2[376], FOXE1[376], NPTX2[376], TFPI2[376], CD1D[377], KCNK12[377], CLEC11A[377], NDRG4 [377], IKZF1[377], PKRCB[377], MUC1[378], MUC2[378], MUC4[378]

MiRNA: MicroRNA; PRSS1: Trypsin-1; CPA5: Carboxypeptidase A5; KLK1: Kallikrein-1; HBD: Hemoglobin Subunit Delta; LAMB3: Laminin subunit beta-3; CFTR: Cystic fibrosis transmembrane conductance regulator; MDR1: Multidrug resistance protein 1; KCNK12: Potassium channel, subfamily K, member 12; CLEC11A: C-Type lectin domain containing 11A; NDRG4: NDRG family member 4; IKZF1: Ikaros family zinc finger protein 1 gene; PKRCB: Protein kinase C beta; FOXE1: Forkhead Box E1; NPTX2: Neuronal pentraxin-2.

Liquid biopsy

Telomerase activity and human telomerase reverse transcriptase: Telomerase activity has previously been deemed a promising marker as it was shown to be elevated in pancreatic juice samples of patients with PC[365-367]. Further to this, a recent meta-analysis assessing the diagnostic utility of the four major altered genes in PC (KRAS/CDKN2A/p16, TP53, and SMAD4/DPC4), telomerase activity, and a combination assay, revealed that the most reliable biomarker in diagnosing PC in pancreatic juice samples was telomerase activity[367]. Human telomerase reverse transcriptase (hTERT) is a catalytic subunit of telomerase, and the detection of mRNA for hTERT has been postulated to aid in the diagnosis of malignancies including PC. hTERT was first detected in 10 of 11 patients diagnosed with invasive PC on pancreatic juice sampling[368]. This was further validated by Nakashima *et al*[369] and was additionally assessed in a recent systematic review assessing the role of hTERT which reported that telomerase reactivation played a significant role in the development of hepatobiliary and pancreatic tumors, along with being a diagnostic biomarker for PC[369,370].

Methylated DNA: Mutations in the KRAS oncogene are present in over 90% of resected PC specimens, with the vast majority of these mutations occurring in KRAS codon 12. A recent meta-analysis published by Patel *et al*[374], encompassing 22 studies aimed to assess the diagnostic accuracy of mutant KRAS detection from pancreatic secretions (mucus, secretions and juice) for the diagnosis of PC. They reported a wide variation in sensitivity (38%-89%) and specificity (13%-100%) for the diagnosis of PC through KRAS mutation testing in pancreatic secretions, with significant heterogeneity in diagnostic accuracy across the included studies. They also assessed whether KRAS mutation detection would be beneficial in diagnosing PC in a screening population, which similarly returned a sensitivity ranging from 21%-86%, however specificity improved remarkably to 82%-100%[374]. In addition to KRAS, Methylated ppENK and p16 were reported to be present in pancreatic juice in 90.9% and 18.2% respectively of patients diagnosed with PC, and due to normal pancreatic juice not containing methylated forms of this DNA, their presence was postulated to suggest the presence of PC[375]. Other markers investigated in single centre studies are shown in Table 5. MUC1 was also assessed in conjunction with MUC2 and MUC4 in 2014. Yokoyama *et al*[378] reported that DNA methylation status of MUC1, MUC2 and MUC4 was useful for the differential diagnosis of human pancreatic neoplasms, with a sensitivity and specificity of 87% and 80% for PC.

PANCREATIC CYST FLUID BIOMARKERS

Pancreatic cysts (PCy) are proving to be a promising area in the field of specimen sampling for biomarker identification. PCy incidence increases with age, with the most common cyst types including IPMN, mucinous cystic neoplasms (MCN), serous cystic neoplasms, and pseudocysts[379-381] (Table 6).

Table 6 Pancreatic cyst fluid biomarker studied in relation to high grade dysplasia and pancreatic cancer diagnosis

Type	Candidate marker
Protein	CEA[383,384,399,402-407], Glucose[385], MUC4[386,412], PGE2[387,388], IL-1B[386,387], PGE synthetase 2[386], IL-4[389], CA72-4[389], sFASL [389], MMP9[389] AREG[390,391], SPINK1[392], mAB Das-1[393,394], IL-10[395], GM-CSF[395], MUC1[413], MUC2[413], MUC5AC[413]
RNA	MiRNA: miR-21[396], miR-221[396], miR-18a[397,398], miR-24[397,398],miR-30a-3p[397,398], miR-92a[397,398], miR-99b[397,398], miR-106b[397, 398], miR-142-3p[397,398], miR-342-3p[397,398], and miR-532-3p[397,398]
Other	DNA based-*KRAS* mutations[399-407,409-411]*GNAS* mutations[409-411]

MiRNA: MicroRNA; CA72-4: Cancer antigen 72-4; sFASL: Soluble Fas; AREG: amphiregulin; SPINK1: serine peptidase inhibitor kazal type 1; GM-CSF: granulocyte macrophage colony-stimulating factor.

Due to IPMNs and MCNs possessing a risk of developing into PC identification of cyst fluid biomarkers in these pre-malignant lesions help to select which patients to proceed to surgery[382]. The cyst fluid is aspirated during EUS (EUS-FNA) under antibiotic cover and the amount of fluid retrieved depends on the size of the cyst therefore highlighting a potential for insufficient sampling during aspiration. Pancreatic cyst fluid analysis was initially focused on proteins isolated for biomarker assessment, however more recently there has been a transition towards the analysis of non-coding RNA, or miRNA in pancreatic cyst fluid to determine their diagnostic capacity for PC[408].

Proteins analyzed on cyst fluid, for the most part, have been reported to lack specificity in the diagnosis of PC, however mucin analysis, CEA level and VEGF-A on cystic fluid has proved to have efficacy in discriminating premalignant and malignant lesions from benign lesions. MUC4 expression has been implicated in PCy, being elevated in MCN, and has been postulated to assist in early detection of PC[412]. In addition to this, MUC1, MUC2 and MUC5AC have been demonstrated to be upregulated in patients with PC on cytology obtained during EUS-FNA but MUC7 is upregulated in PC and also in IPMN and CP, limiting its specificity in the diagnosis of PC[413,414].

Additionally, DNA-based biomarkers, including KRAS and GNAS, have been evaluated in the context of PC diagnosis and IPMN and noted to be elevated in mucin producing cysts. Recently, supervised machine learning techniques were used to develop a test to guide management of PCy based on clinical features, imaging and cyst fluid genetic and biochemical markers (CompCyst)[415]. Due to invasive nature of cyst fluid collection, the authors recommend that future studies should focus on biomarkers and algorithms that can help select which cysts have malignant potential and should proceed to surgery.

SALIVARY BIOMARKERS

Saliva is an emerging interest in the field of biomarker detection as it provides a non-invasive means through which potential diagnostic biomarkers can be sampled. It has previously been validated in the areas of drug abuse, human immunodeficiency virus infection and hormone assessment, along with detection of oral, breast, lung, ovarian and oesophageal cancer, and has been recently named the "diagnostic window to the body"[416-418] (Table 7).

The analysis of salivary fluid as a means for identification and evaluation of diagnostic biomarkers for PC is in its infancy, with proteomic biomarkers scant in the literature and due to the large amounts of salivary amylase, albumin and immunoglobulins present in saliva, their subsequent sensitivity is hampered in PC diagnosis [419,420]. Given this lack of sensitivity, there has been a shift in focus to RNA based biomarkers, namely LncRNA and miRNA . A recent systematic review reported that PC is the most investigated disease in relation to the utilization of salivary miRNA analysis. This is highlighted by 18 miRNA candidates which have been detected and studied in relation to PC, irrespective of stage, through the medium of saliva. Although miRNA analysis in saliva is in its infancy with regard to PC, the reported specificity in the diagnosis of PC is impressive and warrants further validation. Despite this reported specificity, the aforementioned systematic review concluded that there is marked heterogeneity between studies and as such meta-analysis is unachievable, highlighting the need for further research in this area[421-425].

Table 7 Salivary fluid biomarkers studied in relation to pancreatic cancer diagnosis

Type	Candidate marker
RNA	LncRNA: *HOTAIR*[428], *PVT1*[428]; MiRNA: miR-21[286,423,431], miR-23a[423], miR-23b[423], miR-29c[423], miR-1246[422], miR-4644[422], miR-34a[286], miR-155[286], miR-200b[286], miR-376a[286], miR-216[423], miR-940[424], miR-3679-5p[424], miR-17[425], miR-181b[425], miR-196a[425]
Other	Salivary polyamines: Alanine[427], N_1-acetylspermidine[427], 2-oxobutyrate[427], 2-hydroxybutyrate[427]

MiRNA: MicroRNA.

Aside from proteomic and RNA analysis of saliva, polyamine analysis has also emerged as a potential diagnostic biomarker candidate. Abnormalities in tumor-suppressor genes, deemed to play a key role in PC development, accelerate polyamine synthesis and as such, increased levels have been postulated to be a potential biomarker in PC[426]. Only a single study has assessed polyamines in PC detection with modest diagnostic accuracy[427].

BILIARY FLUID BIOMARKERS

Biliary fluid is a potential source for biomarkers, however due to sampling requiring an invasive procedure, ERCP, there are inherent risks with this mode of acquisition and is not routinely used. Currently the literature is limited to protein-based biomarkers, non-coding RNA markers and methylated DNA as a method of liquid biopsy with a recent meta-analysis highlighting minimal literature on biliary miRNA markers utilized in PC diagnosis[445] (Table 8).

There have been mixed results from these studies with a lack of large prospective studies to determine the validity of these biomarkers in clinical use. Although some biomarkers display merit in the early phases of clinical research, their role has also been deemed to be of value in the diagnosis of indeterminate biliary strictures thus highlighting a potential lack of sensitivity in the diagnosis of PC. Given the invasive nature of acquisition, less intrusive methods of biomarker acquisition should be considered for future research.

FAECAL BIOMARKERS

The concept of being able to detect PC biomarkers in stool is due to the large amount of pancreatic juice produced and excreted into the bowel on a daily basis, highlighting the potential that that precancerous or molecular changes indicative of a malignant process can be detected in faeces[447] (Table 9).

Faecal protein biomarkers

Adnab-9: Adnab-9 is a murine monoclonal antibody that has previously been implicated in the diagnosis of gastrointestinal tumors[448,449]. Adnab-9 detection in stools has a sensitivity and specificity of 80% and 87% for detection PC[450,451].

Faecal non-coding RNA

MiRNA: Faecal miRNA detection as a diagnostic biomarker has been utilized in CRC where although the environment has deemed to be more hostile than blood, miRNAs have been demonstrated to remain intact and stable for detection due to being packaged in exosomes. Faecal miRNA detection only requires 1 g of faeces in a sample, therefore presents itself as an efficacious modality as a screening test. Although there is only scant literature describing faecal miRNA analysis as a biomarker in PC[19,452,453], certain candidate markers demonstrate promise however there is heterogeneity between studies. Further studies are required to determine the relationship of faecal miRNA expression in PC to determine whether a candidate marker can be utilized in a screening population.

Faecal liquid biopsy

Faecal mutant *KRAS*: Initially detected in 1994 by Caldas *et al*[454], the presence of *K-ras* mutation in stool in patients with PC has proved to be an area of promise with regard to a non-invasive method of detection, and has also been explored in

Table 8 Biliary fluid diagnostic biomarkers studied with relation to pancreatic cancer

Type	Candidate marker
Protein	VEGF[217,429], CA19-9[431], CA125[432], CA72-4[432], CEA[432,433], sLR11[434], MUC4[435], IGF-1[217,430], NGAL[436-439], CEAM6[436,440], LG3BP[436], MMP7[436], MUC5B[436], MCM5[441,442], Trypsinogen-1[443], Trypsinogen-2[443]
Liquid biopsy	Methylated DNA: *TFP12*[444], *NPTX2*[444], *CCND2*[444]
RNA	MiRNA: miR-10b[292,445], miR-106b[292,445], miR-30c[292,445], miR-155[292,445], miR-212[292,445], miR-1247[446], miR-200a[446], miR-200b[446]

MCM5: Minichromosome maintenance protein 5; NGAL: Neutrophil gelatinase-associated lipocalin; sLR11: Soluble LDL receptor relative with 11 ligand-binding repeats; IGF1: Insulin-like growth factor 1; LG3BP: Galectin-3-binding protein; CEAM6: Carcinoembryonic cell adhesion molecule 6; TFP12: Methylated tissue factor pathway inhibitor 2; NPTX2: Neuronal pentraxin II gene; CCND2: G1/S-specific cyclin-D2; VEGF: Vascular endothelial growth factor; CEA: Carcinoembryonic antigen; MiRNA: MicroRNA.

Table 9 Faecal diagnostic biomarkers implicated in pancreatic cancer

Type	Candidate marker
Protein	Adnab-9[450,451]
RNA	MiRNA: miR-181b[452], miR-210[452], miR-155[453], miR-216a[453], miR-196a[452,453], miR-143[453]
Liquid biopsy	Mutant *KRAS*[454,455], mBMP3[456]

MiRNA: MicroRNA.

combination with methylated bone morphogenetic protein 3 (mBMP3)[454-456].

mBMP3: There is scarce literature regarding the role of BMP3 in the diagnosis of PC with a single study in 2011. Stool mBMP3 use as a biomarker for PC was first assessed in 2012, where it was able to detect 51% of PCs, compared to mutant *KRAS* which detected 50%. The AUC for mBMP3 was 0.73, however when used in combination with mutant *KRAS*, an AUC of 0.85 was achieved highlighting a potential option for non-invasive biomarker testing in a prospective cohort[456].

CONCLUSION

The literature is diverse with regard to biomarkers in the diagnosis of PC, with variation both in the medium utilized (serum, urine, saliva, pancreatic juice, cyst fluid analysis, faeces), along with the type of biomarker detected (miRNA, exosomes, proteins, CTCs, ctDNA) as demonstrated through this review, encompassing over 300 different diagnostic biomarkers in a variety of mediums. The current diagnostic biomarker utilized in the routine diagnostic work-up of PC is CA19-9, however this lacks sensitivity highlighted by phenotypic variation in the Lewis blood group antigen. Current research has focused on miRNA, ctDNA and CTCs in the detection and subsequent diagnosis of PC in experimental or feasibility studies with mixed results so far. Perhaps the most promising area of diagnostic biomarker discovery in the field of PC is the utilisation of diagnostic panels comprising a number of candidate markers rather than a single candidate protein or miRNA. These panels have proved to be efficacious in their diagnostic capacity for PC and as such should be further explored in prospective multi-centre studies to prove generalizability of results across different population groups. Very minimal research has been conducted evaluating biomarkers as a screening tool, with the low incidence of PC in the general population being cited as a barrier. This should be further explored to determine whether these candidate markers can be used as part of a screening program. A small number of studies have assessed the role of biomarkers in high-risk populations part of PC screening programs, however further research is required to determine whether their results can be extended to the general population. Future studies should aim to capitalize on the non-invasive nature of salivary, urinary, faecal and serum testing, as ultimately at a population level these are the most implementable modalities of testing and use cyst analysis and pancreatic juice in undetermined pancreatic lesions when

surgery is contemplated. Although we are yet to find the elusive 'golden ticket' for diagnosing PC, translational research is constantly opening up new doors in the search for a diagnostic biomarker that will help select the patients who need further investigations aimed at detecting PC early, similar to a positive FOBT prompting further assessment with a colonoscopy.

REFERENCES

1 **The Lancet Gastroenterology Hepatology**. Pancreatic cancer: a state of emergency? *Lancet Gastroenterol Hepatol* 2021; **6**: 81 [PMID: 33444531 DOI: 10.1016/S2468-1253(20)30397-6]
2 **Khalaf N**, El-Serag HB, Abrams HR, Thrift AP. Burden of Pancreatic Cancer: From Epidemiology to Practice. *Clin Gastroenterol Hepatol* 2021; **19**: 876-884 [PMID: 32147593 DOI: 10.1016/j.cgh.2020.02.054]
3 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
4 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]
5 **Rawla P**, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. *World J Oncol* 2019; **10**: 10-27 [PMID: 30834048 DOI: 10.14740/wjon1166]
6 **Ghaneh P**, Costello E, Neoptolemos JP. Biology and management of pancreatic cancer. *Gut* 2007; **56**: 1134-1152 [PMID: 17625148 DOI: 10.1136/gut.2006.103333]
7 **Costello E**, Greenhalf W, Neoptolemos JP. New biomarkers and targets in pancreatic cancer and their application to treatment. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 435-444 [PMID: 22733351 DOI: 10.1038/nrgastro.2012.119]
8 **Neoptolemos JP**, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Büchler MW; European Study Group for Pancreatic Cancer. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* 2004; **350**: 1200-1210 [PMID: 15028824 DOI: 10.1056/NEJMoa032295]
9 **Klaiber U**, Leonhardt CS, Strobel O, Tjaden C, Hackert T, Neoptolemos JP. Neoadjuvant and adjuvant chemotherapy in pancreatic cancer. *Langenbecks Arch Surg* 2018; **403**: 917-932 [PMID: 30397779 DOI: 10.1007/s00423-018-1724-8]
10 **Chang MC**, Wu CH, Yang SH, Liang PC, Chen BB, Jan IS, Chang YT, Jeng YM. Pancreatic cancer screening in different risk individuals with family history of pancreatic cancer-a prospective cohort study in Taiwan. *Am J Cancer Res* 2017; **7**: 357-369 [PMID: 28337383]
11 **Henrikson NB**, Aiello Bowles EJ, Blasi PR, Morrison CC, Nguyen M, Pillarisetty VG, Lin JS. Screening for Pancreatic Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2019; **322**: 445-454 [PMID: 31386140 DOI: 10.1001/jama.2019.6190]
12 **Shi C**, Hruban RH, Klein AP. Familial pancreatic cancer. *Arch Pathol Lab Med* 2009; **133**: 365-374 [PMID: 19260742 DOI: 10.5858/133.3.365]
13 **Singhi AD**, Koay EJ, Chari ST, Maitra A. Early Detection of Pancreatic Cancer: Opportunities and Challenges. *Gastroenterology* 2019; **156**: 2024-2040 [PMID: 30721664 DOI: 10.1053/j.gastro.2019.01.259]
14 **Takaori K**, Bassi C, Biankin A, Brunner TB, Cataldo I, Campbell F, Cunningham D, Falconi M, Frampton AE, Furuse J, Giovannini M, Jackson R, Nakamura A, Nealon W, Neoptolemos JP, Real FX, Scarpa A, Sclafani F, Windsor JA, Yamaguchi K, Wolfgang C, Johnson CD; IAP/EPC study group on the clinical managements of pancreatic cancer. International Association of Pancreatology (IAP)/European Pancreatic Club (EPC) consensus review of guidelines for the treatment of pancreatic cancer. *Pancreatology* 2016; **16**: 14-27 [PMID: 26699808 DOI: 10.1016/j.pan.2015.10.013]
15 **Gui JC**, Yan WL, Liu XD. CA19-9 and CA242 as tumor markers for the diagnosis of pancreatic cancer: a meta-analysis. *Clin Exp Med* 2014; **14**: 225-233 [PMID: 23456571 DOI: 10.1007/s10238-013-0234-9]
16 **Herreros-Villanueva M**, Gironella M, Castells A, Bujanda L. Molecular markers in pancreatic cancer diagnosis. *Clin Chim Acta* 2013; **418**: 22-29 [PMID: 23305796 DOI: 10.1016/j.cca.2012.12.025]
17 **Agrawal S**. Potential prognostic biomarkers in pancreatic juice of resectable pancreatic ductal adenocarcinoma. *World J Clin Oncol* 2017; **8**: 255-260 [PMID: 28638795 DOI: 10.5306/wjco.v8.i3.255]
18 **Ishige F**, Hoshino I, Iwatate Y, Chiba S, Arimitsu H, Yanagibashi H, Nagase H, Takayama W. MIR1246 in body fluids as a biomarker for pancreatic cancer. *Sci Rep* 2020; **10**: 8723 [PMID: 32457495 DOI: 10.1038/s41598-020-65695-6]
19 **Yang JY**, Sun YW, Liu DJ, Zhang JF, Li J, Hua R. MicroRNAs in stool samples as potential screening biomarkers for pancreatic ductal adenocarcinoma cancer. *Am J Cancer Res* 2014; **4**: 663-673 [PMID: 25520858]

20 **Jimenez-Luna C**, Torres C, Ortiz R, Dieguez C, Martinez-Galan J, Melguizo C, Prados JC, Caba O. Proteomic biomarkers in body fluids associated with pancreatic cancer. *Oncotarget* 2018; **9**: 16573-16587 [PMID: 29662668 DOI: 10.18632/oncotarget.24654]

21 **Pan S**, Chen R, Crispin DA, May D, Stevens T, McIntosh MW, Bronner MP, Ziogas A, Anton-Culver H, Brentnall TA. Protein alterations associated with pancreatic cancer and chronic pancreatitis found in human plasma using global quantitative proteomics profiling. *J Proteome Res* 2011; **10**: 2359-2376 [PMID: 21443201 DOI: 10.1021/pr101148r]

22 **Goonetilleke KS**, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. *Eur J Surg Oncol* 2007; **33**: 266-270 [PMID: 17097848 DOI: 10.1016/j.ejso.2006.10.004]

23 **Koprowski H**, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Colorectal carcinoma antigens detected by hybridoma antibodies. *Somatic Cell Genet* 1979; **5**: 957-971 [PMID: 94699 DOI: 10.1007/BF01542654]

24 **Ning S**, Wei W, Li J, Hou B, Zhong J, Xie Y, Liu H, Mo X, Chen J, Zhang L. Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 Levels in gastric and colorectal cancer patients. *J Cancer* 2018; **9**: 494-501 [PMID: 29483954 DOI: 10.7150/jca.21562]

25 **Nikolaou S**, Qiu S, Fiorentino F, Rasheed S, Tekkis P, Kontovounisios C. Systematic review of blood diagnostic markers in colorectal cancer. *Tech Coloproctol* 2018; **22**: 481-498 [PMID: 30022330 DOI: 10.1007/s10151-018-1820-3]

26 **Liang B**, Zhong L, He Q, Wang S, Pan Z, Wang T, Zhao Y. Diagnostic Accuracy of Serum CA19-9 in Patients with Cholangiocarcinoma: A Systematic Review and Meta-Analysis. *Med Sci Monit* 2015; **21**: 3555-3563 [PMID: 26576628 DOI: 10.12659/msm.895040]

27 **Ballehaninna UK**, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. *J Gastrointest Oncol* 2012; **3**: 105-119 [PMID: 22811878 DOI: 10.3978/j.issn.2078-6891.2011.021]

28 **Kriz D**, Ansari D, Andersson R. Potential biomarkers for early detection of pancreatic ductal adenocarcinoma. *Clin Transl Oncol* 2020; **22**: 2170-2174 [PMID: 32447642 DOI: 10.1007/s12094-020-02372-0]

29 **Binicier OB**, Pakoz ZB. CA 19-9 levels in patients with acute pancreatitis due to gallstone and metabolic/toxic reasons. *Rev Assoc Med Bras (1992)* 2019; **65**: 965-970 [PMID: 31389506 DOI: 10.1590/1806-9282.65.7.965]

30 **Doğan ÜB**, Gümürdülü Y, Gölge N, Kara B. Relationship of CA 19-9 with choledocholithiasis and cholangitis. *Turk J Gastroenterol* 2011; **22**: 171-177 [PMID: 21796554 DOI: 10.4318/tjg.2011.0186]

31 **Kannagi R**. Carbohydrate antigen sialyl Lewis a--its pathophysiological significance and induction mechanism in cancer progression. *Chang Gung Med J* 2007; **30**: 189-209 [PMID: 17760270]

32 **Fahrmann JF**, Bantis LE, Capello M, Scelo G, Dennison JB, Patel N, Murage E, Vykoukal J, Kundnani DL, Foretova L, Fabianova E, Holcatova I, Janout V, Feng Z, Yip-Schneider M, Zhang J, Brand R, Taguchi A, Maitra A, Brennan P, Max Schmidt C, Hanash S. A Plasma-Derived Protein-Metabolite Multiplexed Panel for Early-Stage Pancreatic Cancer. *J Natl Cancer Inst* 2019; **111**: 372-379 [PMID: 30137376 DOI: 10.1093/jnci/djy126]

33 **Satake K**, Takeuchi T, Homma T, Ozaki H. CA19-9 as a screening and diagnostic tool in symptomatic patients: the Japanese experience. *Pancreas* 1994; **9**: 703-706 [PMID: 7846012 DOI: 10.1097/00006676-199411000-00005]

34 **Kim JE**, Lee KT, Lee JK, Paik SW, Rhee JC, Choi KW. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. *J Gastroenterol Hepatol* 2004; **19**: 182-186 [PMID: 14731128 DOI: 10.1111/j.1440-1746.2004.03219.x]

35 **Chang CY**, Huang SP, Chiu HM, Lee YC, Chen MF, Lin JT. Low efficacy of serum levels of CA 19-9 in prediction of malignant diseases in asymptomatic population in Taiwan. *Hepatogastroenterology* 2006; **53**: 1-4 [PMID: 16506366]

36 **Xing H**, Wang J, Wang Y, Tong M, Hu H, Huang C, Li D. Diagnostic Value of CA 19-9 and Carcinoembryonic Antigen for Pancreatic Cancer: A Meta-Analysis. *Gastroenterol Res Pract* 2018; **2018**: 8704751 [PMID: 30584422 DOI: 10.1155/2018/8704751]

37 **Kaur S**, Smith LM, Patel A, Menning M, Watley DC, Malik SS, Krishn SR, Mallya K, Aithal A, Sasson AR, Johansson SL, Jain M, Singh S, Guha S, Are C, Raimondo M, Hollingsworth MA, Brand RE, Batra SK. A Combination of MUC5AC and CA19-9 Improves the Diagnosis of Pancreatic Cancer: A Multicenter Study. *Am J Gastroenterol* 2017; **112**: 172-183 [PMID: 27845339 DOI: 10.1038/ajg.2016.482]

38 **Liu J**, Gao J, Du Y, Li Z, Ren Y, Gu J, Wang X, Gong Y, Wang W, Kong X. Combination of plasma microRNAs with serum CA19-9 for early detection of pancreatic cancer. *Int J Cancer* 2012; **131**: 683-691 [PMID: 21913185 DOI: 10.1002/ijc.26422]

39 **Staal B**, Liu Y, Barnett D, Hsueh P, He Z, Gao C, Partyka K, Hurd MW, Singhi AD, Drake RR, Huang Y, Maitra A, Brand RE, Haab BB. The sTRA Plasma Biomarker: Blinded Validation of Improved Accuracy Over CA19-9 in Pancreatic Cancer Diagnosis. *Clin Cancer Res* 2019; **25**: 2745-2754 [PMID: 30617132 DOI: 10.1158/1078-0432.CCR-18-3310]

40 **Tang H**, Singh S, Partyka K, Kletter D, Hsueh P, Yadav J, Ensink E, Bern M, Hostetter G, Hartman D, Huang Y, Brand RE, Haab BB. Glycan motif profiling reveals plasma sialyl-lewis x elevations in pancreatic cancers that are negative for sialyl-lewis A. *Mol Cell Proteomics* 2015; **14**: 1323-1333 [PMID: 25733690 DOI: 10.1074/mcp.M114.047837]

41 **Barnett D**, Liu Y, Partyka K, Huang Y, Tang H, Hostetter G, Brand RE, Singhi AD, Drake RR, Haab BB. The CA19-9 and Sialyl-TRA Antigens Define Separate Subpopulations of Pancreatic Cancer Cells. *Sci Rep* 2017; **7**: 4020 [PMID: 28642461 DOI: 10.1038/s41598-017-04164-z]

42 **Hall C**, Clarke L, Pal A, Buchwald P, Eglinton T, Wakeman C, Frizelle F. A Review of the Role of Carcinoembryonic Antigen in Clinical Practice. *Ann Coloproctol* 2019; **35**: 294-305 [PMID: 31937069 DOI: 10.3393/ac.2019.11.13]

43 **Meng Q**, Shi S, Liang C, Liang D, Xu W, Ji S, Zhang B, Ni Q, Xu J, Yu X. Diagnostic and prognostic value of carcinoembryonic antigen in pancreatic cancer: a systematic review and meta-analysis. *Onco Targets Ther* 2017; **10**: 4591-4598 [PMID: 28979147 DOI: 10.2147/OTT.S145708]

44 **Jacobs I**. Screening for ovarian cancer by CA-125 measurement. *Lancet* 1988; **1**: 889 [PMID: 2895400 DOI: 10.1016/s0140-6736(88)91642-x]

45 **Giessen C**, Nagel D, Glas M, Spelsberg F, Lau-Werner U, Modest DP, Michl M, Heinemann V, Stieber P, Schulz C. Evaluation of preoperative serum markers for individual patient prognosis in stage I-III rectal cancer. *Tumour Biol* 2014; **35**: 10237-10248 [PMID: 25027407 DOI: 10.1007/s13277-014-2338-6]

46 **Wu WR**, Shi XD, Zhang R, Zhu MS, Xu LB, Yu XH, Zeng H, Wang J, Liu C. Clinicopathological significance of aberrant Notch receptors in intrahepatic cholangiocarcinoma. *Int J Clin Exp Pathol* 2014; **7**: 3272-3279 [PMID: 25031748]

47 **Luo G**, Xiao Z, Long J, Liu Z, Liu L, Liu C, Xu J, Ni Q, Yu X. CA125 is superior to CA19-9 in predicting the resectability of pancreatic cancer. *J Gastrointest Surg* 2013; **17**: 2092-2098 [PMID: 24146342 DOI: 10.1007/s11605-013-2389-9]

48 **Liu L**, Xu HX, Wang WQ, Wu CT, Xiang JF, Liu C, Long J, Xu J, Fu de L, Ni QX, Houchen CW, Postier RG, Li M, Yu XJ. Serum CA125 is a novel predictive marker for pancreatic cancer metastasis and correlates with the metastasis-associated burden. *Oncotarget* 2016; **7**: 5943-5956 [PMID: 26745601 DOI: 10.18632/oncotarget.6819]

49 **Haglund C**. Tumour marker antigen CA125 in pancreatic cancer: a comparison with CA19-9 and CEA. *Br J Cancer* 1986; **54**: 897-901 [PMID: 3467786 DOI: 10.1038/bjc.1986.259]

50 **Meng Q**, Shi S, Liang C, Xiang J, Liang D, Zhang B, Qin Y, Ji S, Xu W, Xu J, Ni Q, Yu X. Diagnostic Accuracy of a CA125-Based Biomarker Panel in Patients with Pancreatic Cancer: A Systematic Review and Meta-Analysis. *J Cancer* 2017; **8**: 3615-3622 [PMID: 29151947 DOI: 10.7150/jca.18901]

51 **Haglund C**, Lindgren J, Roberts PJ, Kuusela P, Nordling S. Tissue expression of the tumour associated antigen CA242 in benign and malignant pancreatic lesions. A comparison with CA 50 and CA 19-9. *Br J Cancer* 1989; **60**: 845-851 [PMID: 2557879 DOI: 10.1038/bjc.1989.377]

52 **Kuusela P**, Haglund C, Roberts PJ. Comparison of a new tumour marker CA 242 with CA 19-9, CA 50 and carcinoembryonic antigen (CEA) in digestive tract diseases. *Br J Cancer* 1991; **63**: 636-640 [PMID: 2021550 DOI: 10.1038/bjc.1991.146]

53 **Dong D**, Jia L, Zhang L, Ma N, Zhang A, Zhou Y, Ren L. Periostin and CA242 as potential diagnostic serum biomarkers complementing CA19.9 in detecting pancreatic cancer. *Cancer Sci* 2018; **109**: 2841-2851 [PMID: 29945294 DOI: 10.1111/cas.13712]

54 **Dou H**, Sun G, Zhang L. CA242 as a biomarker for pancreatic cancer and other diseases. *Prog Mol Biol Transl Sci* 2019; **162**: 229-239 [PMID: 30905452 DOI: 10.1016/bs.pmbts.2018.12.007]

55 **Zhang Y**, Yang J, Li H, Wu Y, Zhang H, Chen W. Tumor markers CA19-9, CA242 and CEA in the diagnosis of pancreatic cancer: a meta-analysis. *Int J Clin Exp Med* 2015; **8**: 11683-11691 [PMID: 26380005]

56 **Rossi MK**, Gnanamony M, Gondi CS. The 'SPARC' of life: Analysis of the role of osteonectin/SPARC in pancreatic cancer (Review). *Int J Oncol* 2016; **48**: 1765-1771 [PMID: 26983777 DOI: 10.3892/ijo.2016.3417]

57 **Papapanagiotou A**, Sgourakis G, Karkoulias K, Raptis D, Parkin E, Brotzakis P, Panchal S, Papavassiliou AG. Osteonectin as a screening marker for pancreatic cancer: A prospective study. *J Int Med Res* 2018; **46**: 2769-2779 [PMID: 29756486 DOI: 10.1177/0300060518772413]

58 **Koopmann J**, Fedarko NS, Jain A, Maitra A, Iacobuzio-Donahue C, Rahman A, Hruban RH, Yeo CJ, Goggins M. Evaluation of osteopontin as biomarker for pancreatic adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 487-491 [PMID: 15006928]

59 **Poruk KE**, Firpo MA, Scaife CL, Adler DG, Emerson LL, Boucher KM, Mulvihill SJ. Serum osteopontin and tissue inhibitor of metalloproteinase 1 as diagnostic and prognostic biomarkers for pancreatic adenocarcinoma. *Pancreas* 2013; **42**: 193-197 [PMID: 23407481 DOI: 10.1097/MPA.0b013e31825e354d]

60 **Li JJ**, Li HY, Gu F. Diagnostic significance of serum osteopontin level for pancreatic cancer: a meta-analysis. *Genet Test Mol Biomarkers* 2014; **18**: 580-586 [PMID: 24950303 DOI: 10.1089/gtmb.2014.0102]

61 **Rychlíková J**, Vecka M, Jáchymová M, Macášek J, Hrabák P, Zeman M, Vávrová L, Řoupal J, Krechler T, Ák A. Osteopontin as a discriminating marker for pancreatic cancer and chronic pancreatitis. *Cancer Biomark* 2016; **17**: 55-65 [PMID: 27314293 DOI: 10.3233/CBM-160617]

62 **Takasaki H**, Uchida E, Tempero MA, Burnett DA, Metzgar RS, Pour PM. Correlative study on expression of CA 19-9 and DU-PAN-2 in tumor tissue and in serum of pancreatic cancer patients. *Cancer Res* 1988; **48**: 1435-1438 [PMID: 3162196]

63 **Kawa S**, Oguchi H, Kobayashi T, Tokoo M, Furuta S, Kanai M, Homma T. Elevated serum levels of Dupan-2 in pancreatic cancer patients negative for Lewis blood group phenotype. *Br J Cancer* 1991;

64: 899-902 [PMID: 1931612 DOI: 10.1038/bjc.1991.422]

64 **Narimatsu H**, Iwasaki H, Nakayama F, Ikehara Y, Kudo T, Nishihara S, Sugano K, Okura H, Fujita S, Hirohashi S. Lewis and secretor gene dosages affect CA19-9 and DU-PAN-2 serum levels in normal individuals and colorectal cancer patients. *Cancer Res* 1998; **58**: 512-518 [PMID: 9458099]

65 **Chung YS**, Ho JJ, Kim YS, Tanaka H, Nakata B, Hiura A, Motoyoshi H, Satake K, Umeyama K. The detection of human pancreatic cancer-associated antigen in the serum of cancer patients. *Cancer* 1987; **60**: 1636-1643 [PMID: 3476183 DOI: 10.1002/1097-0142(19871001)60:7<1636::aid-cncr2820600736>3.0.co;2-c]

66 **Cooper EH**, Forbes MA, Taylor M. An evaluation of DUPAN-2 in pancreatic cancer and gastrointestinal disease. *Br J Cancer* 1990; **62**: 1004-1005 [PMID: 2257202 DOI: 10.1038/bjc.1990.426]

67 **Satake K**, Chung YS, Umeyama K, Takeuchi T, Kim YS. The possibility of diagnosing small pancreatic cancer (less than 4.0 cm) by measuring various serum tumor markers. A retrospective study. *Cancer* 1991; **68**: 149-152 [PMID: 2049735 DOI: 10.1002/1097-0142(19910701)68:1<149::aid-cncr2820680127>3.0.co;2-9]

68 **Saito S**, Taguchi K, Nishimura N, Watanabe A, Ogoshi K, Niwa M, Furukawa T, Takahashi M. Clinical usefulness of computer-assisted diagnosis using combination assay of tumor markers for pancreatic carcinoma. *Cancer* 1993; **72**: 381-388 [PMID: 8319169 DOI: 10.1002/1097-0142(19930715)72:2<381::aid-cncr2820720212>3.0.co;2-l]

69 **Satake K**, Takeuchi T. Comparison of CA19-9 with other tumor markers in the diagnosis of cancer of the pancreas. *Pancreas* 1994; **9**: 720-724 [PMID: 7846015 DOI: 10.1097/00006676-199411000-00008]

70 **Sunagawa Y**, Yamada S, Sato Y, Morimoto D, Sonohara F, Takami H, Inokawa Y, Hayashi M, Kanda M, Tanaka C, Kobayashi D, Nakayama G, Koike M, Fujiwara M, Fujii T, Kodera Y. Novel Prognostic Implications of DUPAN-2 in the Era of Initial Systemic Therapy for Pancreatic Cancer. *Ann Surg Oncol* 2020; **27**: 2081-2089 [PMID: 31673938 DOI: 10.1245/s10434-019-07981-w]

71 **Badea L**, Herlea V, Dima SO, Dumitrascu T, Popescu I. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. *Hepatogastroenterology* 2008; **55**: 2016-2027 [PMID: 19260470]

72 **Mitsunaga S**, Fujii S, Ishii G, Kinoshita T, Hasebe T, Aoyagi K, Sasaki H, Ochiai A. Nerve invasion distance is dependent on laminin gamma2 in tumors of pancreatic cancer. *Int J Cancer* 2010; **127**: 805-819 [PMID: 20013810 DOI: 10.1002/ijc.25104]

73 **Kosanam H**, Prassas I, Chrystoja CC, Soleas I, Chan A, Dimitromanolakis A, Blasutig IM, Rückert F, Gruetzmann R, Pilarsky C, Maekawa M, Brand R, Diamandis EP. Laminin, gamma 2 (LAMC2): a promising new putative pancreatic cancer biomarker identified by proteomic analysis of pancreatic adenocarcinoma tissues. *Mol Cell Proteomics* 2013; **12**: 2820-2832 [PMID: 23798558 DOI: 10.1074/mcp.M112.023507]

74 **Chan A**, Prassas I, Dimitromanolakis A, Brand RE, Serra S, Diamandis EP, Blasutig IM. Validation of biomarkers that complement CA19.9 in detecting early pancreatic cancer. *Clin Cancer Res* 2014; **20**: 5787-5795 [PMID: 25239611 DOI: 10.1158/1078-0432.CCR-14-0289]

75 **Yang C**, Liu Z, Zeng X, Wu Q, Liao X, Wang X, Han C, Yu T, Zhu G, Qin W, Peng T. Evaluation of the diagnostic ability of laminin gene family for pancreatic ductal adenocarcinoma. *Aging (Albany NY)* 2019; **11**: 3679-3703 [PMID: 31182680 DOI: 10.18632/aging.102007]

76 **Champsaur M**, Lanier LL. Effect of NKG2D ligand expression on host immune responses. *Immunol Rev* 2010; **235**: 267-285 [PMID: 20536569 DOI: 10.1111/j.0105-2896.2010.00893.x]

77 **Unni AM**, Bondar T, Medzhitov R. Intrinsic sensor of oncogenic transformation induces a signal for innate immunosurveillance. *Proc Natl Acad Sci USA* 2008; **105**: 1686-1691 [PMID: 18223157 DOI: 10.1073/pnas.0701675105]

78 **Chang YT**, Wu CC, Shyr YM, Chen TC, Hwang TL, Yeh TS, Chang KP, Liu HP, Liu YL, Tsai MH, Chang YS, Yu JS. Secretome-based identification of ULBP2 as a novel serum marker for pancreatic cancer detection. *PLoS One* 2011; **6**: e20029 [PMID: 21625447 DOI: 10.1371/journal.pone.0020029]

79 **Zhou YF**, Xu LX, Huang LY, Guo F, Zhang F, He XY, Yuan YZ, Yao WY. Combined detection of serum UL16-binding protein 2 and macrophage inhibitory cytokine-1 improves early diagnosis and prognostic prediction of pancreatic cancer. *Oncol Lett* 2014; **8**: 2096-2102 [PMID: 25295097 DOI: 10.3892/ol.2014.2429]

80 **Cui D**, Zhang Y, Lian J, Liu B, Liu F, Han L, Wang Y, Gao S. Value analysis of CA19-9, s-ULBP2 and Dkk1 in the diagnosis of pancreatic cancer. *Shiyong Yixue Zazhi* 2017; **33**: 4086-4089 [DOI: 10.3969/j.issn.1006-5725.2017.24.016]

81 **Kegasawa T**, Tatsumi T, Yoshioka T, Suda T, Ikezawa K, Nakabori T, Yamada R, Kodama T, Shigekawa M, Hikita H, Sakamori R, Takehara T. Soluble UL16-binding protein 2 is associated with a poor prognosis in pancreatic cancer patients. *Biochem Biophys Res Commun* 2019; **517**: 84-88 [PMID: 31303272 DOI: 10.1016/j.bbrc.2019.07.020]

82 **Chung HW**, Lim JB. Clinical significance of elevated serum soluble CD40 ligand levels as a diagnostic and prognostic tumor marker for pancreatic ductal adenocarcinoma. *J Transl Med* 2014; **12**: 102 [PMID: 24745825 DOI: 10.1186/1479-5876-12-102]

83 **Haupt H**, Baudner S. [Isolation and characterization of an unknown, leucine-rich 3.1-S-alpha2-glycoprotein from human serum (author's transl)]. *Hoppe Seylers Z Physiol Chem* 1977; **358**: 639-646 [PMID: 69600]

84 **Furukawa K**, Kawamoto K, Eguchi H, Tanemura M, Tanida T, Tomimaru Y, Akita H, Hama N, Wada H, Kobayashi S, Nonaka Y, Takamatsu S, Shinzaki S, Kumada T, Satomura S, Ito T, Serada S, Naka T, Mori M, Doki Y, Miyoshi E, Nagano H. Clinicopathological Significance of Leucine-Rich α2-Glycoprotein-1 in Sera of Patients With Pancreatic Cancer. *Pancreas* 2015; **44**: 93-98 [PMID: 25058884 DOI: 10.1097/MPA.0000000000000205]

85 **Brodeur SR**, Angelini F, Bacharier LB, Blom AM, Mizoguchi E, Fujiwara H, Plebani A, Notarangelo LD, Dahlback B, Tsitsikov E, Geha RS. C4b-binding protein (C4BP) activates B cells through the CD40 receptor. *Immunity* 2003; **18**: 837-848 [PMID: 12818164 DOI: 10.1016/s1074-7613(03)00149-3]

86 **Sogawa K**, Takano S, Iida F, Satoh M, Tsuchida S, Kawashima Y, Yoshitomi H, Sanda A, Kodera Y, Takizawa H, Mikata R, Ohtsuka M, Shimizu H, Miyazaki M, Yokosuka O, Nomura F. Identification of a novel serum biomarker for pancreatic cancer, C4b-binding protein α-chain (C4BPA) by quantitative proteomic analysis using tandem mass tags. *Br J Cancer* 2016; **115**: 949-956 [PMID: 27657339 DOI: 10.1038/bjc.2016.295]

87 **Wang W**, Eddy R, Condeelis J. The cofilin pathway in breast cancer invasion and metastasis. *Nat Rev Cancer* 2007; **7**: 429-440 [PMID: 17522712 DOI: 10.1038/nrc2148]

88 **Satoh M**, Takano S, Sogawa K, Noda K, Yoshitomi H, Ishibashi M, Mogushi K, Takizawa H, Otsuka M, Shimizu H, Miyazaki M, Nomura F. Immune-complex level of cofilin-1 in sera is associated with cancer progression and poor prognosis in pancreatic cancer. *Cancer Sci* 2017; **108**: 795-803 [PMID: 28161904 DOI: 10.1111/cas.13181]

89 **Peerschke EI**, Ghebrehiwet B. The contribution of gC1qR/p33 in infection and inflammation. *Immunobiology* 2007; **212**: 333-342 [PMID: 17544818 DOI: 10.1016/j.imbio.2006.11.011]

90 **Peerschke EI**, Ghebrehiwet B. cC1qR/CR and gC1qR/p33: observations in cancer. *Mol Immunol* 2014; **61**: 100-109 [PMID: 25044096 DOI: 10.1016/j.molimm.2014.06.011]

91 **Peerschke EI**, Brandwijk RJ, Dembitzer FR, Kinoshita Y, Ghebrehiwet B. Soluble gC1qR in Blood and Body Fluids: Examination in a Pancreatic Cancer Patient Cohort. *Int J Cancer Res Mol Mech* 2015; **1** [PMID: 26973884]

92 **Hedström J**, Haglund C, Haapiainen R, Stenman UH. Serum trypsinogen-2 and trypsin-2-alpha(1)-antitrypsin complex in malignant and benign digestive-tract diseases. Preferential elevation in patients with cholangiocarcinomas. *Int J Cancer* 1996; **66**: 326-331 [PMID: 8621252 DOI: 10.1002/(SICI)1097-0215(19960503)66:3<326::AID-IJC10>3.0.CO;2-9]

93 **Cao J**, Xia C, Cui T, Guo H, Li H, Ren Y, Wang S. Correlations between serum trypsinogen-2 and pancreatic cancer. *Hepatogastroenterology* 2015; **62**: 435-440 [PMID: 25916077]

94 **Osada H**, Tomida S, Yatabe Y, Tatematsu Y, Takeuchi T, Murakami H, Kondo Y, Sekido Y, Takahashi T. Roles of achaete-scute homologue 1 in DKK1 and E-cadherin repression and neuroendocrine differentiation in lung cancer. *Cancer Res* 2008; **68**: 1647-1655 [PMID: 18339843 DOI: 10.1158/0008-5472.CAN-07-5039]

95 **Voorzanger-Rousselot N**, Goehrig D, Journe F, Doriath V, Body JJ, Clézardin P, Garnero P. Increased Dickkopf-1 expression in breast cancer bone metastases. *Br J Cancer* 2007; **97**: 964-970 [PMID: 17876334 DOI: 10.1038/sj.bjc.6603959]

96 **Han SX**, Zhou X, Sui X, He CC, Cai MJ, Ma JL, Zhang YY, Zhou CY, Ma CX, Varela-Ramirez A, Zhu Q. Serum dickkopf-1 is a novel serological biomarker for the diagnosis and prognosis of pancreatic cancer. *Oncotarget* 2015; **6**: 19907-19917 [PMID: 26101916 DOI: 10.18632/oncotarget.4529]

97 **Igbinigie E**, Guo F, Jiang SW, Kelley C, Li J. Dkk1 involvement and its potential as a biomarker in pancreatic ductal adenocarcinoma. *Clin Chim Acta* 2019; **488**: 226-234 [PMID: 30452897 DOI: 10.1016/j.cca.2018.11.023]

98 **Wang X**, Zhang L, Li H, Sun W, Zhang H, Lai M. THBS2 is a Potential Prognostic Biomarker in Colorectal Cancer. *Sci Rep* 2016; **6**: 33366 [PMID: 27632935 DOI: 10.1038/srep33366]

99 **Kim J**, Bamlet WR, Oberg AL, Chaffee KG, Donahue G, Cao XJ, Chari S, Garcia BA, Petersen GM, Zaret KS. Detection of early pancreatic ductal adenocarcinoma with thrombospondin-2 and CA19-9 blood markers. *Sci Transl Med* 2017; **9** [PMID: 28701476 DOI: 10.1126/scitranslmed.aah5583]

100 **Peng HY**, Chang MC, Hu CM, Yang HI, Lee WH, Chang YT. Thrombospondin-2 is a Highly Specific Diagnostic Marker and is Associated with Prognosis in Pancreatic Cancer. *Ann Surg Oncol* 2019; **26**: 807-814 [PMID: 30569296 DOI: 10.1245/s10434-018-07109-6]

101 **Berger AW**, Schwerdel D, Reinacher-Schick A, Uhl W, Algül H, Friess H, Janssen KP, König A, Ghadimi M, Gallmeier E, Bartsch DK, Geissler M, Staib L, Tannapfel A, Kleger A, Beutel A, Schulte LA, Kornmann M, Ettrich TJ, Seufferlein T. A Blood-Based Multi Marker Assay Supports the Differential Diagnosis of Early-Stage Pancreatic Cancer. *Theranostics* 2019; **9**: 1280-1287 [PMID: 30867830 DOI: 10.7150/thno.29247]

102 **Le Large TYS**, Meijer LL, Paleckyte R, Boyd LNC, Kok B, Wurdinger T, Schelfhorst T, Piersma SR, Pham TV, van Grieken NCT, Zonderhuis BM, Daams F, van Laarhoven HWM, Bijlsma MF, Jimenez CR, Giovannetti E, Kazemier G. Combined Expression of Plasma Thrombospondin-2 and CA19-9 for Diagnosis of Pancreatic Cancer and Distal Cholangiocarcinoma: A Proteome Approach. *Oncologist* 2020; **25**: e634-e643 [PMID: 31943574 DOI: 10.1634/theoncologist.2019-0680]

103 **Jenkinson C**, Elliott VL, Evans A, Oldfield L, Jenkins RE, O'Brien DP, Apostolidou S, Gentry-Maharaj A, Fourkala EO, Jacobs IJ, Menon U, Cox T, Campbell F, Pereira SP, Tuveson DA, Park BK, Greenhalf W, Sutton R, Timms JF, Neoptolemos JP, Costello E. Decreased Serum

Thrombospondin-1 Levels in Pancreatic Cancer Patients Up to 24 Months Prior to Clinical Diagnosis: Association with Diabetes Mellitus. *Clin Cancer Res* 2016; **22**: 1734-1743 [PMID: 26573598 DOI: 10.1158/1078-0432.CCR-15-0879]

104 **Fritzsche FR**, Dahl E, Pahl S, Burkhardt M, Luo J, Mayordomo E, Gansukh T, Dankof A, Knuechel R, Denkert C, Winzer KJ, Dietel M, Kristiansen G. Prognostic relevance of AGR2 expression in breast cancer. *Clin Cancer Res* 2006; **12**: 1728-1734 [PMID: 16551856 DOI: 10.1158/1078-0432.CCR-05-2057]

105 **Chen YT**, Ho CL, Chen PK, Chen YL, Chang CF. Anterior gradient 2: a novel sensitive tumor marker for metastatic oral cancer. *Cancer Lett* 2013; **339**: 270-278 [PMID: 23834814 DOI: 10.1016/j.canlet.2013.06.025]

106 **Ramachandran V**, Arumugam T, Wang H, Logsdon CD. Anterior gradient 2 is expressed and secreted during the development of pancreatic cancer and promotes cancer cell survival. *Cancer Res* 2008; **68**: 7811-7818 [PMID: 18829536 DOI: 10.1158/0008-5472.CAN-08-1320]

107 **Dumartin L**, Whiteman HJ, Weeks ME, Hariharan D, Dmitrovic B, Iacobuzio-Donahue CA, Brentnall TA, Bronner MP, Feakins RM, Timms JF, Brennan C, Lemoine NR, Crnogorac-Jurcevic T. AGR2 is a novel surface antigen that promotes the dissemination of pancreatic cancer cells through regulation of cathepsins B and D. *Cancer Res* 2011; **71**: 7091-7102 [PMID: 21948970 DOI: 10.1158/0008-5472.CAN-11-1367]

108 **Makawita S**, Dimitromanolakis A, Soosaipillai A, Soleas I, Chan A, Gallinger S, Haun RS, Blasutig IM, Diamandis EP. Validation of four candidate pancreatic cancer serological biomarkers that improve the performance of CA19.9. *BMC Cancer* 2013; **13**: 404 [PMID: 24007603 DOI: 10.1186/1471-2407-13-404]

109 **Wu H**, Zheng Q, Rengabhashyam P, Zenilman ME. A Brief History of Pancreatic Reg: Implications as to its Clinical Importance. *Einstein Q J Biol Med* 2000; **17**: 178 [PMID: 21687811]

110 **Li Q**, Wang H, Zogopoulos G, Shao Q, Dong K, Lv F, Nwilati K, Gui XY, Cuggia A, Liu JL, Gao ZH. Reg proteins promote acinar-to-ductal metaplasia and act as novel diagnostic and prognostic markers in pancreatic ductal adenocarcinoma. *Oncotarget* 2016; **7**: 77838-77853 [PMID: 27788482 DOI: 10.18632/oncotarget.12834]

111 **Faca VM**, Song KS, Wang H, Zhang Q, Krasnoselsky AL, Newcomb LF, Plentz RR, Gurumurthy S, Redston MS, Pitteri SJ, Pereira-Faca SR, Ireton RC, Katayama H, Glukhova V, Phanstiel D, Brenner DE, Anderson MA, Misek D, Scholler N, Urban ND, Barnett MJ, Edelstein C, Goodman GE, Thornquist MD, McIntosh MW, DePinho RA, Bardeesy N, Hanash SM. A mouse to human search for plasma proteome changes associated with pancreatic tumor development. *PLoS Med* 2008; **5**: e123 [PMID: 18547137 DOI: 10.1371/journal.pmed.0050123]

112 **Oue N**, Mitani Y, Aung PP, Sakakura C, Takeshima Y, Kaneko M, Noguchi T, Nakayama H, Yasui W. Expression and localization of Reg IV in human neoplastic and non-neoplastic tissues: Reg IV expression is associated with intestinal and neuroendocrine differentiation in gastric adenocarcinoma. *J Pathol* 2005; **207**: 185-198 [PMID: 16086444 DOI: 10.1002/path.1827]

113 **Violette S**, Festor E, Pandrea-Vasile I, Mitchell V, Adida C, Dussaulx E, Lacorte JM, Chambaz J, Lacasa M, Lesuffleur T. Reg IV, a new member of the regenerating gene family, is overexpressed in colorectal carcinomas. *Int J Cancer* 2003; **103**: 185-193 [PMID: 12455032 DOI: 10.1002/ijc.10788]

114 **Takehara A**, Eguchi H, Ohigashi H, Ishikawa O, Kasugai T, Hosokawa M, Katagiri T, Nakamura Y, Nakagawa H. Novel tumor marker REG4 detected in serum of patients with resectable pancreatic cancer and feasibility for antibody therapy targeting REG4. *Cancer Sci* 2006; **97**: 1191-1197 [PMID: 16918991 DOI: 10.1111/j.1349-7006.2006.00297.x]

115 **Bishnupuri KS**, Luo Q, Murmu N, Houchen CW, Anant S, Dieckgraefe BK. Reg IV activates the epidermal growth factor receptor/Akt/AP-1 signaling pathway in colon adenocarcinomas. *Gastroenterology* 2006; **130**: 137-149 [PMID: 16401477 DOI: 10.1053/j.gastro.2005.10.001]

116 **Takayama R**, Nakagawa H, Sawaki A, Mizuno N, Kawai H, Tajika M, Yatabe Y, Matsuo K, Uehara R, Ono K, Nakamura Y, Yamao K. Serum tumor antigen REG4 as a diagnostic biomarker in pancreatic ductal adenocarcinoma. *J Gastroenterol* 2010; **45**: 52-59 [PMID: 19789838 DOI: 10.1007/s00535-009-0114-y]

117 **Saukkonen K**, Hagström J, Mustonen H, Lehtinen L, Carpen O, Andersson LC, Seppänen H, Haglund C. Prognostic and diagnostic value of REG4 serum and tissue expression in pancreatic ductal adenocarcinoma. *Tumour Biol* 2018; **40**: 1010428318761494 [PMID: 29542402 DOI: 10.1177/1010428318761494]

118 **Antonin W**, Wagner M, Riedel D, Brose N, Jahn R. Loss of the zymogen granule protein syncollin affects pancreatic protein synthesis and transport but not secretion. *Mol Cell Biol* 2002; **22**: 1545-1554 [PMID: 11839820 DOI: 10.1128/MCB.22.5.1545-1554.2002]

119 **Kalus I**, Hodel A, Koch A, Kleene R, Edwardson JM, Schrader M. Interaction of syncollin with GP-2, the major membrane protein of pancreatic zymogen granules, and association with lipid microdomains. *Biochem J* 2002; **362**: 433-442 [PMID: 11853552 DOI: 10.1042/0264-6021:3620433]

120 **Grønborg M**, Bunkenborg J, Kristiansen TZ, Jensen ON, Yeo CJ, Hruban RH, Maitra A, Goggins MG, Pandey A. Comprehensive proteomic analysis of human pancreatic juice. *J Proteome Res* 2004; **3**: 1042-1055 [PMID: 15473694 DOI: 10.1021/pr0499085]

121 **Saito H**, Papaconstantinou J, Sato H, Goldstein S. Regulation of a novel gene encoding a lysyl oxidase-related protein in cellular adhesion and senescence. *J Biol Chem* 1997; **272**: 8157-8160 [PMID: 9079631 DOI: 10.1074/jbc.272.13.8157]

122 **Cano A**, Santamaría PG, Moreno-Bueno G. LOXL2 in epithelial cell plasticity and tumor progression. *Future Oncol* 2012; **8**: 1095-1108 [PMID: 23030485 DOI: 10.2217/fon.12.105]

123 **Zhang X**, Huang J, You F, Li W, Zou Z. Prognostic and clinicopathological significance of LOXL2 in cancers: A systematic review and meta-analysis. *J Cell Physiol* 2019; **234**: 21369-21379 [PMID: 31032923 DOI: 10.1002/jcp.28746]

124 **Cao J**, Lou S, Ying M, Yang B. DJ-1 as a human oncogene and potential therapeutic target. *Biochem Pharmacol* 2015; **93**: 241-250 [PMID: 25498803 DOI: 10.1016/j.bcp.2014.11.012]

125 **Vasseur S**, Afzal S, Tomasini R, Guillaumond F, Tardivel-Lacombe J, Mak TW, Iovanna JL. Consequences of DJ-1 upregulation following p53 Loss and cell transformation. *Oncogene* 2012; **31**: 664-670 [PMID: 21725356 DOI: 10.1038/onc.2011.268]

126 **He XY**, Liu BY, Yao WY, Zhao XJ, Zheng Z, Li JF, Yu BQ, Yuan YZ. Serum DJ-1 as a diagnostic marker and prognostic factor for pancreatic cancer. *J Dig Dis* 2011; **12**: 131-137 [PMID: 21401899 DOI: 10.1111/j.1751-2980.2011.00488.x]

127 **Zhang Z**, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, Rosenzweig JM, Cameron B, Wang YY, Meng XY, Berchuck A, Van Haaften-Day C, Hacker NF, de Bruijn HW, van der Zee AG, Jacobs IJ, Fung ET, Chan DW. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 2004; **64**: 5882-5890 [PMID: 15313933 DOI: 10.1158/0008-5472.CAN-04-0746]

128 **Jacobsson B**. In situ localization of transthyretin-mRNA in the adult human liver, choroid plexus and pancreatic islets and in endocrine tumours of the pancreas and gut. *Histochemistry* 1989; **91**: 299-304 [PMID: 2659558 DOI: 10.1007/BF00493004]

129 **Ehmann M**, Felix K, Hartmann D, Schnölzer M, Nees M, Vorderwülbecke S, Bogumil R, Büchler MW, Friess H. Identification of potential markers for the detection of pancreatic cancer through comparative serum protein expression profiling. *Pancreas* 2007; **34**: 205-214 [PMID: 17312459 DOI: 10.1097/01.mpa.0000250128.57026.b2]

130 **Chen J**, Chen LJ, Xia YL, Zhou HC, Yang RB, Wu W, Lu Y, Hu LW, Zhao Y. Identification and verification of transthyretin as a potential biomarker for pancreatic ductal adenocarcinoma. *J Cancer Res Clin Oncol* 2013; **139**: 1117-1127 [PMID: 23546595 DOI: 10.1007/s00432-013-1422-4]

131 **Perry JK**, Kannan N, Grandison PM, Mitchell MD, Lobie PE. Are trefoil factors oncogenic? *Trends Endocrinol Metab* 2008; **19**: 74-81 [PMID: 18054496 DOI: 10.1016/j.tem.2007.10.003]

132 **Zhang CX**, Wu CT, Xiao L, Tang SH. The diagnostic and clinicopathological value of trefoil factor 3 in patients with gastric cancer: a systematic review and meta-analysis. *Biomarkers* 2021; **26**: 95-102 [PMID: 33401971 DOI: 10.1080/1354750X.2020.1871411]

133 **Taupin D**, Podolsky DK. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* 2003; **4**: 721-732 [PMID: 14506475 DOI: 10.1038/nrm1203]

134 **Jahan R**, Ganguly K, Smith LM, Atri P, Carmicheal J, Sheinin Y, Rachagani S, Natarajan G, Brand RE, Macha MA, Grandgenett PM, Kaur S, Batra SK. Trefoil factor(s) and CA19.9: A promising panel for early detection of pancreatic cancer. *EBioMedicine* 2019; **42**: 375-385 [PMID: 30956167 DOI: 10.1016/j.ebiom.2019.03.056]

135 **Weterman MA**, Ajubi N, van Dinter IM, Degen WG, van Muijen GN, Ruitter DJ, Bloemers HP. nmb, a novel gene, is expressed in low-metastatic human melanoma cell lines and xenografts. *Int J Cancer* 1995; **60**: 73-81 [PMID: 7814155 DOI: 10.1002/ijc.2910600111]

136 **Onaga M**, Ido A, Hasuike S, Uto H, Moriuchi A, Nagata K, Hori T, Hayash K, Tsubouchi H. Osteoactivin expressed during cirrhosis development in rats fed a choline-deficient, L-amino acid-defined diet, accelerates motility of hepatoma cells. *J Hepatol* 2003; **39**: 779-785 [PMID: 14568261 DOI: 10.1016/s0168-8278(03)00361-1]

137 **Rose AA**, Grosset AA, Dong Z, Russo C, Macdonald PA, Bertos NR, St-Pierre Y, Simantov R, Hallett M, Park M, Gaboury L, Siegel PM. Glycoprotein nonmetastatic B is an independent prognostic indicator of recurrence and a novel therapeutic target in breast cancer. *Clin Cancer Res* 2010; **16**: 2147-2156 [PMID: 20215530 DOI: 10.1158/1078-0432.CCR-09-1611]

138 **Torres C**, Perales S, Alejandre MJ, Iglesias J, Palomino RJ, Martin M, Caba O, Prados JC, Aránega A, Delgado JR, Irigoyen A, Ortuño FM, Rojas I, Linares A. Serum cytokine profile in patients with pancreatic cancer. *Pancreas* 2014; **43**: 1042-1049 [PMID: 24979617 DOI: 10.1097/MPA.0000000000000155]

139 **Cai CY**, Zhai LL, Wu Y, Tang ZG. Expression and clinical value of peroxiredoxin-1 in patients with pancreatic cancer. *Eur J Surg Oncol* 2015; **41**: 228-235 [PMID: 25434328 DOI: 10.1016/j.ejso.2014.11.037]

140 **Lwaleed BA**, Bass PS. Tissue factor pathway inhibitor: structure, biology and involvement in disease. *J Pathol* 2006; **208**: 327-339 [PMID: 16261634 DOI: 10.1002/path.1871]

141 **Balasenthil S**, Huang Y, Liu S, Marsh T, Chen J, Stass SA, KuKuruga D, Brand R, Chen N, Frazier ML, Jack Lee J, Srivastava S, Sen S, McNeill Killary A. A Plasma Biomarker Panel to Identify Surgically Resectable Early-Stage Pancreatic Cancer. *J Natl Cancer Inst* 2017; **109** [PMID: 28376184 DOI: 10.1093/jnci/djw341]

142 **Lee SJ**, Yoo HJ, Bae YS, Kim HJ, Lee ST. TIMP-1 inhibits apoptosis in breast carcinoma cells *via* a pathway involving pertussis toxin-sensitive G protein and c-Src. *Biochem Biophys Res Commun* 2003; **312**: 1196-1201 [PMID: 14652000 DOI: 10.1016/j.bbrc.2003.11.050]

143 **Hayakawa T**, Yamashita K, Tanzawa K, Uchijima E, Iwata K. Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells. A possible new growth factor in serum. *FEBS Lett* 1992; **298**: 29-32 [PMID: 1544418 DOI: 10.1016/0014-5793(92)80015-9]

144 **Joergensen MT**, Brünner N, De Muckadell OB. Comparison of circulating MMP-9, TIMP-1 and CA19-9 in the detection of pancreatic cancer. *Anticancer Res* 2010; **30**: 587-592 [PMID: 20332475]

145 **Mohan S**, Baylink DJ. IGF-binding proteins are multifunctional and act *via* IGF-dependent and -independent mechanisms. *J Endocrinol* 2002; **175**: 19-31 [PMID: 12379487 DOI: 10.1677/joe.0.1750019]

146 **Wolpin BM**, Michaud DS, Giovannucci EL, Schernhammer ES, Stampfer MJ, Manson JE, Cochrane BB, Rohan TE, Ma J, Pollak MN, Fuchs CS. Circulating insulin-like growth factor binding protein-1 and the risk of pancreatic cancer. *Cancer Res* 2007; **67**: 7923-7928 [PMID: 17699799 DOI: 10.1158/0008-5472.CAN-07-0373]

147 **Yoneyama T**, Ohtsuki S, Honda K, Kobayashi M, Iwasaki M, Uchida Y, Okusaka T, Nakamori S, Shimahara M, Ueno T, Tsuchida A, Sata N, Ioka T, Yasunami Y, Kosuge T, Kaneda T, Kato T, Yagihara K, Fujita S, Huang W, Yamada T, Tachikawa M, Terasaki T. Identification of IGFBP2 and IGFBP3 As Compensatory Biomarkers for CA19-9 in Early-Stage Pancreatic Cancer Using a Combination of Antibody-Based and LC-MS/MS-Based Proteomics. *PLoS One* 2016; **11**: e0161009 [PMID: 27579675 DOI: 10.1371/journal.pone.0161009]

148 **Kendrick ZW**, Firpo MA, Repko RC, Scaife CL, Adler DG, Boucher KM, Mulvihill SJ. Serum IGFBP2 and MSLN as diagnostic and prognostic biomarkers for pancreatic cancer. *HPB (Oxford)* 2014; **16**: 670-676 [PMID: 24308545 DOI: 10.1111/hpb.12199]

149 **Kim Y**, Kang M, Han D, Kim H, Lee K, Kim SW, Kim Y, Park T, Jang JY. Biomarker Development for Intraductal Papillary Mucinous Neoplasms Using Multiple Reaction Monitoring Mass Spectrometry. *J Proteome Res* 2016; **15**: 100-113 [PMID: 26561977 DOI: 10.1021/acs.jproteome.5b00553]

150 **Merle NS**, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement System Part II: Role in Immunity. *Front Immunol* 2015; **6**: 257 [PMID: 26074922 DOI: 10.3389/fimmu.2015.00257]

151 **Merle NS**, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement System Part I - Molecular Mechanisms of Activation and Regulation. *Front Immunol* 2015; **6**: 262 [PMID: 26082779 DOI: 10.3389/fimmu.2015.00262]

152 **Wingren C**, Sandström A, Segersvärd R, Carlsson A, Andersson R, Löhr M, Borrebaeck CA. Identification of serum biomarker signatures associated with pancreatic cancer. *Cancer Res* 2012; **72**: 2481-2490 [PMID: 22589272 DOI: 10.1158/0008-5472.CAN-11-2883]

153 **Johnston FM**, Tan MC, Tan BR Jr, Porembka MR, Brunt EM, Linehan DC, Simon PO Jr, Plambeck-Suess S, Eberlein TJ, Hellstrom KE, Hellstrom I, Hawkins WG, Goedegebuure P. Circulating mesothelin protein and cellular antimesothelin immunity in patients with pancreatic cancer. *Clin Cancer Res* 2009; **15**: 6511-6518 [PMID: 19843662 DOI: 10.1158/1078-0432.CCR-09-0565]

154 **Sharon E**, Zhang J, Hollevoet K, Steinberg SM, Pastan I, Onda M, Gaedcke J, Ghadimi BM, Ried T, Hassan R. Serum mesothelin and megakaryocyte potentiating factor in pancreatic and biliary cancers. *Clin Chem Lab Med* 2012; **50**: 721-725 [PMID: 22149739 DOI: 10.1515/CCLM.2011.816]

155 **Kuhlmann KF**, van Till JW, Boermeester MA, de Reuver PR, Tzvetanova ID, Offerhaus GJ, Ten Kate FJ, Busch OR, van Gulik TM, Gouma DJ, Crawford HC. Evaluation of matrix metalloproteinase 7 in plasma and pancreatic juice as a biomarker for pancreatic cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 886-891 [PMID: 17507610 DOI: 10.1158/1055-9965.EPI-06-0779]

156 **Park HD**, Kang ES, Kim JW, Lee KT, Lee KH, Park YS, Park JO, Lee J, Heo JS, Choi SH, Choi DW, Kim S, Lee JK, Lee SY. Serum CA19-9, cathepsin D, and matrix metalloproteinase-7 as a diagnostic panel for pancreatic ductal adenocarcinoma. *Proteomics* 2012; **12**: 3590-3597 [PMID: 23065739 DOI: 10.1002/pmic.201200101]

157 **Kahlert C**, Fiala M, Musso G, Halama N, Keim S, Mazzone M, Lasitschka F, Pecqueux M, Klupp F, Schmidt T, Rahbari N, Schölch S, Pilarsky C, Ulrich A, Schneider M, Weitz J, Koch M. Prognostic impact of a compartment-specific angiogenic marker profile in patients with pancreatic cancer. *Oncotarget* 2014; **5**: 12978-12989 [PMID: 25483099 DOI: 10.18632/oncotarget.2651]

158 **Tsuda E**, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, Higashio K. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochem Biophys Res Commun* 1997; **234**: 137-142 [PMID: 9168977 DOI: 10.1006/bbrc.1997.6603]

159 **Holen I**, Cross SS, Neville-Webbe HL, Cross NA, Balasubramanian SP, Croucher PI, Evans CA, Lippitt JM, Coleman RE, Eaton CL. Osteoprotegerin (OPG) expression by breast cancer cells *in vitro* and breast tumours in vivo--a role in tumour cell survival? *Breast Cancer Res Treat* 2005; **92**: 207-215 [PMID: 16155791 DOI: 10.1007/s10549-005-2419-8]

160 **Brand RE**, Nolen BM, Zeh HJ, Allen PJ, Eloubeidi MA, Goldberg M, Elton E, Arnoletti JP, Christein JD, Vickers SM, Langmead CJ, Landsittel DP, Whitcomb DC, Grizzle WE, Lokshin AE. Serum biomarker panels for the detection of pancreatic cancer. *Clin Cancer Res* 2011; **17**: 805-816 [PMID: 21325298 DOI: 10.1158/1078-0432.CCR-10-0248]

161 **Nolen BM**, Brand RE, Prosser D, Velikokhatnaya L, Allen PJ, Zeh HJ, Grizzle WE, Huang Y, Lomakin A, Lokshin AE. Prediagnostic serum biomarkers as early detection tools for pancreatic cancer in a large prospective cohort study. *PLoS One* 2014; **9**: e94928 [PMID: 24747429 DOI: 10.1371/journal.pone.0094928]

162 **Lee JH**, Welch DR. Suppression of metastasis in human breast carcinoma MDA-MB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. *Cancer Res* 1997; **57**: 2384-2387 [PMID:

9192814]
163 **Bhattacharya M**, Babwah AV. Kisspeptin: beyond the brain. *Endocrinology* 2015; **156**: 1218-1227 [PMID: 25590245 DOI: 10.1210/en.2014-1915]
164 **Ji K**, Ye L, Mason MD, Jiang WG. The Kiss-1/Kiss-1R complex as a negative regulator of cell motility and cancer metastasis (Review). *Int J Mol Med* 2013; **32**: 747-754 [PMID: 23969598 DOI: 10.3892/ijmm.2013.1472]
165 **Loosen SH**, Luedde M, Lurje G, Spehlmann M, Paffenholz P, Ulmer TF, Tacke F, Vucur M, Trautwein C, Neumann UP, Luedde T, Roderburg C. Serum Levels of Kisspeptin Are Elevated in Patients with Pancreatic Cancer. *Dis Markers* 2019; **2019**: 5603474 [PMID: 31772690 DOI: 10.1155/2019/5603474]
166 **Zhao W**, Ajani JA, Sushovan G, Ochi N, Hwang R, Hafley M, Johnson RL, Bresalier RS, Logsdon CD, Zhang Z, Song S. Galectin-3 Mediates Tumor Cell-Stroma Interactions by Activating Pancreatic Stellate Cells to Produce Cytokines via Integrin Signaling. *Gastroenterology* 2018; **154**: 1524-1537.e6 [PMID: 29274868 DOI: 10.1053/j.gastro.2017.12.014]
167 **Lee KM**, Nam K, Oh S, Lim J, Kim YP, Lee JW, Yu JH, Ahn SH, Kim SB, Noh DY, Lee T, Shin I. Extracellular matrix protein 1 regulates cell proliferation and trastuzumab resistance through activation of epidermal growth factor signaling. *Breast Cancer Res* 2014; **16**: 479 [PMID: 25499743 DOI: 10.1186/s13058-014-0479-6]
168 **Arfaoui-Toumi A**, Kria-Ben Mahmoud L, Ben Hmida M, Khalfallah MT, Regaya-Mzabi S, Bouraoui S. Implication of the Galectin-3 in colorectal cancer development (about 325 Tunisian patients). *Bull Cancer* 2010; **97**: E1-E8 [PMID: 20080461 DOI: 10.1684/bdc.2010.1032]
169 **Lin TW**, Chang HT, Chen CH, Lin SW, Hsu TL, Wong CH. Galectin-3 Binding Protein and Galectin-1 Interaction in Breast Cancer Cell Aggregation and Metastasis. *J Am Chem Soc* 2015; **137**: 9685-9693 [PMID: 26168351 DOI: 10.1021/jacs.5b04744]
170 **Xie L**, Ni WK, Chen XD, Xiao MB, Chen BY, He S, Lu CH, Li XY, Jiang F, Ni RZ. The expressions and clinical significances of tissue and serum galectin-3 in pancreatic carcinoma. *J Cancer Res Clin Oncol* 2012; **138**: 1035-1043 [PMID: 22367363 DOI: 10.1007/s00432-012-1178-2]
171 **Yi N**, Zhao X, Ji J, Xu M, Jiao Y, Qian T, Zhu S, Jiang F, Chen J, Xiao M. Serum galectin-3 as a biomarker for screening, early diagnosis, prognosis and therapeutic effect evaluation of pancreatic cancer. *J Cell Mol Med* 2020; **24**: 11583-11591 [PMID: 32886424 DOI: 10.1111/jcmm.15775]
172 **Reid CJ**, Harris A. Developmental expression of mucin genes in the human gastrointestinal system. *Gut* 1998; **42**: 220-226 [PMID: 9536947 DOI: 10.1136/gut.42.2.220]
173 **Wang S**, You L, Dai M, Zhao Y. Mucins in pancreatic cancer: A well-established but promising family for diagnosis, prognosis and therapy. *J Cell Mol Med* 2020; **24**: 10279-10289 [PMID: 32745356 DOI: 10.1111/jcmm.15684]
174 **Balagué C**, Audié JP, Porchet N, Real FX. In situ hybridization shows distinct patterns of mucin gene expression in normal, benign, and malignant pancreas tissues. *Gastroenterology* 1995; **109**: 953-964 [PMID: 7657125 DOI: 10.1016/0016-5085(95)90406-9]
175 **Balagué C**, Gambús G, Carrato C, Porchet N, Aubert JP, Kim YS, Real FX. Altered expression of MUC2, MUC4, and MUC5 mucin genes in pancreas tissues and cancer cell lines. *Gastroenterology* 1994; **106**: 1054-1061 [PMID: 8143972 DOI: 10.1016/0016-5085(94)90767-6]
176 **Gum JR Jr**, Crawley SC, Hicks JW, Szymkowski DE, Kim YS. MUC17, a novel membrane-tethered mucin. *Biochem Biophys Res Commun* 2002; **291**: 466-475 [PMID: 11855812 DOI: 10.1006/bbrc.2002.6475]
177 **Williams SJ**, McGuckin MA, Gotley DC, Eyre HJ, Sutherland GR, Antalis TM. Two novel mucin genes down-regulated in colorectal cancer identified by differential display. *Cancer Res* 1999; **59**: 4083-4089 [PMID: 10463611]
178 **Higuchi T**, Orita T, Nakanishi S, Katsuya K, Watanabe H, Yamasaki Y, Waga I, Nanayama T, Yamamoto Y, Munger W, Sun HW, Falk RJ, Jennette JC, Alcorta DA, Li H, Yamamoto T, Saito Y, Nakamura M. Molecular cloning, genomic structure, and expression analysis of MUC20, a novel mucin protein, up-regulated in injured kidney. *J Biol Chem* 2004; **279**: 1968-1979 [PMID: 14565953 DOI: 10.1074/jbc.M304558200]
179 **Itoh Y**, Kamata-Sakurai M, Denda-Nagai K, Nagai S, Tsuiji M, Ishii-Schrade K, Okada K, Goto A, Fukayama M, Irimura T. Identification and expression of human epiglycanin/MUC21: a novel transmembrane mucin. *Glycobiology* 2008; **18**: 74-83 [PMID: 17977904 DOI: 10.1093/glycob/cwm118]
180 **Wang S**, You L, Dai M, Zhao Y. Quantitative assessment of the diagnostic role of mucin family members in pancreatic cancer: a meta-analysis. *Ann Transl Med* 2021; **9**: 192 [PMID: 33708819 DOI: 10.21037/atm-20-5606]
181 **Kaur S**, Kumar S, Momi N, Sasson AR, Batra SK. Mucins in pancreatic cancer and its microenvironment. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 607-620 [PMID: 23856888 DOI: 10.1038/nrgastro.2013.120]
182 **Yue T**, Maupin KA, Fallon B, Li L, Partyka K, Anderson MA, Brenner DE, Kaul K, Zeh H, Moser AJ, Simeone DM, Feng Z, Brand RE, Haab BB. Enhanced discrimination of malignant from benign pancreatic disease by measuring the CA 19-9 antigen on specific protein carriers. *PLoS One* 2011; **6**: e29180 [PMID: 22220206 DOI: 10.1371/journal.pone.0029180]
183 **Gold DV**, Karanjawala Z, Modrak DE, Goldenberg DM, Hruban RH. PAM4-reactive MUC1 is a biomarker for early pancreatic adenocarcinoma. *Clin Cancer Res* 2007; **13**: 7380-7387 [PMID: 18094420 DOI: 10.1158/1078-0432.CCR-07-1488]

184 **Gold DV**, Lew K, Maliniak R, Hernandez M, Cardillo T. Characterization of monoclonal antibody PAM4 reactive with a pancreatic cancer mucin. *Int J Cancer* 1994; **57**: 204-210 [PMID: 7512537 DOI: 10.1002/ijc.2910570213]

185 **Gold DV**, Cardillo T, Goldenberg DM, Sharkey RM. Localization of pancreatic cancer with radiolabeled monoclonal antibody PAM4. *Crit Rev Oncol Hematol* 2001; **39**: 147-154 [PMID: 11418312 DOI: 10.1016/s1040-8428(01)00114-7]

186 **Shi C**, Merchant N, Newsome G, Goldenberg DM, Gold DV. Differentiation of pancreatic ductal adenocarcinoma from chronic pancreatitis by PAM4 immunohistochemistry. *Arch Pathol Lab Med* 2014; **138**: 220-228 [PMID: 24476519 DOI: 10.5858/arpa.2013-0056-OA]

187 **Gold DV**, Gaedcke J, Ghadimi BM, Goggins M, Hruban RH, Liu M, Newsome G, Goldenberg DM. PAM4 enzyme immunoassay alone and in combination with CA 19-9 for the detection of pancreatic adenocarcinoma. *Cancer* 2013; **119**: 522-528 [PMID: 22898932 DOI: 10.1002/cncr.27762]

188 **Cheng J**, Lv Z, Weng X, Ye S, Shen K, Li M, Qin Y, Hu C, Zhang C, Wu J, Zheng S. Hsp27 Acts as a Master Molecular Chaperone and Plays an Essential Role in Hepatocellular Carcinoma Progression. *Digestion* 2015; **92**: 192-202 [PMID: 26381739 DOI: 10.1159/000431254]

189 **Xu L**, Chen S, Bergan RC. MAPKAPK2 and HSP27 are downstream effectors of p38 MAP kinase-mediated matrix metalloproteinase type 2 activation and cell invasion in human prostate cancer. *Oncogene* 2006; **25**: 2987-2998 [PMID: 16407830 DOI: 10.1038/sj.onc.1209337]

190 **Melle C**, Ernst G, Escher N, Hartmann D, Schimmel B, Bleul A, Thieme H, Kaufmann R, Felix K, Friess HM, Settmacher U, Hommann M, Richter KK, Daffner W, Täubig H, Manger T, Claussen U, von Eggeling F. Protein profiling of microdissected pancreas carcinoma and identification of HSP27 as a potential serum marker. *Clin Chem* 2007; **53**: 629-635 [PMID: 17303689 DOI: 10.1373/clinchem.2006.079194]

191 **Liao WC**, Wu MS, Wang HP, Tien YW, Lin JT. Serum heat shock protein 27 is increased in chronic pancreatitis and pancreatic carcinoma. *Pancreas* 2009; **38**: 422-426 [PMID: 19214136 DOI: 10.1097/MPA.0b013e318198281d]

192 **Gansauge F**, Gansauge S, Parker N, Beger MI, Poch B, Link KH, Safi F, Beger HG. CAM 17.1--a new diagnostic marker in pancreatic cancer. *Br J Cancer* 1996; **74**: 1997-2002 [PMID: 8980403 DOI: 10.1038/bjc.1996.666]

193 **Parker N**, Makin CA, Ching CK, Eccleston D, Taylor OM, Milton JD, Rhodes JM. A new enzyme-linked lectin/mucin antibody sandwich assay (CAM 17.1/WGA) assessed in combination with CA 19-9 and peanut lectin binding assay for the diagnosis of pancreatic cancer. *Cancer* 1992; **70**: 1062-1068 [PMID: 1515982 DOI: 10.1002/1097-0142(19920901)70:5<1062::aid-cncr2820700509>3.0.co;2-p]

194 **Kamada Y**, Kinoshita N, Tsuchiya Y, Kobayashi K, Fujii H, Terao N, Kamihagi K, Koyama N, Yamada S, Daigo Y, Nakamura Y, Taniguchi N, Miyoshi E. Reevaluation of a lectin antibody ELISA kit for measuring fucosylated haptoglobin in various conditions. *Clin Chim Acta* 2013; **417**: 48-53 [PMID: 23262369 DOI: 10.1016/j.cca.2012.12.014]

195 **Miyoshi E**, Kamada Y. Application of glycoscience to the early detection of pancreatic cancer. *Cancer Sci* 2016; **107**: 1357-1362 [PMID: 27418030 DOI: 10.1111/cas.13011]

196 **Yokoi K**, Shih LC, Kobayashi R, Koomen J, Hawke D, Li D, Hamilton SR, Abbruzzese JL, Coombes KR, Fidler IJ. Serum amyloid A as a tumor marker in sera of nude mice with orthotopic human pancreatic cancer and in plasma of patients with pancreatic cancer. *Int J Oncol* 2005; **27**: 1361-1369 [PMID: 16211233]

197 **Bhagwat SV**, Lahdenranta J, Giordano R, Arap W, Pasqualini R, Shapiro LH. CD13/APN is activated by angiogenic signals and is essential for capillary tube formation. *Blood* 2001; **97**: 652-659 [PMID: 11157481 DOI: 10.1182/blood.v97.3.652]

198 **Kido A**, Krueger S, Haeckel C, Roessner A. Inhibitory effect of antisense aminopeptidase N (APN/CD13) cDNA transfection on the invasive potential of osteosarcoma cells. *Clin Exp Metastasis* 2003; **20**: 585-592 [PMID: 14669789 DOI: 10.1023/a:1027383729767]

199 **Curnis F**, Arrigoni G, Sacchi A, Fischetti L, Arap W, Pasqualini R, Corti A. Differential binding of drugs containing the NGR motif to CD13 isoforms in tumor vessels, epithelia, and myeloid cells. *Cancer Res* 2002; **62**: 867-874 [PMID: 11830545]

200 **Pang L**, Zhang N, Xia Y, Wang D, Wang G, Meng X. Serum APN/CD13 as a novel diagnostic and prognostic biomarker of pancreatic cancer. *Oncotarget* 2016; **7**: 77854-77864 [PMID: 27788483 DOI: 10.18632/oncotarget.12835]

201 **Vander Heiden MG**, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; **324**: 1029-1033 [PMID: 19460998 DOI: 10.1126/science.1160809]

202 **Zhu H**, Wu J, Zhang W, Luo H, Shen Z, Cheng H, Zhu X. PKM2 enhances chemosensitivity to cisplatin through interaction with the mTOR pathway in cervical cancer. *Sci Rep* 2016; **6**: 30788 [PMID: 27492148 DOI: 10.1038/srep30788]

203 **Novotný I**, Dítě P, Dastych M, Záková A, Trna J, Novotná H, Nechutová H. Tumor marker M2-pyruvate-kinase in differential diagnosis of chronic pancreatitis and pancreatic cancer. *Hepatogastroenterology* 2008; **55**: 1475-1477 [PMID: 18795715]

204 **Ventrucci M**, Cipolla A, Racchini C, Casadei R, Simoni P, Gullo L. Tumor M2-pyruvate kinase, a new metabolic marker for pancreatic cancer. *Dig Dis Sci* 2004; **49**: 1149-1155 [PMID: 15387337 DOI: 10.1023/b:ddas.0000037803.32013.aa]

205 **Terentes-Printzios D**, Vlachopoulos C, Vyssoulis G, Alexopoulos N, Aznaouridis K, Ioakeimidis

N, Pietri P, Dima I, Samentzas A, Stefanadis C. Lipids, apolipoproteins and their ratios in relation to arterial stiffness in never-treated hypertensives. *Eur Heart J* 2010; **31**: 102-103

206 **Honda K**, Kobayashi M, Okusaka T, Rinaudo JA, Huang Y, Marsh T, Sanada M, Sasajima Y, Nakamori S, Shimahara M, Ueno T, Tsuchida A, Sata N, Ioka T, Yasunami Y, Kosuge T, Miura N, Kamita M, Sakamoto T, Shoji H, Jung G, Srivastava S, Yamada T. Plasma biomarker for detection of early stage pancreatic cancer and risk factors for pancreatic malignancy using antibodies for apolipoprotein-AII isoforms. *Sci Rep* 2015; **5**: 15921 [PMID: 26549697 DOI: 10.1038/srep15921]

207 **Honda K**, Katzke VA, Hüsing A, Okaya S, Shoji H, Onidani K, Olsen A, Tjønneland A, Overvad K, Weiderpass E, Vineis P, Muller D, Tsilidis K, Palli D, Pala V, Tumino R, Naccarati A, Panico S, Aleksandrova K, Boeing H, Bueno-de-Mesquita HB, Peeters PH, Trichopoulou A, Lagiou P, Khaw KT, Wareham N, Travis RC, Merino S, Duell EJ, Rodríguez-Barranco M, Chirlaque MD, Barricarte A, Rebours V, Boutron-Ruault MC, Romana Mancini F, Brennan P, Scelo G, Manjer J, Sund M, Öhlund D, Canzian F, Kaaks R. CA19-9 and apolipoprotein-A2 isoforms as detection markers for pancreatic cancer: a prospective evaluation. *Int J Cancer* 2019; **144**: 1877-1887 [PMID: 30259989 DOI: 10.1002/ijc.31900]

208 **Sato Y**, Kobayashi T, Nishiumi S, Okada A, Fujita T, Sanuki T, Kobayashi M, Asahara M, Adachi M, Sakai A, Shiomi H, Masuda A, Yoshida M, Takeuchi K, Kodama Y, Kutsumi H, Nagashima K, Honda K. Prospective Study Using Plasma Apolipoprotein A2-Isoforms to Screen for High-Risk Status of Pancreatic Cancer. *Cancers (Basel)* 2020; **12** [PMID: 32937962 DOI: 10.3390/cancers12092625]

209 **Takano S**, Yoshitomi H, Togawa A, Sogawa K, Shida T, Kimura F, Shimizu H, Tomonaga T, Nomura F, Miyazaki M. Apolipoprotein C-1 maintains cell survival by preventing from apoptosis in pancreatic cancer cells. *Oncogene* 2008; **27**: 2810-2822 [PMID: 18037960 DOI: 10.1038/sj.onc.1210951]

210 **Xue A**, Chang JW, Chung L, Samra J, Hugh T, Gill A, Butturini G, Baxter RC, Smith RC. Serum apolipoprotein C-II is prognostic for survival after pancreatic resection for adenocarcinoma. *Br J Cancer* 2012; **107**: 1883-1891 [PMID: 23169340 DOI: 10.1038/bjc.2012.458]

211 **Chen J**, Chen LJ, Yang RB, Xia YL, Zhou HC, Wu W, Lu Y, Hu LW, Zhao Y. Expression and clinical significance of apolipoprotein E in pancreatic ductal adenocarcinoma. *Med Oncol* 2013; **30**: 583 [PMID: 23609192 DOI: 10.1007/s12032-013-0583-y]

212 **Yu KH**, Rustgi AK, Blair IA. Characterization of proteins in human pancreatic cancer serum using differential gel electrophoresis and tandem mass spectrometry. *J Proteome Res* 2005; **4**: 1742-1751 [PMID: 16212428 DOI: 10.1021/pr050174l]

213 **Liu X**, Zheng W, Wang W, Shen H, Liu L, Lou W, Wang X, Yang P. A new panel of pancreatic cancer biomarkers discovered using a mass spectrometry-based pipeline. *Br J Cancer* 2017; **117**: 1846-1854 [PMID: 29123261 DOI: 10.1038/bjc.2017.365]

214 **Yako YY**, Kruger D, Smith M, Brand M. Cytokines as Biomarkers of Pancreatic Ductal Adenocarcinoma: A Systematic Review. *PLoS One* 2016; **11**: e0154016 [PMID: 27170998 DOI: 10.1371/journal.pone.0154016]

215 **Zhao J**, Liang Y, Yin Q, Liu S, Wang Q, Tang Y, Cao C. Clinical and prognostic significance of serum transforming growth factor-beta1 levels in patients with pancreatic ductal adenocarcinoma. *Braz J Med Biol Res* 2016; **49** [PMID: 27464025 DOI: 10.1590/1414-431X20165485]

216 **Ai KX**, Lu LY, Huang XY, Chen W, Zhang HZ. Prognostic significance of S100A4 and vascular endothelial growth factor expression in pancreatic cancer. *World J Gastroenterol* 2008; **14**: 1931-1935 [PMID: 18350635 DOI: 10.3748/wjg.14.1931]

217 **Abdel-Razik A**, ElMahdy Y, Hanafy EE, Elhelaly R, Elzehery R, M Tawfik A, Eldars W. Insulin-Like Growth Factor-1 and Vascular Endothelial Growth Factor in Malignant and Benign Biliary Obstructions. *Am J Med Sci* 2016; **351**: 259-264 [PMID: 26992254 DOI: 10.1016/j.amjms.2015.12.013]

218 **Bhushan A**, Itoh N, Kato S, Thiery JP, Czernichow P, Bellusci S, Scharfmann R. Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* 2001; **128**: 5109-5117 [PMID: 11748146]

219 **Nomura S**, Yoshitomi H, Takano S, Shida T, Kobayashi S, Ohtsuka M, Kimura F, Shimizu H, Yoshidome H, Kato A, Miyazaki M. FGF10/FGFR2 signal induces cell migration and invasion in pancreatic cancer. *Br J Cancer* 2008; **99**: 305-313 [PMID: 18594526 DOI: 10.1038/sj.bjc.6604473]

220 **Shaw VE**, Lane B, Jenkinson C, Cox T, Greenhalf W, Halloran CM, Tang J, Sutton R, Neoptolemos JP, Costello E. Serum cytokine biomarker panels for discriminating pancreatic cancer from benign pancreatic disease. *Mol Cancer* 2014; **13**: 114 [PMID: 24884871 DOI: 10.1186/1476-4598-13-114]

221 **Jiang JT**, Wu CP, Deng HF, Lu MY, Wu J, Zhang HY, Sun WH, Ji M. Serum level of TSGF, CA242 and CA19-9 in pancreatic cancer. *World J Gastroenterol* 2004; **10**: 1675-1677 [PMID: 15162550 DOI: 10.3748/wjg.v10.i11.1675]

222 **Chen Y**, Gao SG, Chen JM, Wang GP, Wang ZF, Zhou B, Jin CH, Yang YT, Feng XS. Serum CA242, CA199, CA125, CEA, and TSGF are Biomarkers for the Efficacy and Prognosis of Cryoablation in Pancreatic Cancer Patients. *Cell Biochem Biophys* 2015; **71**: 1287-1291 [PMID: 25486903 DOI: 10.1007/s12013-014-0345-2]

223 **Bootcov MR**, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, Zhang HP, Donnellan M, Mahler S, Pryor K, Walsh BJ, Nicholson RC, Fairlie WD, Por SB, Robbins JM, Breit SN. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci USA* 1997; **94**: 11514-11519 [PMID: 9326641 DOI: 10.1073/pnas.94.21.11514]

224 **Fairlie WD**, Zhang HP, Wu WM, Pankhurst SL, Bauskin AR, Russell PK, Brown PK, Breit SN. The propeptide of the transforming growth factor-beta superfamily member, macrophage inhibitory cytokine-1 (MIC-1), is a multifunctional domain that can facilitate protein folding and secretion. *J Biol Chem* 2001; **276**: 16911-16918 [PMID: 11278594 DOI: 10.1074/jbc.M010000200]

225 **Skipworth RJ**, Deans DA, Tan BH, Sangster K, Paterson-Brown S, Brown DA, Hunter M, Breit SN, Ross JA, Fearon KC. Plasma MIC-1 correlates with systemic inflammation but is not an independent determinant of nutritional status or survival in oesophago-gastric cancer. *Br J Cancer* 2010; **102**: 665-672 [PMID: 20104227 DOI: 10.1038/sj.bjc.6605532]

226 **Kempf T**, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 2007; **50**: 1054-1060 [PMID: 17825714 DOI: 10.1016/j.jacc.2007.04.091]

227 **Yang Y**, Yan S, Tian H, Bao Y. Macrophage inhibitory cytokine-1 *vs* carbohydrate antigen 19-9 as a biomarker for diagnosis of pancreatic cancer: A PRISMA-compliant meta-analysis of diagnostic accuracy studies. *Medicine (Baltimore)* 2018; **97**: e9994 [PMID: 29489701 DOI: 10.1097/MD.0000000000009994]

228 **O'Neill RS**, Emmanuel S, Williams D, Stoita A. Macrophage inhibitory cytokine-1/growth differentiation factor-15 in premalignant and neoplastic tumours in a high-risk pancreatic cancer cohort. *World J Gastroenterol* 2020; **26**: 1660-1673 [PMID: 32327914 DOI: 10.3748/wjg.v26.i14.1660]

229 **Ren C**, Chen Y, Han C, Fu D, Chen H. Plasma interleukin-11 (IL-11) levels have diagnostic and prognostic roles in patients with pancreatic cancer. *Tumour Biol* 2014; **35**: 11467-11472 [PMID: 25123265 DOI: 10.1007/s13277-014-2459-y]

230 **Mroczko B**, Groblewska M, Gryko M, Kedra B, Szmitkowski M. Diagnostic usefulness of serum interleukin 6 (IL-6) and C-reactive protein (CRP) in the differentiation between pancreatic cancer and chronic pancreatitis. *J Clin Lab Anal* 2010; **24**: 256-261 [PMID: 20626020 DOI: 10.1002/jcla.20395]

231 **Okada S**, Okusaka T, Ishii H, Kyogoku A, Yoshimori M, Kajimura N, Yamaguchi K, Kakizoe T. Elevated serum interleukin-6 levels in patients with pancreatic cancer. *Jpn J Clin Oncol* 1998; **28**: 12-15 [PMID: 9491135 DOI: 10.1093/jjco/28.1.12]

232 **Schultz NA**, Christensen IJ, Werner J, Giese N, Jensen BV, Larsen O, Bjerregaard JK, Pfeiffer P, Calatayud D, Nielsen SE, Yilmaz MK, Holländer NH, Wøjdemann M, Bojesen SE, Nielsen KR, Johansen JS. Diagnostic and Prognostic Impact of Circulating YKL-40, IL-6, and CA 19.9 in Patients with Pancreatic Cancer. *PLoS One* 2013; **8**: e67059 [PMID: 23840582 DOI: 10.1371/journal.pone.0067059]

233 **Koca YS**, Bulbul M, Barut I. The Diagnostic Roles of Cytokines in Hepatobiliary Cancers. *Biomed Res Int* 2017; **2017**: 2979307 [PMID: 29410961 DOI: 10.1155/2017/2979307]

234 **Chen Y**, Shi M, Yu GZ, Qin XR, Jin G, Chen P, Zhu MH. Interleukin-8, a promising predictor for prognosis of pancreatic cancer. *World J Gastroenterol* 2012; **18**: 1123-1129 [PMID: 22416189 DOI: 10.3748/wjg.v18.i10.1123]

235 **Sakamoto H**, Kimura H, Sekijima M, Matsumoto K, Arao T, Chikugo T, Yamada Y, Kitano M, Ito A, Takeyama Y, Kudo M, Nishio K. Plasma concentrations of angiogenesis-related molecules in patients with pancreatic cancer. *Jpn J Clin Oncol* 2012; **42**: 105-112 [PMID: 22167663 DOI: 10.1093/jjco/hyr178]

236 **West NR**, Murray JI, Watson PH. Oncostatin-M promotes phenotypic changes associated with mesenchymal and stem cell-like differentiation in breast cancer. *Oncogene* 2014; **33**: 1485-1494 [PMID: 23584474 DOI: 10.1038/onc.2013.105]

237 **Queen MM**, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. *Cancer Res* 2005; **65**: 8896-8904 [PMID: 16204061 DOI: 10.1158/0008-5472.CAN-05-1734]

238 **Benson DD**, Meng X, Fullerton DA, Moore EE, Lee JH, Ao L, Silliman CC, Barnett CC Jr. Activation state of stromal inflammatory cells in murine metastatic pancreatic adenocarcinoma. *Am J Physiol Regul Integr Comp Physiol* 2012; **302**: R1067-R1075 [PMID: 22422663 DOI: 10.1152/ajpregu.00320.2011]

239 **Litman-Zawadzka A**, Łukaszewicz-Zając M, Gryko M, Kulczyńska-Przybik A, Mroczko B. Serum chemokine CXCL8 as a better biomarker for diagnosis and prediction of pancreatic cancer than its specific receptor CXCR2, C-reactive protein, and classic tumor markers CA 19-9 and CEA. *Pol Arch Intern Med* 2018; **128**: 524-531 [PMID: 30057378 DOI: 10.20452/pamw.4307]

240 **Błogowski W**, Deskur A, Budkowska M, Sałata D, Madej-Michniewicz A, Dąbkowski K, Dołęgowska B, Starzyńska T. Selected cytokines in patients with pancreatic cancer: a preliminary report. *PLoS One* 2014; **9**: e97613 [PMID: 24849506 DOI: 10.1371/journal.pone.0097613]

241 **Zhang P**, Zou M, Wen X, Gu F, Li J, Liu G, Dong J, Deng X, Gao J, Li X, Jia X, Dong Z, Chen L, Wang Y, Tian Y. Development of serum parameters panels for the early detection of pancreatic cancer. *Int J Cancer* 2014; **134**: 2646-2655 [PMID: 24615168 DOI: 10.1002/ijc.28584]

242 **Dima SO**, Tanase C, Albulescu R, Herlea V, Chivu-Economescu M, Purnichescu-Purtan R, Dumitrascu T, Duda DG, Popescu I. An exploratory study of inflammatory cytokines as prognostic biomarkers in patients with ductal pancreatic adenocarcinoma. *Pancreas* 2012; **41**: 1001-1007 [PMID: 22722257 DOI: 10.1097/MPA.0b013e3182546e13]

243 **Gabitass RF**, Annels NE, Stocken DD, Pandha HA, Middleton GW. Elevated myeloid-derived

suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. *Cancer Immunol Immunother* 2011; **60**: 1419-1430 [PMID: 21644036 DOI: 10.1007/s00262-011-1028-0]

244 **Miekus K**, Jarocha D, Trzyna E, Majka M. Role of I-TAC-binding receptors CXCR3 and CXCR7 in proliferation, activation of intracellular signaling pathways and migration of various tumor cell lines. *Folia Histochem Cytobiol* 2010; **48**: 104-111 [PMID: 20529825 DOI: 10.2478/v10042-008-0091-7]

245 **Yasuda A**, Sawai H, Takahashi H, Ochi N, Matsuo Y, Funahashi H, Sato M, Okada Y, Takeyama H, Manabe T. The stem cell factor/c-kit receptor pathway enhances proliferation and invasion of pancreatic cancer cells. *Mol Cancer* 2006; **5**: 46 [PMID: 17044945 DOI: 10.1186/1476-4598-5-46]

246 **Mroczko B**, Szmitkowski M, Wereszczyńska-Siemiatkowska U, Jurkowska G. Hematopoietic cytokines in the sera of patients with pancreatic cancer. *Clin Chem Lab Med* 2005; **43**: 146-150 [PMID: 15843207 DOI: 10.1515/CCLM.2005.024]

247 **Mroczko B**, Szmitkowski M, Wereszczynska-Siemiatkowska U, Jurkowska G. Stem cell factor and macrophage-colony stimulating factor in patients with pancreatic cancer. *Clin Chem Lab Med* 2004; **42**: 256-260 [PMID: 15080556 DOI: 10.1515/CCLM.2004.047]

248 **Mroczko B**, Szmitkowski M, Wereszczyńska-Siemiatkowska U, Okulczyk B, Kedra B. Pretreatment serum levels of hematopoietic cytokines in patients with colorectal adenomas and cancer. *Int J Colorectal Dis* 2007; **22**: 33-38 [PMID: 16520929 DOI: 10.1007/s00384-006-0099-4]

249 **Gutierrez-Ramos JC**, Lloyd C, Gonzalo JA. Eotaxin: from an eosinophilic chemokine to a major regulator of allergic reactions. *Immunol Today* 1999; **20**: 500-504 [PMID: 10529777 DOI: 10.1016/s0167-5699(99)01522-4]

250 **Zeh HJ**, Winikoff S, Landsittel DP, Gorelik E, Marrangoni AM, Velikokhatnaya L, Winans MT, Lee K, Moser A, Bartlett D, Lotze MT, Siegfried JM, Whitcomb D, Papacristou G, Slivka A, Bigbee WL, Lokshin AE. Multianalyte profiling of serum cytokines for detection of pancreatic cancer. *Cancer Biomark* 2005; **1**: 259-269 [PMID: 17192050 DOI: 10.3233/cbm-2005-1601]

251 **Gebauer F**, Wicklein D, Horst J, Sundermann P, Maar H, Streichert T, Tachezy M, Izbicki JR, Bockhorn M, Schumacher U. Carcinoembryonic antigen-related cell adhesion molecules (CEACAM) 1, 5 and 6 as biomarkers in pancreatic cancer. *PLoS One* 2014; **9**: e113023 [PMID: 25409014 DOI: 10.1371/journal.pone.0113023]

252 **Arabzadeh A**, Chan C, Nouvion AL, Breton V, Benlolo S, DeMarte L, Turbide C, Brodt P, Ferri L, Beauchemin N. Host-related carcinoembryonic antigen cell adhesion molecule 1 promotes metastasis of colorectal cancer. *Oncogene* 2013; **32**: 849-860 [PMID: 22469976 DOI: 10.1038/onc.2012.112]

253 **Simeone DM**, Ji B, Banerjee M, Arumugam T, Li D, Anderson MA, Bamberger AM, Greenson J, Brand RE, Ramachandran V, Logsdon CD. CEACAM1, a novel serum biomarker for pancreatic cancer. *Pancreas* 2007; **34**: 436-443 [PMID: 17446843 DOI: 10.1097/MPA.0b013e3180333ae3]

254 **Gong DY**, Fu HX, Peng Y, You YQ, Li ZP. [Diagnostic value of serum CEACAM1 in patients with pancreatic cancer]. *Nan Fang Yi Ke Da Xue Xue Bao* 2011; **31**: 164-166 [PMID: 21269981]

255 **Dankner M**, Gray-Owen SD, Huang YH, Blumberg RS, Beauchemin N. CEACAM1 as a multi-purpose target for cancer immunotherapy. *Oncoimmunology* 2017; **6**: e1328336 [PMID: 28811966 DOI: 10.1080/2162402X.2017.1328336]

256 **Chiang WF**, Cheng TM, Chang CC, Pan SH, Changou CA, Chang TH, Lee KH, Wu SY, Chen YF, Chuang KH, Shieh DB, Chen YL, Tu CC, Tsui WL, Wu MH. Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) promotes EGF receptor signaling of oral squamous cell carcinoma metastasis *via* the complex N-glycosylation. *Oncogene* 2018; **37**: 116-127 [PMID: 28892050 DOI: 10.1038/onc.2017.303]

257 **Duxbury MS**, Matros E, Clancy T, Bailey G, Doff M, Zinner MJ, Ashley SW, Maitra A, Redston M, Whang EE. CEACAM6 is a novel biomarker in pancreatic adenocarcinoma and PanIN lesions. *Ann Surg* 2005; **241**: 491-496 [PMID: 15729073 DOI: 10.1097/01.sla.0000154455.86404.e9]

258 **Pandey R**, Zhou M, Islam S, Chen B, Barker NK, Langlais P, Srivastava A, Luo M, Cooke LS, Weterings E, Mahadevan D. Carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6) in Pancreatic Ductal Adenocarcinoma (PDA): An integrative analysis of a novel therapeutic target. *Sci Rep* 2019; **9**: 18347 [PMID: 31797958 DOI: 10.1038/s41598-019-54545-9]

259 **Blumenthal RD**, Leon E, Hansen HJ, Goldenberg DM. Expression patterns of CEACAM5 and CEACAM6 in primary and metastatic cancers. *BMC Cancer* 2007; **7**: 2 [PMID: 17201906 DOI: 10.1186/1471-2407-7-2]

260 **Beauchemin N**, Arabzadeh A. Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) in cancer progression and metastasis. *Cancer Metastasis Rev* 2013; **32**: 643-671 [PMID: 23903773 DOI: 10.1007/s10555-013-9444-6]

261 **Roland CL**, Dineen SP, Toombs JE, Carbon JG, Smith CW, Brekken RA, Barnett CC Jr. Tumor-derived intercellular adhesion molecule-1 mediates tumor-associated leukocyte infiltration in orthotopic pancreatic xenografts. *Exp Biol Med (Maywood)* 2010; **235**: 263-270 [PMID: 20404043 DOI: 10.1258/ebm.2009.009215]

262 **mohamed A**, Saad Y, Saleh D, Elawady R, Eletreby R, Kharalla AS, Badr E. Can Serum ICAM 1 distinguish pancreatic cancer from chronic pancreatitis? *Asian Pac J Cancer Prev* 2016; **17**: 4671-4675 [PMID: 27892682 DOI: 10.22034/apjcp.2016.17.10.4671]

263 **Markocka-Maczka K**. [Concentration of serum soluble forms of ICAM-1 (sVCAM-1) and VCAM-1 (sVCAM-1) in patients with chronic pancreatitis and in patients with pancreatic carcinoma]. *Wiad Lek* 2003; **56**: 147-151 [PMID: 12923961]

264 **Cabili MN**, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev* 2011; **25**: 1915-1927 [PMID: 21890647 DOI: 10.1101/gad.17446611]

265 **McDonel P**, Guttman M. Approaches for Understanding the Mechanisms of Long Noncoding RNA Regulation of Gene Expression. *Cold Spring Harb Perspect Biol* 2019; **11** [PMID: 31791999 DOI: 10.1101/cshperspect.a032151]

266 **Chi Y**, Wang D, Wang J, Yu W, Yang J. Long Non-Coding RNA in the Pathogenesis of Cancers. *Cells* 2019; **8** [PMID: 31480503 DOI: 10.3390/cells8091015]

267 **Lv Y**, Huang S. Role of non-coding RNA in pancreatic cancer. *Oncol Lett* 2019; **18**: 3963-3973 [PMID: 31579086 DOI: 10.3892/ol.2019.10758]

268 **Xiong G**, Liu C, Yang G, Feng M, Xu J, Zhao F, You L, Zhou L, Zheng L, Hu Y, Wang X, Zhang T, Zhao Y. Long noncoding RNA GSTM3TV2 upregulates LAT2 and OLR1 by competitively sponging let-7 to promote gemcitabine resistance in pancreatic cancer. *J Hematol Oncol* 2019; **12**: 97 [PMID: 31514732 DOI: 10.1186/s13045-019-0777-7]

269 **Xiong G**, Feng M, Yang G, Zheng S, Song X, Cao Z, You L, Zheng L, Hu Y, Zhang T, Zhao Y. The underlying mechanisms of non-coding RNAs in the chemoresistance of pancreatic cancer. *Cancer Lett* 2017; **397**: 94-102 [PMID: 28254409 DOI: 10.1016/j.canlet.2017.02.020]

270 **Hu H**, Wang Y, Ding X, He Y, Lu Z, Wu P, Tian L, Yuan H, Liu D, Shi G, Xia T, Yin J, Cai B, Miao Y, Jiang K. Long non-coding RNA XLOC_000647 suppresses progression of pancreatic cancer and decreases epithelial-mesenchymal transition-induced cell invasion by down-regulating NLRP3. *Mol Cancer* 2018; **17**: 18 [PMID: 29386037 DOI: 10.1186/s12943-018-0761-9]

271 **Wang Y**, Zhou L, Lu J, Jiang B, Liu C, Guo J, Xiao GG. Research progress on long non-coding RNAs and their roles as potential biomarkers for diagnosis and prognosis in pancreatic cancer. *Cancer Cell Int* 2020; **20**: 457 [PMID: 32973402 DOI: 10.1186/s12935-020-01550-y]

272 **Yu S**, Li Y, Liao Z, Wang Z, Qian L, Zhao J, Zong H, Kang B, Zou WB, Chen K, He X, Meng Z, Chen Z, Huang S, Wang P. Plasma extracellular vesicle long RNA profiling identifies a diagnostic signature for the detection of pancreatic ductal adenocarcinoma. *Gut* 2020; **69**: 540-550 [PMID: 31562239 DOI: 10.1136/gutjnl-2019-318860]

273 **Baradaran B**, Shahbazi R, Khordadmehr M. Dysregulation of key microRNAs in pancreatic cancer development. *Biomed Pharmacother* 2019; **109**: 1008-1015 [PMID: 30551350 DOI: 10.1016/j.biopha.2018.10.177]

274 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Zen K, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

275 **Weber JA**, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. *Clin Chem* 2010; **56**: 1733-1741 [PMID: 20847327 DOI: 10.1373/clinchem.2010.147405]

276 **Sun X**, Zhou X, Zhang Y, Zhu X, Liu H. Systematic Review and Meta-Analysis of Diagnostic Accuracy of miRNAs in Patients with Pancreatic Cancer. *Dis Markers* 2018; **2018**: 6292396 [PMID: 29887920 DOI: 10.1155/2018/6292396]

277 **Li L**, Zhang GQ, Chen H, Zhao ZJ, Chen HZ, Liu H, Wang G, Jia YH, Pan SH, Kong R, Wang YW, Sun B. Plasma and tumor levels of Linc-pint are diagnostic and prognostic biomarkers for pancreatic cancer. *Oncotarget* 2016; **7**: 71773-71781 [PMID: 27708234 DOI: 10.18632/oncotarget.12365]

278 **Guo XB**, Yin HS, Wang JY. Evaluating the diagnostic and prognostic value of long non-coding RNA SNHG15 in pancreatic ductal adenocarcinoma. *Eur Rev Med Pharmacol Sci* 2018; **22**: 5892-5898 [PMID: 30280769 DOI: 10.26355/eurrev_201809_15917]

279 **Shuai Y**, Ma Z, Lu J, Feng J. LncRNA SNHG15: A new budding star in human cancers. *Cell Prolif* 2020; **53**: e12716 [PMID: 31774607 DOI: 10.1111/cpr.12716]

280 **Lu H**, Ye J, Zhang L, Li M, Lu S, Yang D, Hu W. Downregulation of LINC01638 lncRNA inhibits migration and invasion of pancreatic ductal adenocarcinoma cells by reducing TGFβ signaling. *Mol Med Rep* 2019; **20**: 4533-4539 [PMID: 31702018 DOI: 10.3892/mmr.2019.10699]

281 **Liu Y**, Feng W, Liu W, Kong X, Li L, He J, Wang D, Zhang M, Zhou G, Xu W, Chen W, Gong A, Xu M. Circulating lncRNA ABHD11-AS1 serves as a biomarker for early pancreatic cancer diagnosis. *J Cancer* 2019; **10**: 3746-3756 [PMID: 31333792 DOI: 10.7150/jca.32052]

282 **Takahashi K**, Ota Y, Kogure T, Suzuki Y, Iwamoto H, Yamakita K, Kitano Y, Fujii S, Haneda M, Patel T, Ota T. Circulating extracellular vesicle-encapsulated HULC is a potential biomarker for human pancreatic cancer. *Cancer Sci* 2020; **111**: 98-111 [PMID: 31715081 DOI: 10.1111/cas.14232]

283 **Ou ZL**, Luo Z, Lu YB. Long non-coding RNA HULC as a diagnostic and prognostic marker of pancreatic cancer. *World J Gastroenterol* 2019; **25**: 6728-6742 [PMID: 31857775 DOI: 10.3748/wjg.v25.i46.6728]

284 **Liu P**, Sun QQ, Liu TX, Lu K, Zhang N, Zhu Y, Chen M. Serum lncRNA-UFC1 as a potential biomarker for diagnosis and prognosis of pancreatic cancer. *Int J Clin Exp Pathol* 2019; **12**: 4125-4129 [PMID: 31933809]

285 **Wang J**, Chen J, Chang P, LeBlanc A, Li D, Abbruzzesse JL, Frazier ML, Killary AM, Sen S. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. *Cancer Prev Res (Phila)* 2009; **2**: 807-813 [PMID: 19723895 DOI:

10.1158/1940-6207.CAPR-09-0094]
286 **Alemar B**, Izetti P, Gregório C, Macedo GS, Castro MA, Osvaldt AB, Matte U, Ashton-Prolla P. miRNA-21 and miRNA-34a Are Potential Minimally Invasive Biomarkers for the Diagnosis of Pancreatic Ductal Adenocarcinoma. *Pancreas* 2016; **45**: 84-92 [PMID: 26262588 DOI: 10.1097/MPA.0000000000000383]
287 **Abue M**, Yokoyama M, Shibuya R, Tamai K, Yamaguchi K, Sato I, Tanaka N, Hamada S, Shimosegawa T, Sugamura K, Satoh K. Circulating miR-483-3p and miR-21 is highly expressed in plasma of pancreatic cancer. *Int J Oncol* 2015; **46**: 539-547 [PMID: 25384963 DOI: 10.3892/ijo.2014.2743]
288 **Yu Y**, Tong Y, Zhong A, Wang Y, Lu R, Guo L. Identification of Serum microRNA-25 as a novel biomarker for pancreatic cancer. *Medicine (Baltimore)* 2020; **99**: e23863 [PMID: 33350781 DOI: 10.1097/MD.0000000000023863]
289 **Guz M**, Jeleniewicz W, Cybulski M, Kozicka J, Kurzepa J, Mądro A. Serum miR-210-3p can be used to differentiate between patients with pancreatic ductal adenocarcinoma and chronic pancreatitis. *Biomed Rep* 2021; **14**: 10 [PMID: 33235725 DOI: 10.3892/br.2020.1386]
290 **Dai X**, Pang W, Zhou Y, Yao W, Xia L, Wang C, Chen X, Zen K, Zhang CY, Yuan Y. Altered profile of serum microRNAs in pancreatic cancer-associated new-onset diabetes mellitus. *J Diabetes* 2016; **8**: 422-433 [PMID: 25991015 DOI: 10.1111/1753-0407.12313]
291 **Ho AS**, Huang X, Cao H, Christman-Skieller C, Bennewith K, Le QT, Koong AC. Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. *Transl Oncol* 2010; **3**: 109-113 [PMID: 20360935 DOI: 10.1593/tlo.09256]
292 **Cote GA**, Gore AJ, McElyea SD, Heathers LE, Xu H, Sherman S, Korc M. A pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select miRNA in plasma and bile. *Am J Gastroenterol* 2014; **109**: 1942-1952 [PMID: 25350767 DOI: 10.1038/ajg.2014.331]
293 **Shi Q**, Feng K, Xia L, Wang C, Zhu J. Combined use of Serum miR-499a-5p and CA199 Increases the Diagnostic Sensitivity of Pancreatic Cancer. *Clin Lab* 2019; **65** [PMID: 31710444 DOI: 10.7754/Clin.Lab.2019.190416]
294 **Yan Q**, Hu D, Li M, Chen Y, Wu X, Ye Q, Wang Z, He L, Zhu J. The Serum MicroRNA Signatures for Pancreatic Cancer Detection and Operability Evaluation. *Front Bioeng Biotechnol* 2020; **8**: 379 [PMID: 32411694 DOI: 10.3389/fbioe.2020.00379]
295 **Liu R**, Chen X, Du Y, Yao W, Shen L, Wang C, Hu Z, Zhuang R, Ning G, Zhang C, Yuan Y, Li Z, Zen K, Ba Y, Zhang CY. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. *Clin Chem* 2012; **58**: 610-618 [PMID: 22194634 DOI: 10.1373/clinchem.2011.172767]
296 **Morimura R**, Komatsu S, Ichikawa D, Takeshita H, Tsujiura M, Nagata H, Konishi H, Shiozaki A, Ikoma H, Okamoto K, Ochiai T, Taniguchi H, Otsuji E. Novel diagnostic value of circulating miR-18a in plasma of patients with pancreatic cancer. *Br J Cancer* 2011; **105**: 1733-1740 [PMID: 22045190 DOI: 10.1038/bjc.2011.453]
297 **Johansen JS**, Calatayud D, Albieri V, Schultz NA, Dehlendorff C, Werner J, Jensen BV, Pfeiffer P, Bojesen SE, Giese N, Nielsen KR, Nielsen SE, Yilmaz M, Holländer NH, Andersen KK. The potential diagnostic value of serum microRNA signature in patients with pancreatic cancer. *Int J Cancer* 2016; **139**: 2312-2324 [PMID: 27464352 DOI: 10.1002/ijc.30291]
298 **Duell EJ**, Lujan-Barroso L, Sala N, Deitz McElyea S, Overvad K, Tjonneland A, Olsen A, Weiderpass E, Busund LT, Moi L, Muller D, Vineis P, Aune D, Matullo G, Naccarati A, Panico S, Tagliabue G, Tumino R, Palli D, Kaaks R, Katzke VA, Boeing H, Bueno-de-Mesquita HBA, Peeters PH, Trichopoulou A, Lagiou P, Kotanidou A, Travis RC, Wareham N, Khaw KT, Ramon Quiros J, Rodríguez-Barranco M, Dorronsoro M, Chirlaque MD, Ardanaz E, Severi G, Boutron-Ruault MC, Rebours V, Brennan P, Gunter M, Scelo G, Cote G, Sherman S, Korc M. Plasma microRNAs as biomarkers of pancreatic cancer risk in a prospective cohort study. *Int J Cancer* 2017; **141**: 905-915 [PMID: 28542740 DOI: 10.1002/ijc.30790]
299 **Ganepola GA**, Rutledge JR, Suman P, Yiengpruksawan A, Chang DH. Novel blood-based microRNA biomarker panel for early diagnosis of pancreatic cancer. *World J Gastrointest Oncol* 2014; **6**: 22-33 [PMID: 24578785 DOI: 10.4251/wjgo.v6.i1.22]
300 **Goto T**, Fujiya M, Konishi H, Sasajima J, Fujibayashi S, Hayashi A, Utsumi T, Sato H, Iwama T, Ijiri M, Sakatani A, Tanaka K, Nomura Y, Ueno N, Kashima S, Moriichi K, Mizukami Y, Kohgo Y, Okumura T. An elevated expression of serum exosomal microRNA-191, - 21, -451a of pancreatic neoplasm is considered to be efficient diagnostic marker. *BMC Cancer* 2018; **18**: 116 [PMID: 29385987 DOI: 10.1186/s12885-018-4006-5]
301 **Li A**, Yu J, Kim H, Wolfgang CL, Canto MI, Hruban RH, Goggins M. MicroRNA array analysis finds elevated serum miR-1290 accurately distinguishes patients with low-stage pancreatic cancer from healthy and disease controls. *Clin Cancer Res* 2013; **19**: 3600-3610 [PMID: 23697990 DOI: 10.1158/1078-0432.CCR-12-3092]
302 **Wei J**, Yang L, Wu YN, Xu J. Serum miR-1290 and miR-1246 as Potential Diagnostic Biomarkers of Human Pancreatic Cancer. *J Cancer* 2020; **11**: 1325-1333 [PMID: 32047539 DOI: 10.7150/jca.38048]
303 **Wang WS**, Liu LX, Li GP, Chen Y, Li CY, Jin DY, Wang XL. Combined serum CA19-9 and miR-27a-3p in peripheral blood mononuclear cells to diagnose pancreatic cancer. *Cancer Prev Res (Phila)* 2013; **6**: 331-338 [PMID: 23430754 DOI: 10.1158/1940-6207.CAPR-12-0307]

304 **Zhang J**, Zhao CY, Zhang SH, Yu DH, Chen Y, Liu QH, Shi M, Ni CR, Zhu MH. Upregulation of miR-194 contributes to tumor growth and progression in pancreatic ductal adenocarcinoma. *Oncol Rep* 2014; **31**: 1157-1164 [PMID: 24398877 DOI: 10.3892/or.2013.2960]

305 **Lin MS**, Chen WC, Huang JX, Gao HJ, Sheng HH. Aberrant expression of microRNAs in serum may identify individuals with pancreatic cancer. *Int J Clin Exp Med* 2014; **7**: 5226-5234 [PMID: 25664025]

306 **Komatsu S**, Ichikawa D, Miyamae M, Kawaguchi T, Morimura R, Hirajima S, Okajima W, Ohashi T, Imamura T, Konishi H, Shiozaki A, Ikoma H, Okamoto K, Taniguchi H, Otsuji E. Malignant potential in pancreatic neoplasm; new insights provided by circulating miR-223 in plasma. *Expert Opin Biol Ther* 2015; **15**: 773-785 [PMID: 25819175 DOI: 10.1517/14712598.2015.1029914]

307 **Kim K**, Yoo D, Lee HS, Lee KJ, Park SB, Kim C, Jo JH, Jung DE, Song SY. Identification of potential biomarkers for diagnosis of pancreatic and biliary tract cancers by sequencing of serum microRNAs. *BMC Med Genomics* 2019; **12**: 62 [PMID: 31096984 DOI: 10.1186/s12920-019-0521-8]

308 **Song Z**, Wang S, Liu Y. The diagnostic accuracy of liquid exosomes for lung cancer detection: a meta-analysis. *Onco Targets Ther* 2019; **12**: 181-192 [PMID: 30636881 DOI: 10.2147/OTT.S188832]

309 **Zhu Y**, Zhang H, Chen N, Hao J, Jin H, Ma X. Diagnostic value of various liquid biopsy methods for pancreatic cancer: A systematic review and meta-analysis. *Medicine (Baltimore)* 2020; **99**: e18581 [PMID: 32011436 DOI: 10.1097/MD.0000000000018581]

310 **Lai X**, Wang M, McElyea SD, Sherman S, House M, Korc M. A microRNA signature in circulating exosomes is superior to exosomal glypican-1 Levels for diagnosing pancreatic cancer. *Cancer Lett* 2017; **393**: 86-93 [PMID: 28232049 DOI: 10.1016/j.canlet.2017.02.019]

311 **Que R**, Ding G, Chen J, Cao L. Analysis of serum exosomal microRNAs and clinicopathologic features of patients with pancreatic adenocarcinoma. *World J Surg Oncol* 2013; **11**: 219 [PMID: 24007214 DOI: 10.1186/1477-7819-11-219]

312 **Madhavan B**, Yue S, Galli U, Rana S, Gross W, Müller M, Giese NA, Kalthoff H, Becker T, Büchler MW, Zöller M. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. *Int J Cancer* 2015; **136**: 2616-2627 [PMID: 25388097 DOI: 10.1002/ijc.29324]

313 **Melo SA**, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA, Weitz J, Rahbari N, Reissfelder C, Pilarsky C, Fraga MF, Piwnica-Worms D, Kalluri R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015; **523**: 177-182 [PMID: 26106858 DOI: 10.1038/nature14581]

314 **Castells A**, Puig P, Móra J, Boadas J, Boix L, Urgell E, Solé M, Capellà G, Lluís F, Fernández-Cruz L, Navarro S, Farré A. K-ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance. *J Clin Oncol* 1999; **17**: 578-584 [PMID: 10080602 DOI: 10.1200/JCO.1999.17.2.578]

315 **Yamada T**, Nakamori S, Ohzato H, Oshima S, Aoki T, Higaki N, Sugimoto K, Akagi K, Fujiwara Y, Nishisho I, Sakon M, Gotoh M, Monden M. Detection of K-ras gene mutations in plasma DNA of patients with pancreatic adenocarcinoma: correlation with clinicopathological features. *Clin Cancer Res* 1998; **4**: 1527-1532 [PMID: 9626473]

316 **Maire F**, Micard S, Hammel P, Voitot H, Lévy P, Cugnenc PH, Ruszniewski P, Puig PL. Differential diagnosis between chronic pancreatitis and pancreatic cancer: value of the detection of KRAS2 mutations in circulating DNA. *Br J Cancer* 2002; **87**: 551-554 [PMID: 12189555 DOI: 10.1038/sj.bjc.6600475]

317 **Sefrioui D**, Blanchard F, Toure E, Basile P, Beaussire L, Dolfus C, Perdrix A, Paresy M, Antonietti M, Iwanicki-Caron I, Alhameedi R, Lecleire S, Gangloff A, Schwarz L, Clatot F, Tuech JJ, Frébourg T, Jardin F, Sabourin JC, Sarafan-Vasseur N, Michel P, Di Fiore F. Diagnostic value of CA19.9, circulating tumour DNA and circulating tumour cells in patients with solid pancreatic tumours. *Br J Cancer* 2017; **117**: 1017-1025 [PMID: 28772284 DOI: 10.1038/bjc.2017.250]

318 **Eissa MAL**, Lerner L, Abdelfatah E, Shankar N, Canner JK, Hasan NM, Yaghoobi V, Huang B, Kerner Z, Takaesu F, Wolfgang C, Kwak R, Ruiz M, Tam M, Pisanic TR 2nd, Iacobuzio-Donahue CA, Hruban RH, He J, Wang TH, Wood LD, Sharma A, Ahuja N. Promoter methylation of ADAMTS1 and BNC1 as potential biomarkers for early detection of pancreatic cancer in blood. *Clin Epigenetics* 2019; **11**: 59 [PMID: 30953539 DOI: 10.1186/s13148-019-0650-0]

319 **Ankeny JS**, Court CM, Hou S, Li Q, Song M, Wu D, Chen JF, Lee T, Lin M, Sho S, Rochefort MM, Girgis MD, Yao J, Wainberg ZA, Muthusamy VR, Watson RR, Donahue TR, Hines OJ, Reber HA, Graeber TG, Tseng HR, Tomlinson JS. Circulating tumour cells as a biomarker for diagnosis and staging in pancreatic cancer. *Br J Cancer* 2016; **114**: 1367-1375 [PMID: 27300108 DOI: 10.1038/bjc.2016.121]

320 **Liu H**, Sun B, Wang S, Liu C, Lu Y, Li D, Liu X. Circulating Tumor Cells as a Biomarker in Pancreatic Ductal Adenocarcinoma. *Cell Physiol Biochem* 2017; **42**: 373-382 [PMID: 28558380 DOI: 10.1159/000477481]

321 **Rhim AD**, Thege FI, Santana SM, Lannin TB, Saha TN, Tsai S, Maggs LR, Kochman ML, Ginsberg GG, Lieb JG, Chandrasekhara V, Drebin JA, Ahmad N, Yang YX, Kirby BJ, Stanger BZ. Detection of circulating pancreas epithelial cells in patients with pancreatic cystic lesions. *Gastroenterology* 2014; **146**: 647-651 [PMID: 24333829 DOI: 10.1053/j.gastro.2013.12.007]

322 **Kulemann B**, Pitman MB, Liss AS, Valsangkar N, Fernández-Del Castillo C, Lillemoe KD,

Hoeppner J, Mino-Kenudson M, Warshaw AL, Thayer SP. Circulating tumor cells found in patients with localized and advanced pancreatic cancer. *Pancreas* 2015; **44**: 547-550 [PMID: 25822154 DOI: 10.1097/MPA.0000000000000324]

323 **Zhang Y**, Wang F, Ning N, Chen Q, Yang Z, Guo Y, Xu D, Zhang D, Zhan T, Cui W. Patterns of circulating tumor cells identified by CEP8, CK and CD45 in pancreatic cancer. *Int J Cancer* 2015; **136**: 1228-1233 [PMID: 25042121 DOI: 10.1002/ijc.29070]

324 **Xu Y**, Qin T, Li J, Wang X, Gao C, Xu C, Hao J, Liu J, Gao S, Ren H. Detection of Circulating Tumor Cells Using Negative Enrichment Immunofluorescence and an In Situ Hybridization System in Pancreatic Cancer. *Int J Mol Sci* 2017; **18**: 622 [PMID: 28333072 DOI: 10.3390/ijms18040622]

325 **Ashworth T**. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. *Aust Med J* 1869; **14**: 146

326 **Cheng H**, He W, Yang J, Ye Q, Cheng L, Pan Y, Mao L, Chu X, Lu C, Li G, Qiu Y, He J. Ligand-targeted polymerase chain reaction for the detection of folate receptor-positive circulating tumour cells as a potential diagnostic biomarker for pancreatic cancer. *Cell Prolif* 2020; **53**: e12880 [PMID: 32707596 DOI: 10.1111/cpr.12880]

327 **Nguyen NC**, Taalab K, Osman MM. Decreased blood flow with increased metabolic activity: a novel sign of pancreatic tumor aggressiveness. *Clin Cancer Res* 2010; **16**: 367; author reply 567 [PMID: 20028758 DOI: 10.1158/1078-0432.CCR-09-2512]

328 **Lo YM**, Chiu RW. Genomic analysis of fetal nucleic acids in maternal blood. *Annu Rev Genomics Hum Genet* 2012; **13**: 285-306 [PMID: 22657389 DOI: 10.1146/annurev-genom-090711-163806]

329 **Tsai NW**, Lin TK, Chen SD, Chang WN, Wang HC, Yang TM, Lin YJ, Jan CR, Huang CR, Liou CW, Lu CH. The value of serial plasma nuclear and mitochondrial DNA levels in patients with acute ischemic stroke. *Clin Chim Acta* 2011; **412**: 476-479 [PMID: 21130757 DOI: 10.1016/j.cca.2010.11.036]

330 **Bethel K**, Luttgen MS, Damani S, Kolatkar A, Lamy R, Sabouri-Ghomi M, Topol S, Topol EJ, Kuhn P. Fluid phase biopsy for detection and characterization of circulating endothelial cells in myocardial infarction. *Phys Biol* 2014; **11**: 016002 [PMID: 24406475 DOI: 10.1088/1478-3975/11/1/016002]

331 **Diaz LA Jr**, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol* 2014; **32**: 579-586 [PMID: 24449238 DOI: 10.1200/JCO.2012.45.2011]

332 **Okada T**, Mizukami Y, Ono Y, Sato H, Hayashi A, Kawabata H, Koizumi K, Masuda S, Teshima S, Takahashi K, Katanuma A, Omori Y, Iwano H, Yamada M, Yokochi T, Asahara S, Kawakubo K, Kuwatani M, Sakamoto N, Enomoto K, Goto T, Sasajima J, Fujiya M, Ueda J, Matsumoto S, Taniue K, Sugitani A, Karasaki H, Okumura T. Digital PCR-based plasma cell-free DNA mutation analysis for early-stage pancreatic tumor diagnosis and surveillance. *J Gastroenterol* 2020; **55**: 1183-1193 [PMID: 32939577 DOI: 10.1007/s00535-020-01724-5]

333 **Weeks ME**, Hariharan D, Petronijevic L, Radon TP, Whiteman HJ, Kocher HM, Timms JF, Lemoine NR, Crnogorac-Jurcevic T. Analysis of the urine proteome in patients with pancreatic ductal adenocarcinoma. *Proteomics Clin Appl* 2008; **2**: 1047-1057 [PMID: 21136905 DOI: 10.1002/prca.200780164]

334 **Radon TP**, Massat NJ, Jones R, Alrawashdeh W, Dumartin L, Ennis D, Duffy SW, Kocher HM, Pereira SP, Guarner posthumous L, Murta-Nascimento C, Real FX, Malats N, Neoptolemos J, Costello E, Greenhalf W, Lemoine NR, Crnogorac-Jurcevic T. Identification of a Three-Biomarker Panel in Urine for Early Detection of Pancreatic Adenocarcinoma. *Clin Cancer Res* 2015; **21**: 3512-3521 [PMID: 26240291 DOI: 10.1158/1078-0432.CCR-14-2467]

335 **Roy R**, Zurakowski D, Wischhusen J, Frauenhoffer C, Hooshmand S, Kulke M, Moses MA. Urinary TIMP-1 and MMP-2 levels detect the presence of pancreatic malignancies. *Br J Cancer* 2014; **111**: 1772-1779 [PMID: 25137018 DOI: 10.1038/bjc.2014.462]

336 **Hogendorf P**, Durczyński A, Skulimowski A, Kumor A, Poznańska G, Strzelczyk J. Neutrophil Gelatinase-Associated Lipocalin (NGAL) concentration in urine is superior to CA19-9 and Ca 125 in differentiation of pancreatic mass: Preliminary report. *Cancer Biomark* 2016; **16**: 537-543 [PMID: 27002756 DOI: 10.3233/CBM-160595]

337 **Cui Y**, Shu XO, Li HL, Yang G, Wen W, Gao YT, Cai Q, Rothman N, Yin HY, Lan Q, Xiang YB, Zheng W. Prospective study of urinary prostaglandin E2 metabolite and pancreatic cancer risk. *Int J Cancer* 2017; **141**: 2423-2429 [PMID: 28815606 DOI: 10.1002/ijc.31007]

338 **Chuang LY**, Hung WC, Yang ML, Chang CC, Tsai JF. Urinary epidermal growth factor receptor-binding growth factors in patients with cancers of the digestive tract. *Clin Biochem* 1994; **27**: 485-489 [PMID: 7697894 DOI: 10.1016/0009-9120(94)00053-x]

339 **Juracek J**, Peltanova B, Dolezel J, Fedorko M, Pacik D, Radova L, Vesela P, Svoboda M, Slaby O, Stanik M. Genome-wide identification of urinary cell-free microRNAs for non-invasive detection of bladder cancer. *J Cell Mol Med* 2018; **22**: 2033-2038 [PMID: 29363887 DOI: 10.1111/jcmm.13487]

340 **Debernardi S**, Massat NJ, Radon TP, Sangaralingam A, Banissi A, Ennis DP, Dowe T, Chelala C, Pereira SP, Kocher HM, Young BD, Bond-Smith G, Hutchins R, Crnogorac-Jurcevic T. Noninvasive urinary miRNA biomarkers for early detection of pancreatic adenocarcinoma. *Am J Cancer Res* 2015; **5**: 3455-3466 [PMID: 26807325]

341 **Terasawa H**, Kinugasa H, Ako S, Hirai M, Matsushita H, Uchida D, Tomoda T, Matsumoto K, Horiguchi S, Kato H, Nouso K, Okada H. Utility of liquid biopsy using urine in patients with pancreatic ductal adenocarcinoma. *Cancer Biol Ther* 2019; **20**: 1348-1353 [PMID: 31328611 DOI: 10.1080/15384047.2019.1638685]

342 **Yoshizawa N**, Sugimoto K, Tameda M, Inagaki Y, Ikejiri M, Inoue H, Usui M, Ito M, Takei Y. miR-3940-5p/miR-8069 ratio in urine exosomes is a novel diagnostic biomarker for pancreatic ductal adenocarcinoma. *Oncol Lett* 2020; **19**: 2677-2684 [PMID: 32218818 DOI: 10.3892/ol.2020.11357]

343 **Nissinen SI**, Roine A, Hokkinen L, Karjalainen M, Venäläinen M, Helminen H, Niemi R, Lehtimäki T, Rantanen T, Oksala N. Detection of Pancreatic Cancer by Urine Volatile Organic Compound Analysis. *Anticancer Res* 2019; **39**: 73-79 [PMID: 30591442 DOI: 10.21873/anticanres.13081]

344 **Schilling K**, Larner F, Saad A, Roberts R, Kocher HM, Blyuss O, Halliday AN, Crnogorac-Jurcevic T. Urine metallomics signature as an indicator of pancreatic cancer. *Metallomics* 2020; **12**: 752-757 [PMID: 32211672 DOI: 10.1039/d0mt00061b]

345 **Nishida K**, Tasaki N, Miyagawa H, Yoshikawa T, Kondo M. Estimation of carbohydrate antigen (CA) 19-9 levels in pure pancreatic juice of patients with pancreatic cancer. *Am J Gastroenterol* 1988; **83**: 126-129 [PMID: 3422536]

346 **Wakabayashi T**, Sawabu N, Takemori Y, Satomura Y, Kidani H, Ohta H, Watanabe H, Yamakawa O, Takahashi H, Watanabe K. Diagnostic significance of cancer-associated carbohydrate antigen (CA19-9) concentrations in pancreatic juice: analysis in pure pancreatic juice collected by endoscopic aspiration and immunohistochemical study in chronic pancreatitis. *Pancreas* 1993; **8**: 151-159 [PMID: 8460089 DOI: 10.1097/00006676-199303000-00003]

347 **Matsumoto S**, Harada H, Tanaka J, Ochi K, Seno T, Tsurumi T, Kunichika K. Evaluation of cytology and tumor markers of pure pancreatic juice for the diagnosis of pancreatic cancer at early stages. *Pancreas* 1994; **9**: 741-747 [PMID: 7846018 DOI: 10.1097/00006676-199411000-00012]

348 **Futakawa N**, Kimura W, Yamagata S, Zhao B, Ilsoo H, Inoue T, Sata N, Kawaguchi Y, Kubota Y, Muto T. Significance of K-ras mutation and CEA level in pancreatic juice in the diagnosis of pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2000; **7**: 63-71 [PMID: 10982594 DOI: 10.1007/s005340050156]

349 **Kaur S**, Baine MJ, Guha S, Ochi N, Chakraborty S, Mallya K, Thomas C, Crook J, Wallace MB, Woodward TA, Jain M, Singh S, Sasson AR, Skinner V, Raimondo M, Batra SK. Neutrophil gelatinase-associated lipocalin, macrophage inhibitory cytokine 1, and carbohydrate antigen 19-9 in pancreatic juice: pathobiologic implications in diagnosing benign and malignant disease of the pancreas. *Pancreas* 2013; **42**: 494-501 [PMID: 23146921 DOI: 10.1097/MPA.0b013e31826a8597]

350 **Sharma MP**, Gregg JA, Loewenstein MS, McCabe RP, Zamcheck N. Carcinoembryonic antigen (CEA) activity in pancreatic juice of patients with pancreatic carcinoma and pancreatitis. *Cancer* 1976; **38**: 2457-2461 [PMID: 1000475 DOI: 10.1002/1097-0142(197612)38:6<2457::aid-cncr2820380634>3.0.co;2-3]

351 **Nakaizumi A**, Uehara H, Takenaka A, Uedo N, Sakai N, Yano H, Ohigashi H, Ishikawa O, Ishiguro S, Sugano K, Tatsuta M. Diagnosis of pancreatic cancer by cytology and measurement of oncogene and tumor markers in pure pancreatic juice aspirated by endoscopy. *Hepatogastroenterology* 1999; **46**: 31-37 [PMID: 10228761]

352 **Hayakawa H**, Fukasawa M, Sato T, Takano S, Kadokura M, Shindo H, Takahashi E, Hirose S, Kawakami S, Fukasawa Y, Maekawa S, Inoue T, Yamaguchi T, Nakayama Y, Kawaida H, Kono H, Mochizuki K, Kondo T, Ichikawa D, Enomoto N. Carcinoembryonic antigen level in the pancreatic juice is effective in malignancy diagnosis and prediction of future malignant transformation of intraductal papillary mucinous neoplasm of the pancreas. *J Gastroenterol* 2019; **54**: 1029-1037 [PMID: 31111221 DOI: 10.1007/s00535-019-01592-8]

353 **Porterfield M**, Zhao P, Han H, Cunningham J, Aoki K, Von Hoff DD, Demeure MJ, Pierce JM, Tiemeyer M, Wells L. Discrimination between adenocarcinoma and normal pancreatic ductal fluid by proteomic and glycomic analysis. *J Proteome Res* 2014; **13**: 395-407 [PMID: 24328148 DOI: 10.1021/pr400422g]

354 **Matsumoto K**, Takeda Y, Harada K, Onoyama T, Kawata S, Horie Y, Sakamoto T, Ueki M, Miura N, Murawaki Y. Clinical Impact of the KL-6 Concentration of Pancreatic Juice for Diagnosing Pancreatic Masses. *Biomed Res Int* 2015; **2015**: 528304 [PMID: 26451373 DOI: 10.1155/2015/528304]

355 **Marchegiani G**, Paulo JA, Sahora K, Fernández-Del Castillo C. The proteome of postsurgical pancreatic juice. *Pancreas* 2015; **44**: 574-582 [PMID: 25875796 DOI: 10.1097/MPA.0000000000000304]

356 **Matsunaga T**, Ohtsuka T, Asano K, Kimura H, Ohuchida K, Kitada H, Ideno N, Mori Y, Tokunaga S, Oda Y, Guha S, Raimondo M, Nakamura M, Tanaka M. S100P in Duodenal Fluid Is a Useful Diagnostic Marker for Pancreatic Ductal Adenocarcinoma. *Pancreas* 2017; **46**: 1288-1295 [PMID: 28984789 DOI: 10.1097/MPA.0000000000000940]

357 **Tian M**, Cui YZ, Song GH, Zong MJ, Zhou XY, Chen Y, Han JX. Proteomic analysis identifies MMP-9, DJ-1 and A1BG as overexpressed proteins in pancreatic juice from pancreatic ductal adenocarcinoma patients. *BMC Cancer* 2008; **8**: 241 [PMID: 18706098 DOI: 10.1186/1471-2407-8-241]

358 **Rosty C**, Christa L, Kuzdzal S, Baldwin WM, Zahurak ML, Carnot F, Chan DW, Canto M, Lillemoe KD, Cameron JL, Yeo CJ, Hruban RH, Goggins M. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. *Cancer Res* 2002; **62**: 1868-1875 [PMID: 11912167]

359 **Chen R**, Pan S, Duan X, Nelson BH, Sahota RA, de Rham S, Kozarek RA, McIntosh M, Brentnall

TA. Elevated level of anterior gradient-2 in pancreatic juice from patients with pre-malignant pancreatic neoplasia. *Mol Cancer* 2010; **9**: 149 [PMID: 20550709 DOI: 10.1186/1476-4598-9-149]

360 **Noh KW**, Pungpapong S, Wallace MB, Woodward TA, Raimondo M. Do cytokine concentrations in pancreatic juice predict the presence of pancreatic diseases? *Clin Gastroenterol Hepatol* 2006; **4**: 782-789 [PMID: 16713745 DOI: 10.1016/j.cgh.2006.03.026]

361 **Uno K**, Azuma T, Nakajima M, Yasuda K, Hayakumo T, Mukai H, Sakai T, Kawai K. Clinical significance of cathepsin E in pancreatic juice in the diagnosis of pancreatic ductal adenocarcinoma. *J Gastroenterol Hepatol* 2000; **15**: 1333-1338 [PMID: 11129230]

362 **Sadakari Y**, Ohtsuka T, Ohuchida K, Tsutsumi K, Takahata S, Nakamura M, Mizumoto K, Tanaka M. MicroRNA expression analyses in preoperative pancreatic juice samples of pancreatic ductal adenocarcinoma. *JOP* 2010; **11**: 587-592 [PMID: 21068491]

363 **Wang J**, Raimondo M, Guha S, Chen J, Diao L, Dong X, Wallace MB, Killary AM, Frazier ML, Woodward TA, Wang J, Sen S. Circulating microRNAs in Pancreatic Juice as Candidate Biomarkers of Pancreatic Cancer. *J Cancer* 2014; **5**: 696-705 [PMID: 25258651 DOI: 10.7150/jca.10094]

364 **Watanabe H**, Okada G, Ohtsubo K, Yamaguchi Y, Mouri H, Motoo Y, Wakabayashi T, Sawabu N. Expression of mesothelin mRNA in pure pancreatic juice from patients with pancreatic carcinoma, intraductal papillary mucinous neoplasm of the pancreas, and chronic pancreatitis. *Pancreas* 2005; **30**: 349-354 [PMID: 15841046 DOI: 10.1097/01.mpa.0000160281.56828.76]

365 **Suehara N**, Mizumoto K, Tanaka M, Niiyama H, Yokohata K, Tominaga Y, Shimura H, Muta T, Hamasaki N. Telomerase activity in pancreatic juice differentiates ductal carcinoma from adenoma and pancreatitis. *Clin Cancer Res* 1997; **3**: 2479-2483 [PMID: 9815650]

366 **Myung SJ**, Kim MH, Kim YS, Kim HJ, Park ET, Yoo KS, Lim BC, Wan Seo D, Lee SK, Min YI, Kim JY. Telomerase activity in pure pancreatic juice for the diagnosis of pancreatic cancer may be complementary to K-ras mutation. *Gastrointest Endosc* 2000; **51**: 708-713 [PMID: 10840305 DOI: 10.1067/mge.2000.104654]

367 **Hata T**, Ishida M, Motoi F, Yamaguchi T, Naitoh T, Katayose Y, Egawa S, Unno M. Telomerase activity in pancreatic juice differentiates pancreatic cancer from chronic pancreatitis: A meta-analysis. *Pancreatology* 2016; **16**: 372-381 [PMID: 26899542 DOI: 10.1016/j.pan.2016.01.007]

368 **Hashimoto Y**, Murakami Y, Uemura K, Hayashidani Y, Sudo T, Ohge H, Fukuda E, Sueda T, Hiyama E. Detection of human telomerase reverse transcriptase (hTERT) expression in tissue and pancreatic juice from pancreatic cancer. *Surgery* 2008; **143**: 113-125 [PMID: 18154939 DOI: 10.1016/j.surg.2007.07.042]

369 **Nakashima A**, Murakami Y, Uemura K, Hayashidani Y, Sudo T, Hashimoto Y, Ohge H, Oda M, Sueda T, Hiyama E. Usefulness of human telomerase reverse transcriptase in pancreatic juice as a biomarker of pancreatic malignancy. *Pancreas* 2009; **38**: 527-533 [PMID: 19342980 DOI: 10.1097/MPA.0b013e3181a16d28]

370 **Sansone V**, Le Grazie M, Roselli J, Polvani S, Galli A, Tovoli F, Tarocchi M. Telomerase reactivation is associated with hepatobiliary and pancreatic cancers. *Hepatobiliary Pancreat Dis Int* 2020; **19**: 420-428 [PMID: 32386990 DOI: 10.1016/j.hbpd.2020.04.007]

371 **Zheng J**, Hernandez JM, Doussot A, Bojmar L, Zambirinis CP, Costa-Silva B, van Beek EJAH, Mark MT, Molina H, Askan G, Basturk O, Gonen M, Kingham TP, Allen PJ, D'Angelica MI, DeMatteo RP, Lyden D, Jarnagin WR. Extracellular matrix proteins and carcinoembryonic antigen-related cell adhesion molecules characterize pancreatic duct fluid exosomes in patients with pancreatic cancer. *HPB (Oxford)* 2018; **20**: 597-604 [PMID: 29339034 DOI: 10.1016/j.hpb.2017.12.010]

372 **Osteikoetxea X**, Benke M, Rodriguez M, Pálóczi K, Sódar BW, Szvicsek Z, Szabó-Taylor K, Vukman KV, Kittel Á, Wiener Z, Vékey K, Harsányi L, Szűcs Á, Turiák L, Buzás EI. Detection and proteomic characterization of extracellular vesicles in human pancreatic juice. *Biochem Biophys Res Commun* 2018; **499**: 37-43 [PMID: 29550476 DOI: 10.1016/j.bbrc.2018.03.107]

373 **Nakamura S**, Sadakari Y, Ohtsuka T, Okayama T, Nakashima Y, Gotoh Y, Saeki K, Mori Y, Nakata K, Miyasaka Y, Onishi H, Oda Y, Goggins M, Nakamura M. Pancreatic Juice Exosomal MicroRNAs as Biomarkers for Detection of Pancreatic Ductal Adenocarcinoma. *Ann Surg Oncol* 2019; **26**: 2104-2111 [PMID: 30820789 DOI: 10.1245/s10434-019-07269-z]

374 **Patel N**, Petrinic T, Silva M, Soonawalla Z, Reddy S, Gordon-Weeks A. The Diagnostic Accuracy of Mutant KRAS Detection from Pancreatic Secretions for the Diagnosis of Pancreatic Cancer: A Meta-Analysis. *Cancers (Basel)* 2020; **12** [PMID: 32825312 DOI: 10.3390/cancers12092353]

375 **Fukushima N**, Walter KM, Uek T, Sato N, Matsubayashi H, Cameron JL, Hruban RH, Canto M, Yeo CJ, Goggins M. Diagnosing pancreatic cancer using methylation specific PCR analysis of pancreatic juice. *Cancer Biol Ther* 2003; **2**: 78-83 [PMID: 12673124 DOI: 10.4161/cbt.183]

376 **Matsubayashi H**, Canto M, Sato N, Klein A, Abe T, Yamashita K, Yeo CJ, Kalloo A, Hruban R, Goggins M. DNA methylation alterations in the pancreatic juice of patients with suspected pancreatic disease. *Cancer Res* 2006; **66**: 1208-1217 [PMID: 16424060 DOI: 10.1158/0008-5472.CAN-05-2664]

377 **Kisiel JB**, Raimondo M, Taylor WR, Yab TC, Mahoney DW, Sun Z, Middha S, Baheti S, Zou H, Smyrk TC, Boardman LA, Petersen GM, Ahlquist DA. New DNA Methylation Markers for Pancreatic Cancer: Discovery, Tissue Validation, and Pilot Testing in Pancreatic Juice. *Clin Cancer Res* 2015; **21**: 4473-4481 [PMID: 26023084 DOI: 10.1158/1078-0432.CCR-14-2469]

378 **Yokoyama S**, Kitamoto S, Higashi M, Goto Y, Hara T, Ikebe D, Yamaguchi T, Arisaka Y, Niihara T, Nishimata H, Tanaka S, Takaori K, Batra SK, Yonezawa S. Diagnosis of pancreatic neoplasms

using a novel method of DNA methylation analysis of mucin expression in pancreatic juice. *PLoS One* 2014; **9**: e93760 [PMID: 24714692 DOI: 10.1371/journal.pone.0093760]

379 **de Jong K**, Nio CY, Hermans JJ, Dijkgraaf MG, Gouma DJ, van Eijck CH, van Heel E, Klass G, Fockens P, Bruno MJ. High prevalence of pancreatic cysts detected by screening magnetic resonance imaging examinations. *Clin Gastroenterol Hepatol* 2010; **8**: 806-811 [PMID: 20621679 DOI: 10.1016/j.cgh.2010.05.017]

380 **Elta GH**, Enestvedt BK, Sauer BG, Lennon AM. ACG Clinical Guideline: Diagnosis and Management of Pancreatic Cysts. *Am J Gastroenterol* 2018; **113**: 464-479 [PMID: 29485131 DOI: 10.1038/ajg.2018.14]

381 **Jabbar KS**, Arike L, Verbeke CS, Sadik R, Hansson GC. Highly Accurate Identification of Cystic Precursor Lesions of Pancreatic Cancer Through Targeted Mass Spectrometry: A Phase IIc Diagnostic Study. *J Clin Oncol* 2018; **36**: 367-375 [PMID: 29166170 DOI: 10.1200/JCO.2017.73.7288]

382 **Anand N**, Sampath K, Wu BU. Cyst features and risk of malignancy in intraductal papillary mucinous neoplasms of the pancreas: a meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 913-921; quiz e59 [PMID: 23416279 DOI: 10.1016/j.cgh.2013.02.010]

383 **Park WG**, Mascarenhas R, Palaez-Luna M, Smyrk TC, O'Kane D, Clain JE, Levy MJ, Pearson RK, Petersen BT, Topazian MD, Vege SS, Chari ST. Diagnostic performance of cyst fluid carcinoembryonic antigen and amylase in histologically confirmed pancreatic cysts. *Pancreas* 2011; **40**: 42-45 [PMID: 20966811 DOI: 10.1097/MPA.0b013e3181f69f36]

384 **Ngamruengphong S**, Bartel MJ, Raimondo M. Cyst carcinoembryonic antigen in differentiating pancreatic cysts: a meta-analysis. *Dig Liver Dis* 2013; **45**: 920-926 [PMID: 23790480 DOI: 10.1016/j.dld.2013.05.002]

385 **Carr RA**, Yip-Schneider MT, Simpson RE, Dolejs S, Schneider JG, Wu H, Ceppa EP, Park W, Schmidt CM. Pancreatic cyst fluid glucose: rapid, inexpensive, and accurate diagnosis of mucinous pancreatic cysts. *Surgery* 2018; **163**: 600-605 [PMID: 29241991 DOI: 10.1016/j.surg.2017.09.051]

386 **Maker AV**, Hu V, Kadkol SS, Hong L, Brugge W, Winter J, Yeo CJ, Hackert T, Büchler M, Lawlor RT, Salvia R, Scarpa A, Bassi C, Green S. Cyst Fluid Biosignature to Predict Intraductal Papillary Mucinous Neoplasms of the Pancreas with High Malignant Potential. *J Am Coll Surg* 2019; **228**: 721-729 [PMID: 30794864 DOI: 10.1016/j.jamcollsurg.2019.02.040]

387 **Simpson RE**, Yip-Schneider MT, Flick KF, Wu H, Colgate CL, Schmidt CM. Pancreatic Fluid Interleukin-1β Complements Prostaglandin E2 and Serum Carbohydrate Antigen 19-9 in Prediction of Intraductal Papillary Mucinous Neoplasm Dysplasia. *Pancreas* 2019; **48**: 1026-1031 [PMID: 31404023 DOI: 10.1097/MPA.0000000000001377]

388 **Schmidt CM**, Yip-Schneider MT, Ralstin MC, Wentz S, DeWitt J, Sherman S, Howard TJ, McHenry L, Dutkevitch S, Goggins M, Nakeeb A, Lillemoe KD. PGE(2) in pancreatic cyst fluid helps differentiate IPMN from MCN and predict IPMN dysplasia. *J Gastrointest Surg* 2008; **12**: 243-249 [PMID: 18027059 DOI: 10.1007/s11605-007-0404-8]

389 **Al Efishat MA**, Attiyeh MA, Eaton AA, Gönen M, Prosser D, Lokshin AE, Castillo CF, Lillemoe KD, Ferrone CR, Pergolini I, Mino-Kenudson M, Rezaee N, Dal Molin M, Weiss MJ, Cameron JL, Hruban RH, D'Angelica MI, Kingham TP, DeMatteo RP, Jarnagin WR, Wolfgang CL, Allen PJ. Multi-institutional Validation Study of Pancreatic Cyst Fluid Protein Analysis for Prediction of High-risk Intraductal Papillary Mucinous Neoplasms of the Pancreas. *Ann Surg* 2018; **268**: 340-347 [PMID: 28700444 DOI: 10.1097/SLA.0000000000002421]

390 **Pham K**, Dong A, Wodziak D, Banerjee S, Friedland S, Poultsides GA, Visser B, Norton JA, Lowe AW, Park W. Su1351 Amphiregulin in Pancreatic Cyst Fluid is Elevated in High-Risk Pancreatic Cysts. *Gastroenterology* 2016; **150**: S500-S501

391 **Tun MT**, Pai RK, Kwok S, Dong A, Gupta A, Visser BC, Norton JA, Poultsides GA, Banerjee S, Van Dam J, Chen AM, Friedland S, Scott BA, Verma R, Lowe AW, Park WG. Diagnostic accuracy of cyst fluid amphiregulin in pancreatic cysts. *BMC Gastroenterol* 2012; **12**: 15 [PMID: 22333441 DOI: 10.1186/1471-230X-12-15]

392 **Räty S**, Sand J, Laukkarinen J, Vasama K, Bassi C, Salvia R, Nordback I. Cyst fluid SPINK1 may help to differentiate benign and potentially malignant cystic pancreatic lesions. *Pancreatology* 2013; **13**: 530-533 [PMID: 24075519 DOI: 10.1016/j.pan.2013.06.008]

393 **Das KK**, Xiao H, Geng X, Fernandez-Del-Castillo C, Morales-Oyarvide V, Daglilar E, Forcione DG, Bounds BC, Brugge WR, Pitman MB, Mino-Kenudson M, Das KM. mAb Das-1 is specific for high-risk and malignant intraductal papillary mucinous neoplasm (IPMN). *Gut* 2014; **63**: 1626-1634 [PMID: 24277729 DOI: 10.1136/gutjnl-2013-306219]

394 **Das KK**, Geng X, Brown JW, Morales-Oyarvide V, Huynh T, Pergolini I, Pitman MB, Ferrone C, Al Efishat M, Haviland D, Thompson E, Wolfgang C, Lennon AM, Allen P, Lillemoe KD, Fields RC, Hawkins WG, Liu J, Castillo CF, Das KM, Mino-Kenudson M. Cross Validation of the Monoclonal Antibody Das-1 in Identification of High-Risk Mucinous Pancreatic Cystic Lesions. *Gastroenterology* 2019; **157**: 720-730. e2 [PMID: 31175863 DOI: 10.1053/j.gastro.2019.05.014]

395 **Siu L**, Paredes J, Kurbatov V, Ramachandran R, Serafini F, Grossman E, Gress F, Martello L. Clinical Utility of Cytokine Biomarker Analysis of Pancreatic Cyst Fluid Obtained by Endoscopic Ultrasound Fine Needle Aspiration: A Pilot Study. *Pancreas* 2019; **48**: e60-e61 [PMID: 31425485 DOI: 10.1097/MPA.0000000000001365]

396 **Farrell JJ**, Toste P, Wu N, Li L, Wong J, Malkhassian D, Tran LM, Wu X, Li X, Dawson D, Wu H, Donahue TR. Endoscopically acquired pancreatic cyst fluid microRNA 21 and 221 are associated

with invasive cancer. *Am J Gastroenterol* 2013; **108**: 1352-1359 [PMID: 23752880 DOI: 10.1038/ajg.2013.167]

397 **Matthaei H**, Wylie D, Lloyd MB, Dal Molin M, Kemppainen J, Mayo SC, Wolfgang CL, Schulick RD, Langfield L, Andruss BF, Adai AT, Hruban RH, Szafranska-Schwarzbach AE, Maitra A. miRNA biomarkers in cyst fluid augment the diagnosis and management of pancreatic cysts. *Clin Cancer Res* 2012; **18**: 4713-4724 [PMID: 22723372 DOI: 10.1158/1078-0432.CCR-12-0035]

398 **Utomo WK**, Looijenga LH, Bruno MJ, Hansen BE, Gillis A, Biermann K, Peppelenbosch MP, Fuhler GM, Braat H. A MicroRNA Panel in Pancreatic Cyst Fluid for the Risk Stratification of Pancreatic Cysts in a Prospective Cohort. *Mol Ther Nucleic Acids* 2016; **5**: e350 [PMID: 28131248 DOI: 10.1038/mtna.2016.61]

399 **Khalid A**, McGrath KM, Zahid M, Wilson M, Brody D, Swalsky P, Moser AJ, Lee KK, Slivka A, Whitcomb DC, Finkelstein S. The role of pancreatic cyst fluid molecular analysis in predicting cyst pathology. *Clin Gastroenterol Hepatol* 2005; **3**: 967-973 [PMID: 16234041 DOI: 10.1016/s1542-3565(05)00409-x]

400 **Schoedel KE**, Finkelstein SD, Ohori NP. K-Ras and microsatellite marker analysis of fine-needle aspirates from intraductal papillary mucinous neoplasms of the pancreas. *Diagn Cytopathol* 2006; **34**: 605-608 [PMID: 16900481 DOI: 10.1002/dc.20511]

401 **Khalid A**, Zahid M, Finkelstein SD, LeBlanc JK, Kaushik N, Ahmad N, Brugge WR, Edmundowicz SA, Hawes RH, McGrath KM. Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study. *Gastrointest Endosc* 2009; **69**: 1095-1102 [PMID: 19152896 DOI: 10.1016/j.gie.2008.07.033]

402 **Sawhney MS**, Devarajan S, O'Farrel P, Cury MS, Kundu R, Vollmer CM, Brown A, Chuttani R, Pleskow DK. Comparison of carcinoembryonic antigen and molecular analysis in pancreatic cyst fluid. *Gastrointest Endosc* 2009; **69**: 1106-1110 [PMID: 19249035 DOI: 10.1016/j.gie.2008.08.015]

403 **Shen J**, Brugge WR, Dimaio CJ, Pitman MB. Molecular analysis of pancreatic cyst fluid: a comparative analysis with current practice of diagnosis. *Cancer* 2009; **117**: 217-227 [PMID: 19415731 DOI: 10.1002/cncy.20027]

404 **Sreenarasimhaiah J**, Lara LF, Jazrawi SF, Barnett CC, Tang SJ. A comparative analysis of pancreas cyst fluid CEA and histology with DNA mutational analysis in the detection of mucin producing or malignant cysts. *JOP* 2009; **10**: 163-168 [PMID: 19287110]

405 **Talar-Wojnarowska R**, Pazurek M, Durko L, Degowska M, Rydzewska G, Smigielski J, Janiak A, Olakowski M, Lampe P, Grzelak P, Stefanczyk L, Smolarz B, Malecka-Panas E. A comparative analysis of K-ras mutation and carcinoembryonic antigen in pancreatic cyst fluid. *Pancreatology* 2012; **12**: 417-420 [PMID: 23127529 DOI: 10.1016/j.pan.2012.08.001]

406 **Nikiforova MN**, Khalid A, Fasanella KE, McGrath KM, Brand RE, Chennat JS, Slivka A, Zeh HJ, Zureikat AH, Krasinskas AM, Ohori NP, Schoedel KE, Navina S, Mantha GS, Pai RK, Singhi AD. Integration of KRAS testing in the diagnosis of pancreatic cystic lesions: a clinical experience of 618 pancreatic cysts. *Mod Pathol* 2013; **26**: 1478-1487 [PMID: 23743931 DOI: 10.1038/modpathol.2013.91]

407 **Al-Haddad M**, DeWitt J, Sherman S, Schmidt CM, LeBlanc JK, McHenry L, Coté G, El Chafic AH, Luz L, Stuart JS, Johnson CS, Klochan C, Imperiale TF. Performance characteristics of molecular (DNA) analysis for the diagnosis of mucinous pancreatic cysts. *Gastrointest Endosc* 2014; **79**: 79-87 [PMID: 23845445 DOI: 10.1016/j.gie.2013.05.026]

408 **Thiruvengadam N**, Park WG. Systematic Review of Pancreatic Cyst Fluid Biomarkers: The Path Forward. *Clin Transl Gastroenterol* 2015; **6**: e88 [PMID: 26065716 DOI: 10.1038/ctg.2015.17]

409 **Wu J**, Matthaei H, Maitra A, Dal Molin M, Wood LD, Eshleman JR, Goggins M, Canto MI, Schulick RD, Edil BH, Wolfgang CL, Klein AP, Diaz LA Jr, Allen PJ, Schmidt CM, Kinzler KW, Papadopoulos N, Hruban RH, Vogelstein B. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. *Sci Transl Med* 2011; **3**: 92ra66 [PMID: 21775669 DOI: 10.1126/scitranslmed.3002543]

410 **Siddiqui AA**, Kowalski TE, Kedika R, Roy A, Loren DE, Ellsworth E, Adler D, Finkelstein SD. EUS-guided pancreatic fluid aspiration for DNA analysis of KRAS and GNAS mutations for the evaluation of pancreatic cystic neoplasia: a pilot study. *Gastrointest Endosc* 2013; **77**: 669-670 [PMID: 23498145 DOI: 10.1016/j.gie.2012.11.009]

411 **Singhi AD**, Nikiforova MN, Fasanella KE, McGrath KM, Pai RK, Ohori NP, Bartholow TL, Brand RE, Chennat JS, Lu X, Papachristou GI, Slivka A, Zeh HJ, Zureikat AH, Lee KK, Tsung A, Mantha GS, Khalid A. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. *Clin Cancer Res* 2014; **20**: 4381-4389 [PMID: 24938521 DOI: 10.1158/1078-0432.CCR-14-0513]

412 **Etekpo A**, Alghawalby A, Alghawalby M, Soliman A, Hablas A. Differences in MUC4 Expression in Pancreatic Cancers and Pancreatic Cysts in Egypt. *J Carcinog Mutagen* 2018; **9**: 2 [DOI: 10.4172/2157-2518.1000312]

413 **Wang Y**, Gao J, Li Z, Jin Z, Gong Y, Man X. Diagnostic value of mucins (MUC1, MUC2 and MUC5AC) expression profile in endoscopic ultrasound-guided fine-needle aspiration specimens of the pancreas. *Int J Cancer* 2007; **121**: 2716-2722 [PMID: 17708554 DOI: 10.1002/ijc.22997]

414 **Carrara S**, Cangi MG, Arcidiacono PG, Perri F, Petrone MC, Mezzi G, Boemo C, Talarico A, Cin ED, Grassini G, Doglioni C, Testoni PA. Mucin expression pattern in pancreatic diseases: findings from EUS-guided fine-needle aspiration biopsies. *Am J Gastroenterol* 2011; **106**: 1359-1363 [PMID: 21647207 DOI: 10.1038/ajg.2011.22]

415 **Springer S**, Masica DL, Dal Molin M, Douville C, Thoburn CJ, Afsari B, Li L, Cohen JD,

Thompson E, Allen PJ, Klimstra DS, Schattner MA, Schmidt CM, Yip-Schneider M, Simpson RE, Fernandez-Del Castillo C, Mino-Kenudson M, Brugge W, Brand RE, Singhi AD, Scarpa A, Lawlor R, Salvia R, Zamboni G, Hong SM, Hwang DW, Jang JY, Kwon W, Swan N, Geoghegan J, Falconi M, Crippa S, Doglioni C, Paulino J, Schulick RD, Edil BH, Park W, Yachida S, Hijioka S, van Hooft J, He J, Weiss MJ, Burkhart R, Makary M, Canto MI, Goggins MG, Ptak J, Dobbyn L, Schaefer J, Sillman N, Popoli M, Klein AP, Tomasetti C, Karchin R, Papadopoulos N, Kinzler KW, Vogelstein B, Wolfgang CL, Hruban RH, Lennon AM. A multimodality test to guide the management of patients with a pancreatic cyst. *Sci Transl Med* 2019; **11** [PMID: 31316009 DOI: 10.1126/scitranslmed.aav4772]

416 **Lee YH**, Wong DT. Saliva: an emerging biofluid for early detection of diseases. *Am J Dent* 2009; **22**: 241-248 [PMID: 19824562]

417 **Xie ZJ**, Chen G, Zhang XC, Li DF, Huang J, Li ZJ. Saliva supernatant miR-21: a novel potential biomarker for esophageal cancer detection. *Asian Pac J Cancer Prev* 2012; **13**: 6145-6149 [PMID: 23464420 DOI: 10.7314/apjcp.2012.13.12.6145]

418 **Zhang Y**, Sun J, Lin CC, Abemayor E, Wang MB, Wong DT. The emerging landscape of salivary diagnostics. *Periodontol 2000* 2016; **70**: 38-52 [PMID: 26662481 DOI: 10.1111/prd.12099]

419 **Vitorino R**, Lobo MJ, Ferrer-Correira AJ, Dubin JR, Tomer KB, Domingues PM, Amado FM. Identification of human whole saliva protein components using proteomics. *Proteomics* 2004; **4**: 1109-1115 [PMID: 15048992 DOI: 10.1002/pmic.200300638]

420 **Hu S**, Xie Y, Ramachandran P, Ogorzalek Loo RR, Li Y, Loo JA, Wong DT. Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry. *Proteomics* 2005; **5**: 1714-1728 [PMID: 15800970 DOI: 10.1002/pmic.200401037]

421 **Setti G**, Pezzi ME, Viani MV, Pertinhez TA, Cassi D, Magnoni C, Bellini P, Musolino A, Vescovi P, Meleti M. Salivary MicroRNA for Diagnosis of Cancer and Systemic Diseases: A Systematic Review. *Int J Mol Sci* 2020; **21** [PMID: 32019170 DOI: 10.3390/ijms21030907]

422 **Machida T**, Tomofuji T, Maruyama T, Yoneda T, Ekuni D, Azuma T, Miyai H, Mizuno H, Kato H, Tsutsumi K, Uchida D, Takaki A, Okada H, Morita M. miR1246 and miR4644 in salivary exosome as potential biomarkers for pancreatobiliary tract cancer. *Oncol Rep* 2016; **36**: 2375-2381 [PMID: 27573701 DOI: 10.3892/or.2016.5021]

423 **Humeau M**, Vignolle-Vidoni A, Sicard F, Martins F, Bournet B, Buscail L, Torrisani J, Cordelier P. Salivary MicroRNA in Pancreatic Cancer Patients. *PLoS One* 2015; **10**: e0130996 [PMID: 26121640 DOI: 10.1371/journal.pone.0130996]

424 **Xie Z**, Yin X, Gong B, Nie W, Wu B, Zhang X, Huang J, Zhang P, Zhou Z, Li Z. Salivary microRNAs show potential as a noninvasive biomarker for detecting resectable pancreatic cancer. *Cancer Prev Res (Phila)* 2015; **8**: 165-173 [PMID: 25538087 DOI: 10.1158/1940-6207.CAPR-14-0192]

425 **Gao S**, Chen LY, Wang P, Liu LM, Chen Z. MicroRNA expression in salivary supernatant of patients with pancreatic cancer and its relationship with ZHENG. *Biomed Res Int* 2014; **2014**: 756347 [PMID: 25126577 DOI: 10.1155/2014/756347]

426 **Gerner EW**, Meyskens FL Jr. Polyamines and cancer: old molecules, new understanding. *Nat Rev Cancer* 2004; **4**: 781-792 [PMID: 15510159 DOI: 10.1038/nrc1454]

427 **Asai Y**, Itoi T, Sugimoto M, Sofuni A, Tsuchiya T, Tanaka R, Tonozuka R, Honjo M, Mukai S, Fujita M, Yamamoto K, Matsunami Y, Kurosawa T, Nagakawa Y, Kaneko M, Ota S, Kawachi S, Shimazu M, Soga T, Tomita M, Sunamura M. Elevated Polyamines in Saliva of Pancreatic Cancer. *Cancers (Basel)* 2018; **10** [PMID: 29401744 DOI: 10.3390/cancers10020043]

428 **Xie Z**, Chen X, Li J, Guo Y, Li H, Pan X, Jiang J, Liu H, Wu B. Salivary HOTAIR and PVT1 as novel biomarkers for early pancreatic cancer. *Oncotarget* 2016; **7**: 25408-25419 [PMID: 27028998 DOI: 10.18632/oncotarget.8323]

429 **Navaneethan U**, Gutierrez NG, Jegadeesan R, Venkatesh PG, Poptic E, Liu X, Sanaka MR, Jang S, Vargo JJ, Parsi MA. Vascular endothelial growth factor levels in bile distinguishes pancreatic cancer from other etiologies of biliary stricture: a pilot study. *Dig Dis Sci* 2013; **58**: 2986-2992 [PMID: 23828141 DOI: 10.1007/s10620-013-2764-0]

430 **Alvaro D**, Macarri G, Mancino MG, Marzioni M, Bragazzi M, Onori P, Corradini SG, Invernizzi P, Franchitto A, Attili AF, Gaudio E, Benedetti A. Serum and biliary insulin-like growth factor I and vascular endothelial growth factor in determining the cause of obstructive cholestasis. *Ann Intern Med* 2007; **147**: 451-459 [PMID: 17909206 DOI: 10.7326/0003-4819-147-7-200710020-00003]

431 **Brockmann J**, Emparan C, Hernandez CA, Sulkowski U, Dietl KH, Menzel J, Wolters H, Glodny B, Senninger N. Gallbladder bile tumor marker quantification for detection of pancreato-biliary malignancies. *Anticancer Res* 2000; **20**: 4941-4947 [PMID: 11326643]

432 **Natsios A**, Vezakis A, Kaparos G, Fragulidis G, Karakostas N, Kouskouni E, Logothetis E, Polydorou A. Significance of serum and bile tumor markers in the diagnostic approach of patients with malignant pancreatobiliary disease. *J BUON* 2015; **20**: 1030-1036 [PMID: 26416052]

433 **Joo KR**, Kim DH, Park JH, Bang SJ, Shin JW, Park NH. The role of bile carcinoembryonic antigen in diagnosing bile duct cancer. *J Korean Med Sci* 2003; **18**: 855-858 [PMID: 14676443 DOI: 10.3346/jkms.2003.18.6.855]

434 **Terai K**, Jiang M, Tokuyama W, Murano T, Takada N, Fujimura K, Ebinuma H, Kishimoto T, Hiruta N, Schneider WJ, Bujo H. Levels of soluble LR11/SorLA are highly increased in the bile of patients with biliary tract and pancreatic cancers. *Clin Chim Acta* 2016; **457**: 130-136 [PMID:

27079357 DOI: 10.1016/j.cca.2016.04.010]

435 **Matull WR**, Andreola F, Loh A, Adiguzel Z, Deheragoda M, Qureshi U, Batra SK, Swallow DM, Pereira SP. MUC4 and MUC5AC are highly specific tumour-associated mucins in biliary tract cancer. *Br J Cancer* 2008; **98**: 1675-1681 [PMID: 18475301 DOI: 10.1038/sj.bjc.6604364]

436 **Adrait A**, Dumonceau JM, Delhaye M, Annessi-Ramseyer I, Frossard JL, Couté Y, Farina A. Liquid Biopsy of Bile based on Targeted Mass Spectrometry for the Diagnosis of Malignant Biliary Strictures. *Clin Transl Sci* 2021; **14**: 148-152 [PMID: 33048472 DOI: 10.1111/cts.12890]

437 **Budzynska A**, Nowakowska-Dulawa E, Marek T, Boldys H, Nowak A, Hartleb M. Differentiation of pancreatobiliary cancer from benign biliary strictures using neutrophil gelatinase-associated lipocalin. *J Physiol Pharmacol* 2013; **64**: 109-114 [PMID: 23568978]

438 **Zabron AA**, Horneffer-van der Sluis VM, Wadsworth CA, Laird F, Gierula M, Thillainayagam AV, Vlavianos P, Westaby D, Taylor-Robinson SD, Edwards RJ, Khan SA. Elevated levels of neutrophil gelatinase-associated lipocalin in bile from patients with malignant pancreatobiliary disease. *Am J Gastroenterol* 2011; **106**: 1711-1717 [PMID: 21670771 DOI: 10.1038/ajg.2011.187]

439 **Roli L**, Pecoraro V, Trenti T. Can NGAL be employed as prognostic and diagnostic biomarker in human cancers? *Int J Biol Markers* 2017; **32**: e53-e61 [PMID: 28106227 DOI: 10.5301/jbm.5000245]

440 **Farina A**, Dumonceau JM, Antinori P, Annessi-Ramseyer I, Frossard JL, Hochstrasser DF, Delhaye M, Lescuyer P. Bile carcinoembryonic cell adhesion molecule 6 (CEAM6) as a biomarker of malignant biliary stenoses. *Biochim Biophys Acta* 2014; **1844**: 1018-1025 [PMID: 23806607 DOI: 10.1016/j.bbapap.2013.06.010]

441 **Ayaru L**, Stoeber K, Webster GJ, Hatfield AR, Wollenschlaeger A, Okoturo O, Rashid M, Williams G, Pereira SP. Diagnosis of pancreaticobiliary malignancy by detection of minichromosome maintenance protein 5 in bile aspirates. *Br J Cancer* 2008; **98**: 1548-1554 [PMID: 18414413 DOI: 10.1038/sj.bjc.6604342]

442 **Keane MG**, Huggett MT, Chapman MH, Johnson GJ, Webster GJ, Thorburn D, Mackay J, Pereira SP. Diagnosis of pancreaticobiliary malignancy by detection of minichromosome maintenance protein 5 in biliary brush cytology. *Br J Cancer* 2017; **116**: 349-355 [PMID: 28081547 DOI: 10.1038/bjc.2016.447]

443 **Hedström J**, Haglund C, Leinonen J, Nordling S, Stenman UH. Trypsinogen-1, -2 and tumour-associated trypsin-inhibitor in bile and biliary tract tissues from patients with biliary tract diseases and pancreatic carcinomas. *Scand J Clin Lab Invest* 2001; **61**: 111-118 [PMID: 11347977 DOI: 10.1080/00365510151097584]

444 **Parsi MA**, Li A, Li CP, Goggins M. DNA methylation alterations in endoscopic retrograde cholangiopancreatography brush samples of patients with suspected pancreaticobiliary disease. *Clin Gastroenterol Hepatol* 2008; **6**: 1270-1278 [PMID: 18995218 DOI: 10.1016/j.cgh.2008.07.007]

445 **Pei Z**, Liu SM, Huang JT, Zhang X, Yan D, Xia Q, Ji C, Chen W, Xu J, Wang J. Clinically relevant circulating microRNA profiling studies in pancreatic cancer using meta-analysis. *Oncotarget* 2017; **8**: 22616-22624 [PMID: 28186984 DOI: 10.18632/oncotarget.15148]

446 **Ohtsubo K**, Miyake K, Arai S, Fukuda K, Yanagimura N, Suzuki C, Otani S, Adachi Y, Tanimoto A, Nishiyama A, Yamashita K, Takeuchi S, Notohara K, Yoshimura K, Yano S. Aberrant Methylation of Tumor Suppressive miRNAs in Bile from Patients With Pancreaticobiliary Diseases. *Anticancer Res* 2019; **39**: 5449-5459 [PMID: 31570439 DOI: 10.21873/anticanres.13738]

447 **Haug U**, Wente MN, Seiler CM, Jesenofsky R, Brenner H. Stool testing for the early detection of pancreatic cancer: rationale and current evidence. *Expert Rev Mol Diagn* 2008; **8**: 753-759 [PMID: 18999925 DOI: 10.1586/14737159.8.6.753]

448 **Tobi M**, Elitsur Y, Moyer MP, Halline A, Deutsch M, Nochomovitz L, Luk GD. Mucosal origin and shedding of an early colonic tumor marker defined by Adnab-9 monoclonal antibody. *Scand J Gastroenterol* 1993; **28**: 1025-1034 [PMID: 8303203 DOI: 10.3109/00365529309098304]

449 **Qiao SX**, Yuan M, Liu YL, Lin XS, Zhang XP, Tobi M. Detection of gastric cancer and premalignant lesions by novel marker glycoprotein 87 using monoclonal antibody Adnab-9. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 1095-1099 [PMID: 14578149]

450 **Tobi M**, Kim M, Weinstein DH, Rambus MA, Hatfield J, Adsay NV, Levi E, Evans D, Lawson MJ, Fligiel S. Prospective markers for early diagnosis and prognosis of sporadic pancreatic ductal adenocarcinoma. *Dig Dis Sci* 2013; **58**: 744-750 [PMID: 23001406 DOI: 10.1007/s10620-012-2387-x]

451 **Tobi M**, Hatfield J, Adsay V, Galagan K, Kozarek R, Inagaki M, Kasai S, Tokusashi Y, Obara T, Hruban R, Lough J, Barkun A, Jabbari M, Sheikh R, Ruebner B, Lawson M, Ben-Josef E, Fligiel S. Prognostic Significance of the Labeling of Adnab-9 in Pancreatic Intraductal Papillary Mucinous Neoplasms. *Int J Gastrointest Cancer* 2001; **29**: 141-150 [PMID: 12754384]

452 **Ren Y**, Gao J, Liu JQ, Wang XW, Gu JJ, Huang HJ, Gong YF, Li ZS. Differential signature of fecal microRNAs in patients with pancreatic cancer. *Mol Med Rep* 2012; **6**: 201-209 [PMID: 22504911 DOI: 10.3892/mmr.2012.862]

453 **Link A**, Becker V, Goel A, Wex T, Malfertheiner P. Feasibility of fecal microRNAs as novel biomarkers for pancreatic cancer. *PLoS One* 2012; **7**: e42933 [PMID: 22905187 DOI: 10.1371/journal.pone.0042933]

454 **Caldas C**, Hahn SA, Hruban RH, Redston MS, Yeo CJ, Kern SE. Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia. *Cancer Res* 1994; **54**: 3568-3573 [PMID: 8012983]

455 **Haug U**, Hillebrand T, Bendzko P, Löw M, Rothenbacher D, Stegmaier C, Brenner H. Mutant-enriched PCR and allele-specific hybridization reaction to detect K-ras mutations in stool DNA: high prevalence in a large sample of older adults. *Clin Chem* 2007; **53**: 787-790 [PMID: 17317884 DOI: 10.1373/clinchem.2006.078188]

456 **Kisiel JB**, Yab TC, Taylor WR, Chari ST, Petersen GM, Mahoney DW, Ahlquist DA. Stool DNA testing for the detection of pancreatic cancer: assessment of methylation marker candidates. *Cancer* 2012; **118**: 2623-2631 [PMID: 22083596 DOI: 10.1002/cncr.26558]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4088-4103

DOI: 10.3748/wjg.v27.i26.4088

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Non-occlusive mesenteric ischemia: Diagnostic challenges and perspectives in the era of artificial intelligence

Simon Bourcier, Julian Klug, Lee S Nguyen

ORCID number: Simon Bourcier 0000-0003-4358-2330; Julian Klug 0000-0002-4849-9811; Lee S Nguyen 0000-0002-6014-6269.

Author contributions: Bourcier S wrote the manuscript; Klug J significantly contributed to the manuscript; Nguyen LS co-wrote and supervised this work.

Conflict-of-interest statement: The authors have no conflict of interest regarding this work.

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: France

Simon Bourcier, Department of Intensive Care Medicine, University Hospital of Geneva, Geneva 1201, Switzerland

Julian Klug, Department of Internal Medicine, Groupement Hospitalier de l'Ouest Lémanique, Nyon 1260, Switzerland

Lee S Nguyen, Department of Intensive Care Medicine, CMC Ambroise Paré, Neuilly-sur-Seine 92200, France

Corresponding author: Lee S Nguyen, MD, PhD, Doctor, Senior Researcher, Department of Intensive Care Medicine, CMC Ambroise Paré, 25 Boulevard Victor Hugo, Neuilly-sur-Seine 92200, France. nguyen.lee@icloud.com

Abstract

Acute mesenteric ischemia (AMI) is a severe condition associated with poor prognosis, ultimately leading to death due to multiorgan failure. Several mechanisms may lead to AMI, and non-occlusive mesenteric ischemia (NOMI) represents a particular form of AMI. NOMI is prevalent in intensive care units in critically ill patients. In NOMI management, promptness and accuracy of diagnosis are paramount to achieve decisive treatment, but the last decades have been marked by failure to improve NOMI prognosis, due to lack of tools to detect this condition. While real-life diagnostic management relies on a combination of physical examination, several biomarkers, imaging, and endoscopy to detect the possibility of several grades of NOMI, research studies only focus on a few elements at a time. In the era of artificial intelligence (AI), which can aggregate thousands of variables in complex longitudinal models, the prospect of achieving accurate diagnosis through machine-learning-based algorithms may be sought. In the following work, we bring you a state-of-the-art literature review regarding NOMI, its presentation, its mechanics, and the pitfalls of routine work-up diagnostic exams including biomarkers, imaging, and endoscopy, we raise the perspectives of new biomarker exams, and finally we discuss what AI may add to the field, after summarizing what this technique encompasses.

Key Words: Mesenteric ischemia; Biomarkers; Critically ill; Machine learning; Artificial intelligence

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

Received: January 28, 2021
Peer-review started: January 28, 2021
First decision: March 7, 2021
Revised: March 25, 2021
Accepted: June 18, 2021
Article in press: June 18, 2021
Published online: July 14, 2021

P-Reviewer: Shirai T, Wang Y
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Liu JH

Core Tip: In this review we focus on non-occlusive mesenteric ischemia and discuss the challenges of a reliable diagnosis, which requires several simultaneous elements, including physical examination, biomarkers, and imaging elements. While taken individually these elements do not provide sufficient diagnostic accuracy, a multimodal approach relying on artificial intelligent algorithms may increase speed and accuracy in recognizing this rare but severe condition.

Citation: Bourcier S, Klug J, Nguyen LS. Non-occlusive mesenteric ischemia: Diagnostic challenges and perspectives in the era of artificial intelligence. *World J Gastroenterol* 2021; 27(26): 4088-4103
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4088.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4088

INTRODUCTION

Acute mesenteric ischemia (AMI), due to inadequacy between oxygen demand and supply in the digestive tract, is a life-threatening emergency[1]. This term encompasses several entities that differ regarding their initial trigger of gut ischemia but ultimately converge towards digestive and systemic complications such as tissue necrosis, perforation, bacterial translocation, and eventually, death due to multiorgan failure. Contrary to most conditions, AMI is associated with a poor prognosis, which has not improved in the last decades. Mortality ranges around 80% and mostly depends on early diagnosis and adequate treatment.

Diagnosis of AMI secondary to large vessels occlusion mainly relies on imaging, including contrast-enhanced abdominal computed tomography (CT) scan, allowing the identification of the occluded vessel (or vessels) in order to choose between different revascularization options (interventional, surgical, or medical treatment).

While obstructive AMI has been reported at length, and their management is supported by evidence-based guidelines[2], AMI occurring in the absence of major vascular occlusion, non-occlusive mesenteric ischemia (NOMI), frequently raises diagnostic and therapeutic challenges. Indeed, NOMI often occurs as the consequence of a critical condition[3]. The diagnosis is often suspected in the intensive care unit (ICU) in the context of a patient's clinical condition worsening after a prior episode of profound and acute circulatory failure, such as a successfully resuscitated cardiac arrest, cardiopulmonary bypass surgery, as well as septic, hypovolemic, or cardiogenic shock. Reported mortality rates are extremely high, and time to diagnosis represents a key factor for improving its associated prognosis[4,5].

Several leads have been pursued to achieve this goal, including the development of new biomarkers as well as new multimodal tools. In the last decade, the advent of artificial intelligence (AI) allowed the facilitation of complex diagnoses relying on imaging.

In the following work, we bring you a state-of-the-art literature review regarding NOMI, its presentation, its mechanics, the pitfalls of routine work-up diagnostic exams, and perspectives in new biomarker exams and finally discuss what AI may add to the field. For brevity, we did not cover therapeutic management.

EPIDEMIOLOGY, MECHANISMS, AND MANAGEMENT OF NOMI

In contrast to AMI secondary to large vessels occlusion, NOMI was initially poorly understood. Nearly 80 years ago, first reports of NOMI described intestinal gangrene secondary to low cardiac output but without evidence of either arterial or venous occlusion[6,7]. As of today, only case-series and retrospective cohort studies report these severe events and in a selected population. One exception reports epidemiological data in a general population[8]. This Swedish population-based study was performed between 1970 and 1982 and suggested a population-based incidence of fatal NOMI of 2/100000 person-years. From 23446 systematic autopsies, 62 fatal NOMI cases were identified. After clinical data records were investigated, these patients were more likely to have suffered from fatal cardiac failure, atrial fibrillation, and recent

surgery. Of note, necroptic examination often showed concomitant infarction of other visceral organs such as liver, spleen, and kidneys suggesting a state of global organ hypoperfusion. Through non-recent retrospective monocentric surgical case-series, NOMI ranges between 4% and 60% of AMI causes, depending on the case-mix[9,10].

Several other smaller cohorts also reported hemodialysis as a setting associated with a risk of NOMI[11]. In a retrospective study of 57 cases occurring in the first 12 h after the last hemodialysis session, all cases were preceded by an episode of hypotension during hemodialysis, and investigations found diffuse (≥ 3) ischemic areas, in 20% of cases[12]. Vasculitis was also reported as an occasional cause of NOMI, especially polyarteritis nodosa[13].

In the ICU, while described for decades, interest in NOMI is growing; as shown by an increase in reporting in the last few years[2,3,14,15]. To date, the largest retrospective multicenter study gathered 780 AMI diagnoses in ICU patients, reporting an in-ICU mortality of 58%[15]. Of note, the occlusive or non-occlusive origin of AMI was not investigated. When AMI occurs in the ICU, NOMI appears prevalent: 91% of cases in a study of 101 AMI patients, with similar rates in other cohorts[4,16]. This increased prevalence in ICU may be explained by the fact that many conditions leading to ICU admission may be associated with a NOMI onset.

Several studies reported NOMI as a complication of cardiopulmonary bypass surgery, occurring in less than 1% of patients, often in patients with peripheral artery disease[4,17-19]. As a result, NOMI should be suspected in patients suffering from multiple organ failure after cardiac surgery; as suggested by Guillaume *et al*[4] in a cohort study of 320 patients in which NOMI rate was 10%[4]. In this study, the incidence of NOMI was not immediate: The authors reported a median of 7 d between cardiac surgery and NOMI diagnosis.

NOMI may also occur in patients admitted for successfully resuscitated cardiac arrest[5,20]. According to a recent report of a cardiac arrest center, NOMI may affect 2.5% to 6% of patients after cardiac arrest, mortality being 96%[5]. Factors reflecting the severity of the ischemia-reperfusion syndrome, such as higher admission lactate, low flow > 17 min, and higher inotropic score, were associated with NOMI diagnosis. Furthermore, NOMI represents a cause of secondary worsening in septic shock. Investigating the cause of death in septic shock according to time since ICU admission, Daviaud *et al*[21] identified NOMI respectively as the second and third causes of early (≤ 3 d) and late (> 3 d) death.

Mechanisms of AMI

The pathophysiological mechanical concept of AMI relies on an imbalance between oxygen supply and demand of the intestinal tissues. Ischemic lesions first begin to appear in the intestinal mucosa and subsequently may progress to irreversible transmural necrosis[22,23]. Complications include intestinal perforation, peritonitis, bacteriemia due to rupture of the gut barrier, inflammation leading to further non-mesenteric organ dysfunction, and shock. An essential contribution to the field was the historic work from Chiu *et al*[24] demonstrating how decreased mesenteric flow generates mucosal lesions. In an animal model, superior mesenteric artery blood flow was modulated, serial biopsies of the small intestine were performed, and ischemic intestinal mucosal lesions were detailed. The authors observed two observations of high importance. First, mucosal lesions appeared very early after the start of the experience. Second, the rapidity and the severity of mucosal lesions were correlated with the importance of decrease in blood flow. This experience highlights how urgent it is to make the diagnosis of AMI and proceed to treatment since vital and functional complications evolve quickly. A parallel can be drawn with acute myocardial infarction and stroke. Hence, intestinal stroke centers allowing early multimodal management have been suggested, and first reports showed increased survival[25].

Although the experiments performed by Chiu *et al*[24] strongly support the hypothesis of a supply-demand imbalance as a primary step towards NOMI, other complex processes may be involved, ultimately leading to the progression towards intestinal necrosis. These processes include the promotion of remote multiorgan failure through complex inflammatory pathways after a first insult in the form of transient hypoperfusion of the main mesenteric arteries[3,22,26]. Other mechanisms include impaired tissue perfusion responsible for gut barrier failure and endotoxin translocation, endothelial dysfunction and ischemia-reperfusion injury with increased local cytokine production, which may vary according to the primary cause of intestinal hypoxia[3,27-29].

In septic shock, tissue perfusion may be altered at the microcirculation level; despite seemingly optimized global hemodynamic parameters and these microcirculatory abnormalities are directly linked to organ failure[30]. Notably, Dubin *et al*[31]

demonstrated persistence of altered intestinal microcirculation disorders in deceased animals after correction of arterial hypotension in a model of septic shock[31]. Moreover, in sepsis, other mechanisms may participate to tissue dysoxia: Cellular and metabolic disorders[32,33]. Lobo *et al*[34] showed possible "cytopathic hypoxia" without impairment of oxygen delivery in the development of gut mucosal injury during endotoxic shock[34]. Therefore, a primary transient main mesenteric arteries hypoperfusion may not be a mandatory step in NOMI related to sepsis.

Although NOMI is thought to represent the worst stage of acute gastrointestinal injury in critically ill patients[35], the exact pathophysiology is still poorly understood, and the definition of this concept remains unclear[36]. According to a working group of the European Society of Intensive Medicine, NOMI is one of the possible facets of acute gastrointestinal dysfunction. In a recent update, acute gastrointestinal dysfunction is outlined as the consequence of a multitude of interacting pathophysiological mechanisms, resulting in other life-threating conditions such as Ogilvie's syndrome, sepsis, gastrointestinal tract perforation or bleeding, and acute compartment syndrome[36].

Additionally, deleterious therapeutic interventions may add to the incidence of NOMI by worsening tissue dysoxia in ICU patients. Experimental and observational studies suggest that the use of vasopressors such as norepinephrine and epinephrine might result in impaired mucosal perfusion[37-39]. Other pharmacological agents such as vasopressin and digoxin[3] as well as acute profound hypovolemia could also worsen ischemic lesions. Lastly, the role of enteral nutrition in critically ill patients is controversial and depends on several factors such as the dose of enteral nutrition, the metabolic phase, and the severity of the patients. In the recent randomized controlled trial "NUTRIREA 2"[40], enteral nutrition was compared to parenteral nutrition with a normocaloric target (*i.e.* 20-25 kcal/kg *per* day) during the first days of admission (*i.e.* catabolic phase) in mechanically ventilated patients with shock. Mortality did not differ between the two groups, but a significantly higher rate of bowel ischemia was reported in the enteral group [19 (2%) patients *vs* 5 (< 1%) patients]. However, an ancillary study focused on citrulline and intestinal-fatty acid binding protein (I-FABP) biomarkers showed possible protective effects of enteral nutrition on enterocyte mass, raising an interest for further investigation[41]. In particular, some hypothesized that a lower dose of enteral nutrition in these patients may yield a protective effect[42].

Compared with occlusive AMI, NOMI reported mortality is higher, ranging between 70% and 100% depending on the series[4,5,16]. While complications of NOMI are similar to those of occlusive AMI (including necrosis, perforation with peritonitis, bacteremia secondary to digestive translocations, acute compartment syndrome, vasoplegic shock, multi organ failure leading to death), their prognosis is indeed different[2]. In survivor patients, late AMI complications classically include short bowel syndrome, undernourishment, and need of total parental nutrition[43,44]. Of note, late outcomes in NOMI patients (*e.g.*, long-term mortality, quality of life) are currently unknown and should be investigated.

Several reasons may explain this poor prognosis in NOMI as compared to occlusive AMI. To start, in the former, patients are in a critically state, due to an earlier severe aggression, and NOMI represents a "second-hit" added on top of the reason for ICU admission. Secondly, treatment options do not allow a rapid reversal of the causal insult (as opposed to a revascularization of an occluded vessel), leading to late treatment and thus worse outcomes. Thirdly, surgical treatment is complex because of the lack of clear delimitation between viable and necrotic tissue: Lesions are often diffuse or patchy and extensive resections are then performed, when deemed relevant, which often is not the case after laparoscopic evaluation. Lastly, diagnosis is complex and requires multimodal approaches, leading to delays, as compared to obstructive AMI causes[3,45].

DIAGNOSTIC PITFALLS AND CHALLENGES FOR FUTURE RESEARCH

Facing a rapidly evolving disorder, the diagnostic process should provide answers to two important issues. First of all, diagnosing AMI early is essential to avoid progression to transmural necrosis and the associated complications[3,46]. Secondly, reliable information on the presence or absence of intestinal necrosis, and ideally on the intestinal location involved, is needed to guide decision regarding surgical treatment, according to the location and indication of surgery and patients' condition. The importance of this issue is summarized in Figure 1.

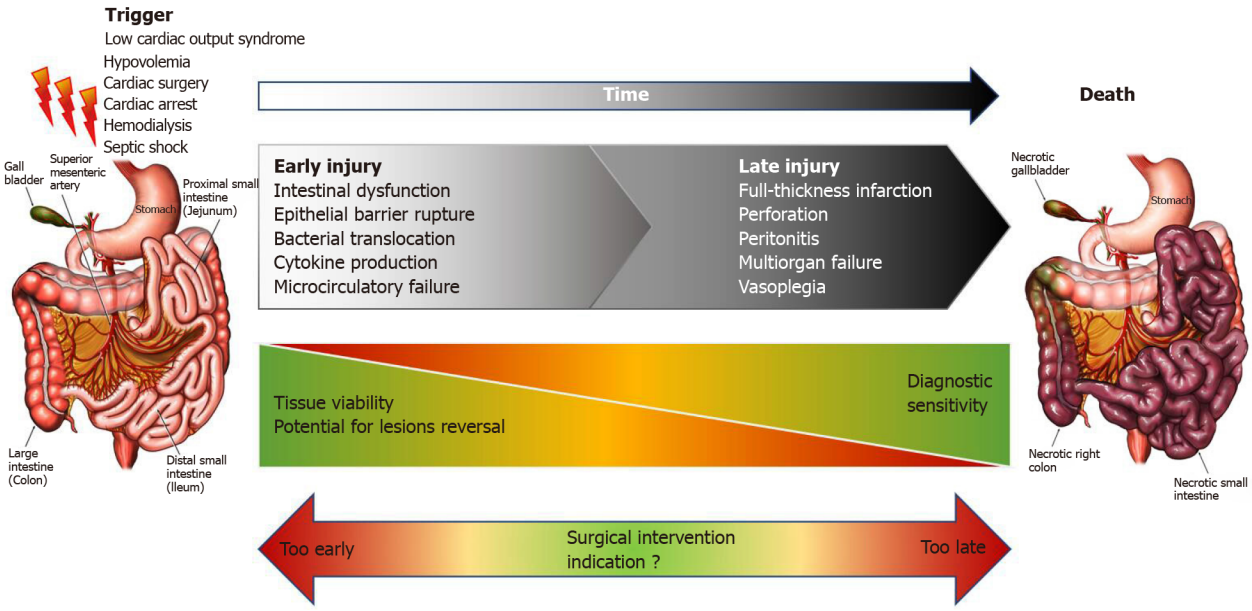

Figure 1 Timing of events in non-occlusive mesenteric ischemia.

As of today, these vital questions remain unanswered in the setting of NOMI and are often diagnosed too late. At time of diagnosis in NOMI studies, severity biological parameters are usually markedly high, and necrosis is frequently observed in comparison to recent occlusive AMI cohorts[4,5,16,47]. This severity, possibly associated with diagnostic delay, could partially explain the discrepancies between observed mortality rates.

Clinical examination

NOMI has always been presented as a challenging diagnosis due in particular to clinical signs considered to be non-specific[45,46]. Diagnosis is usually suspected in front of novel or worsening of circulatory failure in evocative contexts (*e.g.*, cardiac surgery or after cardiac resuscitation) and is discussed as a differential diagnosis of secondary infections[3]. Digestive signs, similar to that of an occlusive AMI presentation, might then evoke NOMI. These signs include possibly "brutal" abdominal pain, diarrhea, upper or lower digestive hemorrhage, and vomiting. However, a notable difference is that the beginning of occlusive AMI is often brutal allowing to pinpoint the exact onset time; in contrast, in the ICU setting, patients may be sedated and curarized, abdominal exam may not yield much, even if other non-specific digestive signs might suggest NOMI (abnormal gastric residual volume under enteral nutrition, ileus, increase of abdominal perimeter, increased intra-abdominal pressure).

Biomarkers

Biomarkers represent additive tools to this difficult diagnosis. Routine biomarkers, among those reflecting tissue ischemia, plasma lactate, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), can reinforce NOMI suspicion[45,48].

D-dimers have also been reported as highly sensitive for AMI diagnosis with plausible negative predictive value[49-51]. However, no study focused on a selected NOMI population in which it is likely to find multiple concurring causes for an increase in D-dimer levels, resulting in low specificity, and its dosage is not recommended[2]. Similarly, this conclusion can be drawn with LDH levels and leukocyte count[16,52,53].

After cardiac surgery, there has been interest in AST[53,54]. A value ≥ 100 IU/L was independently associated with AMI diagnosis in patients with multi organ failure[4]. Interestingly, AST was also associated with mortality in 780 AMI patients from various causes[15]. Thus, despite obvious lack of specificity, elevation of AST may reinforce clinical suspicion.

Procalcitonin has also been shown to be associated with mortality in AMI patients [55]. A threshold value of 2.47 ng/mL was suggested in a monocentric retrospective study of 128 AMI patients[56]. However, in a NOMI subgroup, procalcitonin may be less accurate given the high prevalence of acute renal failure and infections[57].

Serum lactate, a long-time marker of tissue ischemia, is usually associated with mortality in the AMI setting[15]. Despite a lack of specificity, lactate could be useful to predict necrosis when associated with other parameters. A prospective study of 67 selected patients with AMI identified three parameters associated with necrosis: Presence of organ failure, serum lactate levels > 2 mmol/L, and bowel dilation on computed tomography (CT) imaging[47]. When all three parameters were present, necrosis requiring surgical resection was highly likely. However, there was only one patient with NOMI in this cohort, and, in the setting of NOMI, an increase in plasma lactate levels is consistent with numerous possible etiologies.

Yet, clinical exam and routine laboratory tests are of only little value to make an early reliable diagnosis and to differentiate suspicion from confirmed NOMI[1,16,58].

Perspectives in biomarkers

Research is in progress to identify candidate AMI biomarkers. One of the most promising is I-FABP. Preliminary studies suggest a potential interest in I-FABP, a small cytosolic protein specific to small bowel released in the context of intestinal ischemia[59]. Experimental studies demonstrated early increase of I-FABP after onset of gut ischemia[60]. Thuijls *et al*[52] studied plasma and urinary I-FABP accuracy in 46 patients with a suspicion of AMI, of which 22 AMI cases were finally confirmed[52]. The area under the receiver-operating curve (AUC) for urinary I-FABP was 0.93, performing better than plasma I-FABP (AUC = 0.70). Notably, the increase in I-FABP was greater in patients with ischemia of the ileum, which is the main source of I-FABP production. However, in critical illness and particularly in NOMI, acute renal failure is highly prevalent, and urine samples might not be available. Further studies are needed to refine plasma I-FABP accuracy.

Interestingly, Matsumoto *et al*[53] found an AUC of 0.88 for AMI diagnosis including 15 cases of NOMI and 9 arterial occlusions[53]. The authors also highlighted that I-FABP is increased in various non-vascular intestinal ischemia etiologies such strangulated bowel obstruction, incarcerated hernia, and volvulus. While promising, the integration of plasma I-FABP to the routine monitoring of intestinal ischemia is probably too early at this point and should be further explored. In adults with septic shock, Sekino *et al*[61] measured daily plasma I-FABP in a monocentric observational study and found a higher incidence of NOMI when I-FABP levels were superior to a threshold of 19.0 ng/mL[61]. Importantly, I-FABP thresholds for AMI diagnosis are not consensual[29], and differences in accuracy of I-FABP dosage according to enzyme-linked immunosorbent assay kits lead to further limitations[62].

Plasmatic citrulline, an amino acid synthesized from glutamine by small bowel enterocytes and metabolized into arginine by the kidney, reflects functional enterocyte mass and has been proposed as a marker of acute intestinal failure in critically ill patients[63]. However, the high prevalence of acute renal failure in the ICU population may lead to high plasma citrulline concentrations despite a reduction of enterocyte mass[64]. Further studies are needed to precise its performance in critical illness and NOMI diagnosis. To a lesser extent, the ability of endothelin-1 to predict NOMI has been investigated in 78 post cardiac surgery patients and revealed high specificity (94%) but poor sensitivity (51%)[65].

Imaging

From clinical suspicion of NOMI to certitude, diagnosis relies on imaging. Historically, angiography was considered pivotal by some experts, as it was considered an efficient treatment for NOMI[66,67]. Angiographic observations of NOMI included the visualization of absence of large artery occlusion and vasoconstriction of small intestinal arteries. Subsequently, angiography enabled the *in situ* administration of a continuous infusion of vasodilatory drugs like papaverine. Small cohort studies reported efficacy, suggesting this treatment may be associated with fewer progression to necrosis, and improved survival[26]; the effectiveness of this strategy may not be warranted if NOMI is diagnosed at the stage of intestinal necrosis requiring surgical treatment. Moreover, tolerance of vasodilatory drugs in hemodynamically unstable patients is unclear, and given the low availability of the technique, it remains reserved for expert centers. Hence, evidence diagnostic and therapeutic angiography interventions in NOMI remain low, for now.

On the other hand, while contrast-enhanced abdominal CT scan plays a central role in occlusive AMI[68], indicating the occluded vessel and eventually guiding revascularization possibilities, its performance in NOMI is disappointing. A monocentric study compared the classical CT signs evoking AMI of 75 patients with NOMI, with 39 patients in which NOMI was suspected but subsequently ruled out, when compared to macroscopic diagnosis considered as reference[16]. Portal venous gas, pneumatosis

intestinalis, and abnormal contrast-induced bowel wall enhancement exhibited good specificities (respectively 95%, 85%, and 71%) but were poorly sensitive to the point, that one quarter of patients exhibited mesenteric ischemia without any suggestive radiological signs.

Abdominal ultrasound has been recently proposed for the investigation of acute gastrointestinal injury, emphasizing the possibilities to measure gastrointestinal diameter, mucosal thickness, peristalsis, and blood flow[69]. As of today, data on ultrasound performance for NOMI diagnosis are scarce, despite the evident advantage of being performed at the bedside non-invasively and the ability to diagnose bowel dilation, intramural, or portal venous gas[70,71].

Endoscopy

Finally, endoscopy is widely used in the ICU setting and presents the advantages of direct visualization of intestinal mucosa at the bedside. Given the relatively low negative predictive value of CT imaging, endoscopy is frequently performed and allows to diagnose a significant number of NOMI cases in the ICU. In post cardiac arrest patients, hemorrhagic or necrotic lesions are likely to be found during gut endoscopy in the presence of clinical signs of gastrointestinal dysfunction[72]. However, its disadvantages are numerous: A large part of the intestines (*i.e.* small bowel) are inaccessible, the observed mucosal necrosis does not always correspond to transmural necrosis, there exists an inherent risk of perforation in weakened tissues, and availability is dependent on the operators.

Hence, given the numerous pitfalls of the current diagnostic approach for the diagnosis of NOMI, a high index of suspicion is required in populations at risk, such as post cardiac or aortic surgery, hemodialyzed patients, and critically ill patients[23,73]. Research is encouraged to identify or validate new biomarkers and imagery tools and increase knowledge on the pathophysiological understanding of NOMI genesis, especially in critically ill patients[36]. Specific accuracy of these new biomarkers should be further evaluated in the future. However, well designed studies are incredibly difficult due to numerous issues. Importantly, the low incidence of NOMI requires an appropriate selection of the study population with consideration of the pre-test probability. Additionally, patients in which NOMI has been ruled out are difficult to define given the low negative predictive value of CT imaging. Methodological difficulties originate from the lack of knowledge of the physiopathology and the important variability of NOMI time course due to differences in the intensity and duration of the aggression at the origin of NOMI. Furthermore, a working group of the European Society of Intensive Care Medicine stated the need for a consensus definition of NOMI in order to improve the current knowledge, study epidemiology and suggest interventions[36].

Overview of AI in healthcare

AI is a vague term reflecting the use of computers to perform tasks that are thought to require unique skills, often in ways that are hard to pin-point and that evolve with time. For example, although basic game algorithms such as those initially developed for chess, were considered as such 50 years ago, they are now part of every personal computer, and we know how to break them down into discrete steps and feel we understand them[74].

Later, AI encompassed the field of image recognition[75]. Although we humans perform this task naturally, we often cannot articulate exactly how this is done. This lack of supervision is one of the features of machine learning (ML), a subset of AI. It is the study of algorithms that learn from experience without being explicitly programmed for their task. ML incorporates a broad range of statistical methods ranging from linear regression to support vector machines, decision trees, or neural networks that make use of new datapoints to update the function they approximate [76].

As introduced, this field is subdivided into supervised methods, which learn from labeled samples, and unsupervised methods that attempt to find patterns in the data themselves[76,77]. The main applications of supervised models are classification, in other words, choosing to which predefined class an observation belongs, and regression, in which a value is derived from given observations. In medical imagery, these two applications often amount to diagnosis (classification) and prognostication (regression). Clustering, in which observations are grouped in classes that are not predefined, and dimensionality reduction used for data structuring or visualization are the most common applications of unsupervised learning. Finally, in reinforcement learning, a model interacts with its environment, performs actions, and learns in a trial-and-error fashion. The main applications of reinforcement learning lie in decision

support tools and autonomous agents[78].

During the development of a ML system, the parameters of a model, termed weights, are gradually adjusted to fit a training dataset[79]. In a second step, the model is validated on a separate dataset. An evaluation on the initial dataset would result in overly optimistic results, dubbed the overfitting effect. This common phenomenon occurs when the ML model adapts itself too much to the training dataset and then fails to generalize on other datasets. In other terms, the model remembers the examples seen in the training phase but does not learn any relevant features that are applicable to future observations.

The simplest way to derive a model from a set of observations is variable thresholding. When combining multiple features, linear and logistic regression are the most frequently used techniques in healthcare but require the assumption of normality [80]. Methods capable of using non-linear discriminant functions such as support vector machines as well as methods relying on multiple linear boundaries such as decisions trees have been elaborated. While simple to implement, these models are limited in their ability to process raw data, such as images or time course data, as they struggle to model the relationships of large amounts of variables in multiple dimensions[81]. Instead, the elaboration of such models often needs to rely on considerable domain expertise to extract relevant traits from the raw data, yet, arbitrarily selected features and the underlying physiologic assumptions may fail to capture a specific individual's response. This is especially true for the analysis of medical images, in which every voxel represents an individual variable influenced by location, tissue type, and surrounding structures as well as time-sensitive data such as those recorded in standardized electronic healthcare records (EHRs).

Specific ML algorithms that are built in a multi-layered fashion, termed deep learning (DL) algorithms, circumvent this limitation by automatically encoding multiple levels of inner representations of relevant features. This is achieved by composing simple non-linear units that sequentially transform the representation, starting from the raw data, into a slightly more abstract representation at a deeper level. Visually, when analyzing images, the first two layers often represent edges and particular arrangements of edges. Subsequent deeper layers then assemble the motifs encoded in the prior layers into larger combinations representing parts of patterns featured in the raw data. The main advantage of this process is that the features are learnt by a general-purpose learning algorithm without any direct human intervention. This allows for the rapid development of models able to discover intricate features in multi-dimensional data. In the last decade, DL has led to major improvements in performance in the fields of computer vision[75,82] and natural language processing[83,84]. The main model architectures used in these domains are convolutional neural networks, recurrent neural networks (RNNs), auto-encoders, and transformers. Even if DL methods have been able to produce spectacular results, it is important to realize that these methods are still in their early days, and their performance does not always exceed that of conventional techniques using hand-selected features[85]. DL works well with large datasets but often requires specific computational infrastructure for the training process, whereas conventional ML methods have advantages for smaller datasets and can be created with classical processors.

The advantages brought by DL-powered data analyses have rapidly been taken over into the medical domain with first translations to radiology[86], ophthalmology [87] and pathology. Although the implementation of such algorithms in a clinical setting remains challenging[74], this progression has culminated in the approval of the first insurance reimbursement for AI augmented medical care[88] for the CT-based detection of large vessel occlusion in stroke. Modern ICUs generate vast streams of data stored in EHRs and current in-silico research has yielded successful DL tools to improve the prediction of mortality[89-93] and to guide clinical decisions[94]. A major focus has been the prediction of sepsis, which, analogously to NOMI, lacks a distinctive marker for an accurate and timely diagnosis. In recent years, multiple ML methods have emerged to diagnose sepsis in real-time or to predict its occurrence. The most prominent models relied on RNNs[95,96], custom hazard models[97] or a combination of multiple models, known as ensembles. Although clinical validation studies are often still lacking, these automated methods offer new possibilities for the early detection of sepsis based on objective variables extracted from EHR data[98,99]. Similarly, the lack of a gold standard non-invasive definition of NOMI and the need for rapid detection make for an excellent opportunity for the application of ML.

Diagnostic approaches in NOMI

Likewise, the diagnosis and management of NOMI highly depends on information obtained from imaging studies, clinical variables, and biological findings. Yet, no single marker allows for the accurate detection of intestinal ischemia. The expertise of gastroenterologists, intensivists, radiologists, and surgeons remains mandatory, but their availability and the time needed to process all these complex data may delay timely surgical intervention.

In NOMI prediction, multivariate logistic regressions models have been described several times[16,100-102]. When applying a threshold to a linear combination of weighted clinical variables, these have been used for the prediction of NOMI in 865 patients after cardiac surgery, of which 78 were angiographically confirmed to have developed mesenteric ischemia[19]. According to the authors, this linear discriminant analysis yielded a sensitivity of 76.9% and specificity of 93.8%. The interpretation of these results remains, however, limited as variables and weights were derived and tested in the same cohort. A follow-up logistic regression model used preoperative, intraoperative, and postoperative risk factors derived from 4449 patients after cardiac surgery to predict the occurrence of NOMI[103]. The authors report an AUC of 0.91 in their control cohort (n = 4299). Although these are encouraging results, the evaluation of these models suffers from methodological flaws as derivation and validation datasets of the model weights were not distinct.

Future models may benefit from more advanced algorithms such as those employed for the prediction of sepsis as discussed above. Furthermore, using continuous data streams instead of single timepoints as input would result in models with closer resemblance to clinical and physiologic reality. Long short-term memory networks in particular, a specific form of RNN, have shown promising results on temporal sequences sampled from ICU EHRs[93,95,96]. The so-called transformer, a successor model to long short-term memory networks integrating the concept of selective attention, has since emerged from the natural language processing domain[104]. Although the application of transformer models to medical EHRs is only beginning [105,106], it is possible model architecture will be prominent in the coming years.

Abdominal CT findings can reveal intestinal ischemia, although inter-rater agreement often remains limited. A multivariate combination of radiological signs has been identified through a logistic regression model in a cohort of 68 patients requiring cardiopulmonary bypass during surgery[107]. The resulting model was not accurately validated but performed well on the training cohort (AUC = 0.84). A model for the detection of transmural intestinal infarction confirmed on laparotomy has been elaborated on CT scans of 207 patients with superior mesenteric venous thrombosis [108]. A follow-up validation on an external cohort (n = 89), demonstrated satisfying performance (AUC = 0.84) and led to the development of a nomogram. Although this model has been developed in a different patient population, it remains one of the most accurately validated models for the detection of intestinal ischemia.

The use of image-based models could strongly improve performance and usability as they do not depend on the detection of a few selected findings and may integrate holistic imaging features, using convolutional neural networks such as those used in abdominal CT scans for the detection of acute appendicitis[109,110]. It is of note that although feature-based models (such as presence of pneumatosis intestinalis or abnormal bowel wall enhancement) for the detection of intestinal ischemia developed on patients with occlusion may translate to patients without occlusion, the features used by image-based models are often hidden to the user and will inadvertently rely on findings extracted from the site of occlusion. Much attention should therefore be paid to a careful selection of the study population and a clear restriction of use-cases for the developed models.

Perspectives in NOMI prediction

The availability of diverse and complex data points makes the use of AI for the detection and prognostication of NOMI in the ICU a valuable clinical opportunity. Currently developed ML models show encouraging results but lack rigorous statistical validation. Moreover, the use of state-of-the-art DL methods is likely to benefit model performance. As such, algorithms can encode an inner representation of relevant patterns and can approximate more complex non-linear functions, their use would forego the need of handcrafted features. This is especially relevant for the analysis of temporal sequences extracted from EHRs and abdominal CT scans. Indeed, although many signs of bowel ischemia have been identified, their performance[16] and inter-rater agreement remain limited[107,111]. Recent advances in fusion models leveraging both imaging and EHR data have performed well in the detection of pulmonary

embolism[112] and may be tested in NOMI. To fast-forward the development of future models there is a clear need for the collection and release of datasets incorporating patients with suspicion and definite diagnosis of NOMI. The diagnosis of NOMI in such datasets should ideally be verified pathologically or surgically, be it *via* laparotomy or laparoscopy, to obtain a clean target definition for model development. Moreover, it is essential to obtain clinical validation through prospective studies of not only the performance of developed models but also the ease of implementation into the ICU setting and their clinical utility following recently published guidelines[113, 114]. While most attention is often directed to increasing predictive performance, future AI solutions should account for their predictions to lead to wider clinical applicability and acceptance. Ideally, future AI systems should therefore strive to achieve ease-of-use, interpretability, and diagnostic performance.

It is likely that the results achieved by ML will continue to improve as the computational power at disposition increases, collected datasets grow, and more performant and adequate algorithms are developed. When applied to EHR data and medical imaging with statistical rigor, ML models could refine the accuracy and speed of diagnosis of NOMI in critically ill patients. Used appropriately, this emerging technology could further be leveraged to identify and explore new disease mechanisms and single-out yet unrealized connections between datapoints, paving the way for a deeper understanding of the intricated interactions leading to NOMI in the ICU.

CONCLUSION

NOMI is associated with poor prognosis due to lack of accurate diagnostic tools. While taken individually, several biomarkers and imagery modalities exist, their combination and the study of their variation through time, which requires sheer computational power that may be provided by artificial intelligent tools, is bound to increase diagnostic performance in NOMI and improve therapeutic management.

ACKNOWLEDGEMENTS

We thank Pr Alain Cariou for participating in the inception of this work.

REFERENCES

1 **Clair DG**, Beach JM. Mesenteric Ischemia. *N Engl J Med* 2016; **374**: 959-968 [PMID: 26962730 DOI: 10.1056/NEJMra1503884]

2 **Björck M**, Koelemay M, Acosta S, Bastos Goncalves F, Kölbel T, Kolkman JJ, Lees T, Lefevre JH, Menyhei G, Oderich G; Esvs Guidelines Committee, Kolh P, de Borst GJ, Chakfe N, Debus S, Hinchliffe R, Kakkos S, Koncar I, Sanddal Lindholt J, Vega de Ceniga M, Vermassen F, Verzini F, Document Reviewers, Geelkerken B, Gloviczki P, Huber T, Naylor R. Editor's Choice - Management of the Diseases of Mesenteric Arteries and Veins: Clinical Practice Guidelines of the European Society of Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg* 2017; **53**: 460-510 [PMID: 28359440 DOI: 10.1016/j.ejvs.2017.01.010]

3 **Oldenburg WA**, Lau LL, Rodenberg TJ, Edmonds HJ, Burger CD. Acute mesenteric ischemia: a clinical review. *Arch Intern Med* 2004; **164**: 1054-1062 [PMID: 15159262 DOI: 10.1001/archinte.164.10.1054]

4 **Guillaume A**, Pili-Floury S, Chocron S, Delabrousse E, De Parseval B, Koch S, Samain E, Capellier G, Piton G. Acute Mesenteric Ischemia Among Postcardiac Surgery Patients Presenting with Multiple Organ Failure. *Shock* 2017; **47**: 296-302 [PMID: 28195969 DOI: 10.1097/SHK.0000000000000720]

5 **Paul M**, Bougouin W, Legriel S, Charpentier J, Jaubert P, Savary G, Bourcier S, Pène F, Dumas F, Grimaldi D, Cariou A. Frequency, risk factors, and outcomes of non-occlusive mesenteric ischaemia after cardiac arrest. *Resuscitation* 2020; **157**: 211-218 [PMID: 33027618 DOI: 10.1016/j.resuscitation.2020.09.028]

6 **Ende N**. Infarction of the Bowel in Cardiac Failure. *N Engl J Med* 1958; **258**: 879-881 [DOI: 10.1056/NEJM195805012581804]

7 **Wilcox MG**, Howard TJ, Plaskon LA, Unthank JL, Madura JA. Current theories of pathogenesis and treatment of nonocclusive mesenteric ischemia. *Dig Dis Sci* 1995; **40**: 709-716 [PMID: 7720458 DOI: 10.1007/BF02064966]

8 **Acosta S**, Ogren M, Sternby NH, Bergqvist D, Björck M. Fatal nonocclusive mesenteric ischaemia:

population-based incidence and risk factors. *J Intern Med* 2006; **259**: 305-313 [PMID: 16476108 DOI: 10.1111/j.1365-2796.2006.01613.x]
9 **Sitges-Serra A**, Mas X, Roqueta F, Figueras J, Sanz F. Mesenteric infarction: an analysis of 83 patients with prognostic studies in 44 cases undergoing a massive small-bowel resection. *Br J Surg* 1988; **75**: 544-548 [PMID: 3395820 DOI: 10.1002/bjs.1800750614]
10 **Endean ED**, Barnes SL, Kwolek CJ, Minion DJ, Schwarcz TH, Mentzer RM Jr. Surgical management of thrombotic acute intestinal ischemia. *Ann Surg* 2001; **233**: 801-808 [PMID: 11407335 DOI: 10.1097/00000658-200106000-00010]
11 **Zeier M**, Wiesel M, Rambausek M, Ritz E. Non-occlusive mesenteric infarction in dialysis patients: the importance of prevention and early intervention. *Nephrol Dial Transplant* 1995; **10**: 771-773 [PMID: 7566602]
12 **Quiroga B**, Verde E, Abad S, Vega A, Goicoechea M, Reque J, López-Gómez JM, Luño J. Detection of patients at high risk for non-occlusive mesenteric ischemia in hemodialysis. *J Surg Res* 2013; **180**: 51-55 [PMID: 23122519 DOI: 10.1016/j.jss.2012.10.008]
13 **Hatemi I**, Hatemi G, Çelik AF. Systemic vasculitis and the gut. *Curr Opin Rheumatol* 2017; **29**: 33-38 [PMID: 27684357 DOI: 10.1097/BOR.0000000000000344]
14 **Björck M**, Wanhainen A. Nonocclusive mesenteric hypoperfusion syndromes: recognition and treatment. *Semin Vasc Surg* 2010; **23**: 54-64 [PMID: 20298950 DOI: 10.1053/j.semvascsurg.2009.12.009]
15 **Leone M**, Bechis C, Baumstarck K, Ouattara A, Collange O, Augustin P, Annane D, Arbelot C, Asehnoune K, Baldési O, Bourcier S, Delapierre L, Demory D, Hengy B, Ichai C, Kipnis E, Brasdefer E, Lasocki S, Legrand M, Mimoz O, Rimmelé T, Aliane J, Bertrand PM, Bruder N, Klasen F, Friou E, Lévy B, Martinez O, Peytel E, Piton A, Richter E, Toufik K, Vogler MC, Wallet F, Boufi M, Allaouchiche B, Constantin JM, Martin C, Jaber S, Lefrant JY. Outcome of acute mesenteric ischemia in the intensive care unit: a retrospective, multicenter study of 780 cases. *Intensive Care Med* 2015; **41**: 667-676 [PMID: 25731634 DOI: 10.1007/s00134-015-3690-8]
16 **Bourcier S**, Oudjit A, Goudard G, Charpentier J, Leblanc S, Coriat R, Gouya H, Dousset B, Mira JP, Pène F. Diagnosis of non-occlusive acute mesenteric ischemia in the intensive care unit. *Ann Intensive Care* 2016; **6**: 112 [PMID: 27858375 DOI: 10.1186/s13613-016-0213-x]
17 **Björck M**, Bergqvist D, Troëng T. Incidence and clinical presentation of bowel ischaemia after aortoiliac surgery--2930 operations from a population-based registry in Sweden. *Eur J Vasc Endovasc Surg* 1996; **12**: 139-144 [PMID: 8760974 DOI: 10.1016/s1078-5884(96)80098-0]
18 **Nilsson J**, Hansson E, Andersson B. Intestinal ischemia after cardiac surgery: analysis of a large registry. *J Cardiothorac Surg* 2013; **8**: 156 [PMID: 23777600 DOI: 10.1186/1749-8090-8-156]
19 **Groesdonk HV**, Klingele M, Schlempp S, Bomberg H, Schmied W, Minko P, Schäfers HJ. Risk factors for nonocclusive mesenteric ischemia after elective cardiac surgery. *J Thorac Cardiovasc Surg* 2013; **145**: 1603-1610 [PMID: 23219496 DOI: 10.1016/j.jtcvs.2012.11.022]
20 **Wurm R**, Cho A, Arfsten H, van Tulder R, Wallmüller C, Steininger P, Sterz F, Tendl K, Balassy C, Distelmaier K, Hülsmann M, Heinz G, Adlbrecht C. Non-occlusive mesenteric ischaemia in out of hospital cardiac arrest survivors. *Eur Heart J Acute Cardiovasc Care* 2018; **7**: 450-458 [PMID: 28045326 DOI: 10.1177/2048872616687096]
21 **Daviaud F**, Grimaldi D, Dechartres A, Charpentier J, Geri G, Marin N, Chiche JD, Cariou A, Mira JP, Pène F. Timing and causes of death in septic shock. *Ann Intensive Care* 2015; **5**: 16 [PMID: 26092499 DOI: 10.1186/s13613-015-0058-8]
22 **Reilly PM**, Wilkins KB, Fuh KC, Haglund U, Bulkley GB. The mesenteric hemodynamic response to circulatory shock: an overview. *Shock* 2001; **15**: 329-343 [PMID: 11336191 DOI: 10.1097/00024382-200115050-00001]
23 **Reintam Blaser A**, Acosta S, Arabi YM. A clinical approach to acute mesenteric ischemia. *Curr Opin Crit Care* 2021; **27**: 183-192 [PMID: 33395084 DOI: 10.1097/MCC.0000000000000802]
24 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483 [PMID: 5457245 DOI: 10.1001/archsurg.1970.01340280030009]
25 **Corcos O**, Castier Y, Sibert A, Gaujoux S, Ronot M, Joly F, Paugam C, Bretagnol F, Abdel-Rehim M, Francis F, Bondjemah V, Ferron M, Zappa M, Amiot A, Stefanescu C, Leseche G, Marmuse JP, Belghiti J, Ruszniewski P, Vilgrain V, Panis Y, Mantz J, Bouhnik Y. Effects of a multimodal management strategy for acute mesenteric ischemia on survival and intestinal failure. *Clin Gastroenterol Hepatol* 2013; **11**: 158-65. e2 [PMID: 23103820 DOI: 10.1016/j.cgh.2012.10.027]
26 **Klotz S**, Vestring T, Rötker J, Schmidt C, Scheld HH, Schmid C. Diagnosis and treatment of nonocclusive mesenteric ischemia after open heart surgery. *Ann Thorac Surg* 2001; **72**: 1583-1586 [PMID: 11722048 DOI: 10.1016/s0003-4975(01)03179-4]
27 **Grimaldi D**, Guivarch E, Neveux N, Fichet J, Pène F, Marx JS, Chiche JD, Cynober L, Mira JP, Cariou A. Markers of intestinal injury are associated with endotoxemia in successfully resuscitated patients. *Resuscitation* 2013; **84**: 60-65 [PMID: 22743354 DOI: 10.1016/j.resuscitation.2012.06.010]
28 **Piton G**, Cypriani B, Regnard J, Patry C, Puyraveau M, Capellier G. Catecholamine use is associated with enterocyte damage in critically ill patients. *Shock* 2015; **43**: 437-442 [PMID: 25565647 DOI: 10.1097/SHK.0000000000000327]
29 **Piton G**, Capellier G. Biomarkers of gut barrier failure in the ICU. *Curr Opin Crit Care* 2016; **22**: 152-160 [PMID: 26808138 DOI: 10.1097/MCC.0000000000000283]
30 **Ait-Oufella H**, Bourcier S, Lehoux S, Guidet B. Microcirculatory disorders during septic shock.

Curr Opin Crit Care 2015; **21**: 271-275 [PMID: 26103150 DOI: 10.1097/MCC.0000000000000217]

31 **Dubin A**, Edul VS, Pozo MO, Murias G, Canullán CM, Martins EF, Ferrara G, Canales HS, Laporte M, Estenssoro E, Ince C. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med* 2008; **36**: 535-542 [PMID: 18216603 DOI: 10.1097/01.CCM.0000300083.74726.43]

32 **Singer M**, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; **315**: 801-810 [PMID: 26903338 DOI: 10.1001/jama.2016.0287]

33 **Abraham E**, Singer M. Mechanisms of sepsis-induced organ dysfunction. *Crit Care Med* 2007; **35**: 2408-2416 [PMID: 17948334 DOI: 10.1097/01.ccm.0000282072.56245.91]

34 **Lobo SM**, De Backer D, Sun Q, Tu Z, Dimopoulos G, Preiser JC, Nagy N, Vray B, Vercruy V, Terzi RG, Vincent JL. Gut mucosal damage during endotoxic shock is due to mechanisms other than gut ischemia. *J Appl Physiol (1985)* 2003; **95**: 2047-2054 [PMID: 12923122 DOI: 10.1152/japplphysiol.00925.2002]

35 **Reintam Blaser A**, Malbrain ML, Starkopf J, Fruhwald S, Jakob SM, De Waele J, Braun JP, Poeze M, Spies C. Gastrointestinal function in intensive care patients: terminology, definitions and management. Recommendations of the ESICM Working Group on Abdominal Problems. *Intensive Care Med* 2012; **38**: 384-394 [PMID: 22310869 DOI: 10.1007/s00134-011-2459-y]

36 **Reintam Blaser A**, Preiser JC, Fruhwald S, Wilmer A, Wernerman J, Benstoem C, Casaer MP, Starkopf J, van Zanten A, Rooyackers O, Jakob SM, Loudet CI, Bear DE, Elke G, Kott M, Lautenschläger I, Schäper J, Gunst J, Stoppe C, Nobile L, Fuhrmann V, Berger MM, Oudemans-van Straaten HM, Arabi YM, Deane AM; Working Group on Gastrointestinal Function within the Section of Metabolism, Endocrinology and Nutrition (MEN Section) of ESICM. Gastrointestinal dysfunction in the critically ill: a systematic scoping review and research agenda proposed by the Section of Metabolism, Endocrinology and Nutrition of the European Society of Intensive Care Medicine. *Crit Care* 2020; **24**: 224 [PMID: 32414423 DOI: 10.1186/s13054-020-02889-4]

37 **Meier-Hellmann A**, Reinhart K, Bredle DL, Specht M, Spies CD, Hannemann L. Epinephrine impairs splanchnic perfusion in septic shock. *Crit Care Med* 1997; **25**: 399-404 [PMID: 9118653 DOI: 10.1097/00003246-199703000-00005]

38 **De Backer D**, Creteur J, Silva E, Vincent JL. Effects of dopamine, norepinephrine, and epinephrine on the splanchnic circulation in septic shock: which is best? *Crit Care Med* 2003; **31**: 1659-1667 [PMID: 12794401 DOI: 10.1097/01.CCM.0000063045.77339.B6]

39 **Krychtiuk KA**, Richter B, Lenz M, Hohensinner PJ, Huber K, Hengstenberg C, Wojta J, Heinz G, Speidl WS. Epinephrine treatment but not time to ROSC is associated with intestinal injury in patients with cardiac arrest. *Resuscitation* 2020; **155**: 32-38 [PMID: 32522698 DOI: 10.1016/j.resuscitation.2020.05.046]

40 **Reignier J**, Boisramé-Helms J, Brisard L, Lascarrou JB, Ait Hssain A, Anguel N, Argaud L, Asehnoune K, Asfar P, Bellec F, Botoc V, Bretagnol A, Bui HN, Canet E, Da Silva D, Darmon M, Das V, Devaquet J, Djibre M, Ganster F, Garrouste-Orgeas M, Gaudry S, Gontier O, Guérin C, Guidet B, Guitton C, Herbrecht JE, Lacherade JC, Letocart P, Martino F, Maxime V, Mercier E, Mira JP, Nseir S, Piton G, Quenot JP, Richecoeur J, Rigaud JP, Robert R, Rolin N, Schwebel C, Sirodot M, Tinturier F, Thévenin D, Giraudeau B, Le Gouge A; NUTRIREA-2 Trial Investigators; Clinical Research in Intensive Care and Sepsis (CRICS) group. Enteral versus parenteral early nutrition in ventilated adults with shock: a randomised, controlled, multicentre, open-label, parallel-group study (NUTRIREA-2). *Lancet* 2018; **391**: 133-143 [PMID: 29128300 DOI: 10.1016/S0140-6736(17)32146-3]

41 **Piton G**, Le Gouge A, Brulé N, Cypriani B, Lacherade JC, Nseir S, Mira JP, Mercier E, Sirodot M, Rigaud JP, Malaquin S, Soum E, Djibre M, Gaudry S, Thévenin D, Reignier J. Impact of the route of nutrition on gut mucosa in ventilated adults with shock: an ancillary of the NUTRIREA-2 trial. *Intensive Care Med* 2019; **45**: 948-956 [PMID: 31143999 DOI: 10.1007/s00134-019-05649-3]

42 **Singer P**, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer MP, Hiesmayr M, Mayer K, Montejo JC, Pichard C, Preiser JC, van Zanten ARH, Oczkowski S, Szczeklik W, Bischoff SC. ESPEN guideline on clinical nutrition in the intensive care unit. *Clin Nutr* 2019; **38**: 48-79 [PMID: 30348463 DOI: 10.1016/j.clnu.2018.08.037]

43 **Huisman-de Waal G**, Schoonhoven L, Jansen J, Wanten G, van Achterberg T. The impact of home parenteral nutrition on daily life-a review. *Clin Nutr* 2007; **26**: 275-288 [PMID: 17161888 DOI: 10.1016/j.clnu.2006.10.002]

44 **Gupta PK**, Natarajan B, Gupta H, Fang X, Fitzgibbons RJ Jr. Morbidity and mortality after bowel resection for acute mesenteric ischemia. *Surgery* 2011; **150**: 779-787 [PMID: 22000191 DOI: 10.1016/j.surg.2011.07.079]

45 **van den Heijkant TC**, Aerts BA, Teijink JA, Buurman WA, Luyer MD. Challenges in diagnosing mesenteric ischemia. *World J Gastroenterol* 2013; **19**: 1338-1341 [PMID: 23538325 DOI: 10.3748/wjg.v19.i9.1338]

46 **Howard TJ**, Plaskon LA, Wiebke EA, Wilcox MG, Madura JA. Nonocclusive mesenteric ischemia remains a diagnostic dilemma. *Am J Surg* 1996; **171**: 405-408 [PMID: 8604831 DOI: 10.1016/S0002-9610(97)89619-5]

47 **Nuzzo A**, Maggiori L, Ronot M, Becq A, Plessier A, Gault N, Joly F, Castier Y, Vilgrain V, Paugam

C, Panis Y, Bouhnik Y, Cazals-Hatem D, Corcos O. Predictive Factors of Intestinal Necrosis in Acute Mesenteric Ischemia: Prospective Study from an Intestinal Stroke Center. *Am J Gastroenterol* 2017; **112**: 597-605 [PMID: 28266590 DOI: 10.1038/ajg.2017.38]

48 **Montagnana M**, Danese E, Lippi G. Biochemical markers of acute intestinal ischemia: possibilities and limitations. *Ann Transl Med* 2018; **6**: 341 [PMID: 30306080 DOI: 10.21037/atm.2018.07.22]

49 **Acosta S**, Nilsson TK, Björck M. D-dimer testing in patients with suspected acute thromboembolic occlusion of the superior mesenteric artery. *Br J Surg* 2004; **91**: 991-994 [PMID: 15286959 DOI: 10.1002/bjs.4645]

50 **Akyildiz H**, Akcan A, Oztürk A, Sozuer E, Kucuk C, Karahan I. The correlation of the D-dimer test and biphasic computed tomography with mesenteric computed tomography angiography in the diagnosis of acute mesenteric ischemia. *Am J Surg* 2009; **197**: 429-433 [PMID: 19324109 DOI: 10.1016/j.amjsurg.2008.02.011]

51 **Chiu YH**, Huang MK, How CK, Hsu TF, Chen JD, Chern CH, Yen DH, Huang CI. D-dimer in patients with suspected acute mesenteric ischemia. *Am J Emerg Med* 2009; **27**: 975-979 [PMID: 19857417 DOI: 10.1016/j.ajem.2009.06.006]

52 **Thuijls G**, van Wijck K, Grootjans J, Derikx JP, van Bijnen AA, Heineman E, Dejong CH, Buurman WA, Poeze M. Early diagnosis of intestinal ischemia using urinary and plasma fatty acid binding proteins. *Ann Surg* 2011; **253**: 303-308 [PMID: 21245670 DOI: 10.1097/SLA.0b013e318207a767]

53 **Matsumoto S**, Sekine K, Funaoka H, Yamazaki M, Shimizu M, Hayashida K, Kitano M. Diagnostic performance of plasma biomarkers in patients with acute intestinal ischaemia. *Br J Surg* 2014; **101**: 232-238 [PMID: 24402763 DOI: 10.1002/bjs.9331]

54 **Mitsuyoshi A**, Obama K, Shinkura N, Ito T, Zaima M. Survival in nonocclusive mesenteric ischemia: early diagnosis by multidetector row computed tomography and early treatment with continuous intravenous high-dose prostaglandin E(1). *Ann Surg* 2007; **246**: 229-235 [PMID: 17667501 DOI: 10.1097/01.sla.0000263157.59422.76]

55 **Merle C**, Lepouse C, De Garine A, Frayssinet N, Leymarie F, Leon A, Jolly D. Surgery for mesenteric infarction: prognostic factors associated with early death within 72 hours. *J Cardiothorac Vasc Anesth* 2004; **18**: 734-741 [PMID: 15650983 DOI: 10.1053/j.jvca.2004.08.011]

56 **Cosse C**, Sabbagh C, Browet F, Mauvais F, Rebibo L, Zogheib E, Chatelain D, Kamel S, Regimbeau JM. Serum value of procalcitonin as a marker of intestinal damages: type, extension, and prognosis. *Surg Endosc* 2015; **29**: 3132-3139 [PMID: 25701059 DOI: 10.1007/s00464-014-4038-0]

57 **Leone M**, Lefrant JY, Martin C, Constantin JM. Acute mesenteric ischemia, procalcitonin, and intensive care unit. *Intensive Care Med* 2015; **41**: 1378 [PMID: 26077064 DOI: 10.1007/s00134-015-3867-1]

58 **Bourcier S**, Combes A. The NUTRIREA-2 study. *Lancet* 2019; **393**: 1501-1502 [PMID: 30983584 DOI: 10.1016/S0140-6736(18)33198-2]

59 **Kanda T**, Fujii H, Tani T, Murakami H, Suda T, Sakai Y, Ono T, Hatakeyama K. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology* 1996; **110**: 339-343 [PMID: 8566578 DOI: 10.1053/gast.1996.v110.pm8566578]

60 **Schellekens DH**, Grootjans J, Dello SA, van Bijnen AA, van Dam RM, Dejong CH, Derikx JP, Buurman WA. Plasma intestinal fatty acid-binding protein levels correlate with morphologic epithelial intestinal damage in a human translational ischemia-reperfusion model. *J Clin Gastroenterol* 2014; **48**: 253-260 [PMID: 24100750 DOI: 10.1097/MCG.0b013e3182a87e3e]

61 **Sekino M**, Funaoka H, Sato S, Okada K, Inoue H, Yano R, Matsumoto S, Ichinomiya T, Higashijima U, Hara T. Intestinal fatty acid-binding protein level as a predictor of 28-day mortality and bowel ischemia in patients with septic shock: A preliminary study. *J Crit Care* 2017; **42**: 92-100 [PMID: 28710988 DOI: 10.1016/j.jcrc.2017.07.012]

62 **Treskes N**, Persoon AM, van Zanten ARH. Diagnostic accuracy of novel serological biomarkers to detect acute mesenteric ischemia: a systematic review and meta-analysis. *Intern Emerg Med* 2017; **12**: 821-836 [PMID: 28478489 DOI: 10.1007/s11739-017-1668-y]

63 **Piton G**, Manzon C, Monnet E, Cypriani B, Barbot O, Navellou JC, Carbonnel F, Capellier G. Plasma citrulline kinetics and prognostic value in critically ill patients. *Intensive Care Med* 2010; **36**: 702-706 [PMID: 20084502 DOI: 10.1007/s00134-010-1751-6]

64 **Piton G**, Manzon C, Cypriani B, Carbonnel F, Capellier G. Acute intestinal failure in critically ill patients: is plasma citrulline the right marker? *Intensive Care Med* 2011; **37**: 911-917 [PMID: 21400011 DOI: 10.1007/s00134-011-2172-x]

65 **Groesdonk HV**, Raffel M, Speer T, Bomberg H, Schmied W, Klingele M, Schäfers HJ. Elevated endothelin-1 level is a risk factor for nonocclusive mesenteric ischemia. *J Thorac Cardiovasc Surg* 2015; **149**: 1436-42. e2 [PMID: 25623906 DOI: 10.1016/j.jtcvs.2014.12.019]

66 **Brandt LJ**, Boley SJ. AGA technical review on intestinal ischemia. American Gastrointestinal Association. *Gastroenterology* 2000; **118**: 954-968 [PMID: 10784596 DOI: 10.1016/s0016-5085(00)70183-1]

67 **Trompeter M**, Brazda T, Remy CT, Vestring T, Reimer P. Non-occlusive mesenteric ischemia: etiology, diagnosis, and interventional therapy. *Eur Radiol* 2002; **12**: 1179-1187 [PMID: 11976865 DOI: 10.1007/s00330-001-1220-2]

68 ID, Kroeker MA, Greenberg HM. Biphasic CT with mesenteric CT angiography in the evaluation of acute mesenteric ischemia: initial experience. *Radiology* 2003; **229**: 91-98 [PMID: 12944600 DOI: 10.1148/radiol.2291020991]

69 **Perez-Calatayud AA**, Carrillo-Esper R, Anica-Malagon ED, Briones-Garduño JC, Arch-Tirado E, Wise R, Malbrain MLNG. Point-of-care gastrointestinal and urinary tract sonography in daily evaluation of gastrointestinal dysfunction in critically ill patients (GUTS Protocol). *Anaesthesiol Intensive Ther* 2018; **50**: 40-48 [PMID: 29303209 DOI: 10.5603/AIT.a2017.0073]

70 **McCarthy E**, Little M, Briggs J, Sutcliffe J, Tapping CR, Patel R, Bratby MJ, Uberoi R. Radiology and mesenteric ischaemia. *Clin Radiol* 2015; **70**: 698-705 [PMID: 25812475 DOI: 10.1016/j.crad.2015.02.012]

71 **Piton G**, Capellier G, Delabrousse E. Echography of the Portal Vein in a Patient With Shock. *Crit Care Med* 2016; **44**: e443-e445 [PMID: 26646463 DOI: 10.1097/CCM.0000000000001476]

72 **L'Her E**, Cassaz C, Le Gal G, Cholet F, Renault A, Boles JM. Gut dysfunction and endoscopic lesions after out-of-hospital cardiac arrest. *Resuscitation* 2005; **66**: 331-334 [PMID: 16039032 DOI: 10.1016/j.resuscitation.2005.03.016]

73 **Kougias P**, Lau D, El Sayed HF, Zhou W, Huynh TT, Lin PH. Determinants of mortality and treatment outcome following surgical interventions for acute mesenteric ischemia. *J Vasc Surg* 2007; **46**: 467-474 [PMID: 17681712 DOI: 10.1016/j.jvs.2007.04.045]

74 **Meskó B**, Görög M. A short guide for medical professionals in the era of artificial intelligence. *NPJ Digit Med* 2020; **3**: 126 [PMID: 33043150 DOI: 10.1038/s41746-020-00333-z]

75 **Krizhevsky A**, Sutskever I, Hinton GE. ImageNet Classification with Deep Convolutional Neural Networks. *Adv Neural Inf Process Syst* 2012; **25**: 1097-1105

76 **Mitchell TM**. Machine Learning. McGraw-Hill, 2003

77 **Hinton G**, Sejnowski TJ. Unsupervised Learning: Foundations of Neural Computation. MIT Press, 1999

78 **Andrew G**, Barto RSS. Reinforcement Learning: An Introduction. A Bradford Book, 1998

79 **Hinton G**. Deep Learning-A Technology With the Potential to Transform Health Care. *JAMA* 2018; **320**: 1101-1102 [PMID: 30178065 DOI: 10.1001/jama.2018.11100]

80 **Schmidt AF**, Finan C. Linear regression and the normality assumption. *J Clin Epidemiol* 2018; **98**: 146-151 [PMID: 29258908 DOI: 10.1016/j.jclinepi.2017.12.006]

81 **LeCun Y**, Bengio Y, Hinton G. Deep learning. *Nature* 2015; **521**: 436-444 [PMID: 26017442 DOI: 10.1038/nature14539]

82 **Farabet C**, Couprie C, Najman L, Lecun Y. Learning hierarchical features for scene labeling. *IEEE Trans Pattern Anal Mach Intell* 2013; **35**: 1915-1929 [PMID: 23787344 DOI: 10.1109/TPAMI.2012.231]

83 **Howard J**, Ruder S. Universal Language Model Fine-tuning for Text Classification. 2018 Preprint. Available from: arXiv:180106146

84 **Peters ME**, Neumann M, Iyyer M, Gardner M, Clark C, Lee K, Zettlemoyer L. Deep contextualized word representations. 2018 Preprint. Available from: arXiv:180205365

85 **Liu X**, Faes L, Kale AU, Wagner SK, Fu DJ, Bruynseels A, Mahendiran T, Moraes G, Shamdas M, Kern C, Ledsam JR, Schmid MK, Balaskas K, Topol EJ, Bachmann LM, Keane PA, Denniston AK. A comparison of deep learning performance against health-care professionals in detecting diseases from medical imaging: a systematic review and meta-analysis. *Lancet Digit Health* 2019; **1**: e271-e297 [PMID: 33323251 DOI: 10.1016/S2589-7500(19)30123-2]

86 **Rajpurkar P**, Irvin J, Zhu K, Yang B, Mehta H, Duan T, Ding D, Bagul A, Langlotz C, Shpanskaya K, Lungren MP, Ng AY. CheXNet: Radiologist-Level Pneumonia Detection on Chest X-Rays with Deep Learning. 2017 Preprint. Available from: arXiv:171105225

87 **De Fauw J**, Ledsam JR, Romera-Paredes B, Nikolov S, Tomasev N, Blackwell S, Askham H, Glorot X, O'Donoghue B, Visentin D, van den Driessche G, Lakshminarayanan B, Meyer C, Mackinder F, Bouton S, Ayoub K, Chopra R, King D, Karthikesalingam A, Hughes CO, Raine R, Hughes J, Sim DA, Egan C, Tufail A, Montgomery H, Hassabis D, Rees G, Back T, Khaw PT, Suleyman M, Cornebise J, Keane PA, Ronneberger O. Clinically applicable deep learning for diagnosis and referral in retinal disease. *Nat Med* 2018; **24**: 1342-1350 [PMID: 30104768 DOI: 10.1038/s41591-018-0107-6]

88 **Federal Register**. Medicare Program; Hospital Inpatient Prospective Payment Systems for Acute Care Hospitals and the Long-Term Care Hospital Prospective Payment System and Final Policy Changes and Fiscal Year 2021 Rates; Quality Reporting and Medicare and Medicaid Promoting Interoperability Programs Requirements for Eligible Hospitals and Critical Access Hospitals. [cited 10 January 2021]. Available from: https://www.federalregister.gov/documents/2020/09/18/2020-19637/medicare-program-hospital-inpatient-prospective-payment-systems-for-acute-care-hospitals-and-the

89 **Saria S**, Rajani AK, Gould J, Koller D, Penn AA. Integration of early physiological responses predicts later illness severity in preterm infants. *Sci Transl Med* 2010; **2**: 48ra65 [PMID: 20826840 DOI: 10.1126/scitranslmed.3001304]

90 **Johnson AE**, Ghassemi MM, Nemati S, Niehaus KE, Clifton DA, Clifford GD. Machine Learning and Decision Support in Critical Care. *Proc IEEE Inst Electr Electron Eng* 2016; **104**: 444-466 [PMID: 27765959 DOI: 10.1109/JPROC.2015.2501978]

91 **Aczon M**, Ledbetter D, Ho L, Gunny A, Flynn A, Williams J, Wetzel R. Dynamic Mortality Risk Predictions in Pediatric Critical Care Using Recurrent Neural Networks. 2017 Preprint. Available from: arXiv:170106675

92 **Nielsen AB**, Thorsen-Meyer HC, Belling K, Nielsen AP, Thomas CE, Chmura PJ, Lademann M, Moseley PL, Heimann M, Dybdahl L, Spangsege L, Hulsen P, Perner A, Brunak S. Survival

prediction in intensive-care units based on aggregation of long-term disease history and acute physiology: a retrospective study of the Danish National Patient Registry and electronic patient records. *Lancet Digit Health* 2019; **1**: e78-e89 [PMID: 33323232 DOI: 10.1016/S2589-7500(19)30024-X]

93 **Thorsen-Meyer HC**, Nielsen AB, Nielsen AP, Kaas-Hansen BS, Toft P, Schierbeck J, Strøm T, Chmura PJ, Heimann M, Dybdahl L, Spangsege L, Hulsen P, Belling K, Brunak S, Perner A. Dynamic and explainable machine learning prediction of mortality in patients in the intensive care unit: a retrospective study of high-frequency data in electronic patient records. *Lancet Digit Health* 2020; **2**: e179-e191 [PMID: 33328078 DOI: 10.1016/S2589-7500(20)30018-2]

94 **Komorowski M**, Celi LA, Badawi O, Gordon AC, Faisal AA. The Artificial Intelligence Clinician learns optimal treatment strategies for sepsis in intensive care. *Nat Med* 2018; **24**: 1716-1720 [PMID: 30349085 DOI: 10.1038/s41591-018-0213-5]

95 **Kam HJ**, Kim HY. Learning representations for the early detection of sepsis with deep neural networks. *Comput Biol Med* 2017; **89**: 248-255 [PMID: 28843829 DOI: 10.1016/j.compbiomed.2017.08.015]

96 **Lauritsen SM**, Kalør ME, Kongsgaard EL, Lauritsen KM, Jørgensen MJ, Lange J, Thiesson B. Early detection of sepsis utilizing deep learning on electronic health record event sequences. *Artif Intell Med* 2020; **104**: 101820 [PMID: 32498999 DOI: 10.1016/j.artmed.2020.101820]

97 **Henry KE**, Hager DN, Pronovost PJ, Saria S. A targeted real-time early warning score (TREWScore) for septic shock. *Sci Transl Med* 2015; **7**: 299ra122 [PMID: 26246167 DOI: 10.1126/scitranslmed.aab3719]

98 **Saria S**, Henry KE. Too Many Definitions of Sepsis: Can Machine Learning Leverage the Electronic Health Record to Increase Accuracy and Bring Consensus? *Crit Care Med* 2020; **48**: 137-141 [PMID: 31939780 DOI: 10.1097/CCM.0000000000004144]

99 **Fleuren LM**, Klausch TLT, Zwager CL, Schoonmade LJ, Guo T, Roggeveen LF, Swart EL, Girbes ARJ, Thoral P, Ercole A, Hoogendoorn M, Elbers PWG. Machine learning for the prediction of sepsis: a systematic review and meta-analysis of diagnostic test accuracy. *Intensive Care Med* 2020; **46**: 383-400 [PMID: 31965266 DOI: 10.1007/s00134-019-05872-y]

100 **Ghosh S**, Roberts N, Firmin RK, Jameson J, Spyt TJ. Risk factors for intestinal ischaemia in cardiac surgical patients. *Eur J Cardiothorac Surg* 2002; **21**: 411-416 [PMID: 11888756 DOI: 10.1016/s1010-7940(02)00015-5]

101 **Chaudhuri N**, James J, Sheikh A, Grayson AD, Fabri BM. Intestinal ischaemia following cardiac surgery: a multivariate risk model. *Eur J Cardiothorac Surg* 2006; **29**: 971-977 [PMID: 16675235 DOI: 10.1016/j.ejcts.2006.03.014]

102 **Stahl K**, Busch M, Maschke SK, Schneider A, Manns MP, Fuge J, Wiesner O, Meyer BC, Hoeper MM, Hinrichs JB, David S. A Retrospective Analysis of Nonocclusive Mesenteric Ischemia in Medical and Surgical ICU Patients: Clinical Data on Demography, Clinical Signs, and Survival. *J Intensive Care Med* 2020; **35**: 1162-1172 [PMID: 30909787 DOI: 10.1177/0885066619837911]

103 **Bomberg H**, Stroeder J, Karrenbauer K, Groesdonk HV, Wagenpfeil S, Klingele M, Bücker A, Schäfers HJ, Minko P. Establishment of Predictive Models for Nonocclusive Mesenteric Ischemia Comparing 8,296 Control with 452 Study Patients. *J Cardiothorac Vasc Anesth* 2019; **33**: 1290-1297 [PMID: 30245114 DOI: 10.1053/j.jvca.2018.08.194]

104 **Vaswani A**, Shazeer N, Parmar N, Uszkoreit J, Jones L, Gomez AN, Kaiser L, Polosukhin I. Attention Is All You Need. 2017 Preprint. Available from: arXiv:170603762

105 **Li Y**, Rao S, Solares JRA, Hassaine A, Ramakrishnan R, Canoy D, Zhu Y, Rahimi K, Salimi-Khorshidi G. BEHRT: Transformer for Electronic Health Records. *Sci Rep* 2020; **10**: 7155 [PMID: 32346050 DOI: 10.1038/s41598-020-62922-y]

106 **Choi E**, Xu Z, Li Y, Dusenberry MW, Flores G, Xue Y, Dai AM. Learning the Graphical Structure of Electronic Health Records with Graph Convolutional Transformer. 2020 Preprint. Available from: arXiv:190604716

107 **Barrett T**, Upponi S, Benaglia T, Tasker AD. Multidetector CT findings in patients with mesenteric ischaemia following cardiopulmonary bypass surgery. *Br J Radiol* 2013; **86**: 20130277 [PMID: 23966376 DOI: 10.1259/bjr.20130277]

108 **Jiang M**, Li CL, Pan CQ, Lv WZ, Ren YF, Cui XW, Dietrich CF. Nomogram for predicting transmural bowel infarction in patients with acute superior mesenteric venous thrombosis. *World J Gastroenterol* 2020; **26**: 3800-3813 [PMID: 32774059 DOI: 10.3748/wjg.v26.i26.3800]

109 **Park JJ**, Kim KA, Nam Y, Choi MH, Choi SY, Rhie J. Convolutional-neural-network-based diagnosis of appendicitis *via* CT scans in patients with acute abdominal pain presenting in the emergency department. *Sci Rep* 2020; **10**: 9556 [PMID: 32533053 DOI: 10.1038/s41598-020-66674-7]

110 **Rajpurkar P**, Park A, Irvin J, Chute C, Bereket M, Mastrodicasa D, Langlotz CP, Lungren MP, Ng AY, Patel BN. AppendiXNet: Deep Learning for Diagnosis of Appendicitis from A Small Dataset of CT Exams Using Video Pretraining. *Sci Rep* 2020; **10**: 3958 [PMID: 32127625 DOI: 10.1038/s41598-020-61055-6]

111 **Copin P**, Ronot M, Nuzzo A, Maggiori L, Bouhnik Y, Corcos O, Vilgrain V; SURVI team. Inter-reader agreement of CT features of acute mesenteric ischemia. *Eur J Radiol* 2018; **105**: 87-95 [PMID: 30017304 DOI: 10.1016/j.ejrad.2018.05.027]

112 **Huang SC**, Pareek A, Zamanian R, Banerjee I, Lungren MP. Multimodal fusion with deep neural networks for leveraging CT imaging and electronic health record: a case-study in pulmonary

embolism detection. *Sci Rep* 2020; **10**: 22147 [PMID: 33335111 DOI: 10.1038/s41598-020-78888-w]

113 **Cruz Rivera S**, Liu X, Chan AW, Denniston AK, Calvert MJ; SPIRIT-AI and CONSORT-AI Working Group; SPIRIT-AI and CONSORT-AI Steering Group; SPIRIT-AI and CONSORT-AI Consensus Group. Guidelines for clinical trial protocols for interventions involving artificial intelligence: the SPIRIT-AI extension. *Nat Med* 2020; **26**: 1351-1363 [PMID: 32908284 DOI: 10.1038/s41591-020-1037-7]

114 **Liu X**, Rivera SC, Moher D, Calvert MJ, Denniston AK; SPIRIT-AI and CONSORT-AI Working Group. Reporting guidelines for clinical trial reports for interventions involving artificial intelligence: the CONSORT-AI Extension. *BMJ* 2020; **370**: m3164 [PMID: 32909959 DOI: 10.1136/bmj.m3164]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4104-4142

DOI: 10.3748/wjg.v27.i26.4104

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Hepatocellular carcinoma in patients with renal dysfunction: Pathophysiology, prognosis, and treatment challenges

Hsuan Yeh, Chun-Cheng Chiang, Tzung-Hai Yen

ORCID number: Hsuan Yeh 0000-0002-4926-8433; Chun-Cheng Chiang 0000-0001-8105-2512; Tzung-Hai Yen 0000-0002-0907-1505.

Author contributions: Yeh H and Chiang CC contributed equally to this study, and both contributed to data collection and manuscript writing; Yen TH contributed to study design and supervision.

Supported by Chang Gung Memorial Hospital, No. CORPG3K0191, No. CMRPG3G0871, and No. CMRPG3G0872.

Conflict-of-interest statement: The authors declare no competing financial interests.

Hsuan Yeh, Tzung-Hai Yen, Department of Nephrology, Chang Gung Memorial Hospital and Chang Gung University, Taipei 105, Taiwan

Chun-Cheng Chiang, Department of Gastroenterology and Hepatology, Chang Gung Memorial Hospital, Taoyuan 333, Taiwan

Corresponding author: Tzung-Hai Yen, MD, PhD, Doctor, Professor, Department of Nephrology, Chang Gung Memorial Hospital, No. 199 Tung Hwa North Road, Taipei 105, Taiwan. m19570@adm.cgmh.org.tw

Abstract

The population of patients with hepatocellular carcinoma (HCC) overlaps to a high degree with those for chronic kidney disease (CKD) and end-stage renal disease (ESRD). The degrees of renal dysfunction vary, from the various stages of CKD to dialysis-dependent ESRD, which often affects the prognosis and treatment choice of patients with HCC. In addition, renal dysfunction makes treatment more difficult and may negatively affect treatment outcomes. This study summarized the possible causes of the high comorbidity of HCC and renal dysfunction. The possible mechanisms of CKD causing HCC involve uremia itself, long-term dialysis status, immunosuppressive agents for postrenal transplant status, and miscellaneous factors such as hormone alterations and dysbiosis. The possible mechanisms of HCC affecting renal function include direct tumor invasion and hepatorenal syndrome. Finally, we categorized the risk factors that could lead to both HCC and CKD into four categories: Environmental toxins, viral hepatitis, metabolic syndrome, and vasoactive factors. Both CKD and ESRD have been reported to negatively affect HCC prognosis, but more research is warranted to confirm this. Furthermore, ESRD status itself ought not to prevent patients receiving aggressive treatments. This study then adopted the well-known Barcelona Clinic Liver Cancer guidelines as a framework to discuss the indicators for each stage of HCC treatment, treatment-related adverse renal effects, and concerns that are specific to patients with pre-existing renal dysfunction when undergoing aggressive treatments against CKD and ESRD. Such aggressive treatments include liver resection, simultaneous liver kidney transplantation, radiofrequency ablation, and transarterial chemoembolization. Finally, focusing on patients unable to receive active treatment, this study compiled information on the latest systemic pharmacological therapies, including targeted and immunotherapeutic drugs. Based on available clinical studies and Food and Drug Administration labels, this study details the drug indications, side effects, and

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Taiwan

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

Received: January 28, 2021
Peer-review started: January 28, 2021
First decision: March 6, 2021
Revised: March 17, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 14, 2021

P-Reviewer: Zhu J
S-Editor: Fan JR
L-Editor: A
P-Editor: Xing YX

dose adjustments for patients with renal dysfunction. It also provides a comprehensive review of information on HCC patients with renal dysfunction from disease onset to treatment.

Key Words: Hepatocellular carcinoma; Chronic kidney disease; End-stage renal disease; Hemodialysis; Cancer prognosis; Cancer therapeutics

Core Tip: The varying degrees of renal dysfunction, from the various stages of chronic kidney disease to dialysis-dependent end-stage renal disease, often affect the choice of treatment and prognosis of patients with hepatocellular carcinoma (HCC). This complicates HCC treatment. This review encompasses the presumptive causes of the high degree of comorbidity of HCC and renal dysfunction, the impact of renal dysfunction on HCC prognosis, and the concerns that are specific to patients with pre-existing renal dysfunction for each stage of HCC treatment.

Citation: Yeh H, Chiang CC, Yen TH. Hepatocellular carcinoma in patients with renal dysfunction: Pathophysiology, prognosis, and treatment challenges. *World J Gastroenterol* 2021; 27(26): 4104-4142
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4104.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4104

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy worldwide and accounts for substantial morbidity and mortality[1,2]. However, the etiology, incidence, and mortality of HCC are geographically uneven. Most HCC cases are found in East Asia and Sub-Saharan Africa. Although the incidence rates are relatively lower in Western countries, the mortality rates remain high[1]. The major risk factors for HCC include chronic hepatitis B virus (HBV), chronic hepatitis C virus (HCV), cirrhosis, alcoholic liver disease, and nonalcoholic steatohepatitis (NASH)[3]. Another leading cause of morbidity and mortality worldwide is renal dysfunction, which includes chronic kidney disease (CKD) and end-stage renal disease (ESRD). Caring for patients with renal diseases has greatly burdened health care systems[4]. Notably, patients with renal dysfunction have a higher prevalence of cancer, including liver cancer, compared with the general population[5,6]. Furthermore, such patients—especially those on maintenance dialysis for ESRD—were reported to have a higher prevalence of viral hepatitis compared with the general population[7]. In certain regions where both renal dysfunction and HCC are highly prevalent, the two conditions are highly comorbid [8]. In the present study, we searched and organized the available evidence-based literature to provide a comprehensive review guided by the following research questions: (1) Does any correlation or causality exist between renal dysfunction and the development of HCC? (2) Would renal dysfunction, including CKD and ESRD status, affect the prognosis and treatment outcomes of HCC? And (3) What are the challenges of treating HCC in patients with renal dysfunction in all categories of the Barcelona Clinic Liver Cancer (BCLC) system algorithm?

Despite the lack of a validated international consensus on the management of patients with both renal dysfunction and HCC, we aimed to summarize information that is critical to the development of preventive and therapeutic strategies for this specific population.

ASSOCIATION AND POSSIBLE PATHOPHYSIOLOGICAL MECHANISMS BETWEEN RENAL DYSFUNCTION AND HCC

A high incidence of cancer has been reported in patients with renal dysfunction. Both CKD and ESRD were reported to be bidirectionally connected to cancer: Renal

dysfunction can serve as a risk factor for cancers including HCC, whereas cancer and related treatments can directly or indirectly lead to or aggravate renal dysfunction[6]. In addition to the mutual relationship between renal dysfunction and cancer, renal dysfunction and HCC share common risk factors that complicate the association between the two diseases. These risk factors can be categorized into vasoactive factors and those related to environmental toxins, viral hepatitis, and metabolic diseases (Table 1). The complex pathways linking renal dysfunction and HCC are depicted in Figure 1. The following subsections summarize the studies that provide evidence for each of these links.

Renal dysfunction as a cause of or risk factor for HCC

Cancer risk has been reported to be elevated in patients with renal dysfunction. Numerous studies have demonstrated an increased risk of liver cancer in patients with ESRD on dialysis[9-11]. Limited data are available on whether less advanced CKD, where dialysis is not needed, can increase the risk of liver cancer[12,13]. Several hypotheses have been proposed for these correlations, including a dysregulated immune system, defective DNA repair mechanism, impaired antioxidant defense, accumulation of carcinogenic compounds caused by reduced renal elimination, and the uremia milieu[14]. Because it is well-established that kidney transplant recipients have an increased cancer risk because of the aggressive administration of immunosuppressive agents[15], it is reasonable to have a separate discussion for post-kidney-transplantation status.

CKD without dialysis − accumulation of uremic toxins and elevated serum levels of cytokines: The well-documented phenomenon of patients with impaired renal function having increased cancer risk raises the following question: Could uremic toxin accumulation, one of the direct consequences of renal dysfunction, be carcinogenic?

P-Cresyl sulfate (PCS), a protein-bound uremic toxin prototype that cannot be efficiently removed through routine dialysis procedures, has been found to be fibrogenic in the kidney and vascular system of mice through epithelial-to-mesenchymal transition (EMT)[16-18]. EMT is an irreversible process through which epithelial cells lose their cell polarity and cell–cell adhesion and acquire migratory properties to become mesenchymal cells. EMT has also been implicated in the development of liver fibrosis and cirrhosis[19-22]. It has been widely accepted that transforming growth factor-beta (TGF-β) plays a crucial role in hepatic EMT through stellate cell activation and excessive matrix synthesis[19,20,23,24]. The sequential progression from chronic liver fibrosis to cirrhosis culminates in the development of HCC, which is a major cause of death in patients with compensated cirrhosis[25,26]. A study in Taiwan found that PCS increased the incidence of liver fibrosis in people with HBV and HCV[27]. Although limited data exist on whether the PCS can directly induce EMT in the liver, we postulate that PCS accumulation secondary to renal dysfunction influences liver fibrosis, cirrhosis, and eventually the risk of HCC. Hwang *et al*[28] conducted a population-based study to examine the mechanism behind the high incidence of HCC in ESRD, and they found PCS to be positively correlated with HCC occurrence[28]. However, in that study, ESRD was no longer associated with a higher incidence of HCC than in the general population after matching was conducted for hepatitis and liver cirrhosis. Those authors concluded that the high incidence of HCC in patients with ESRD was caused by a high viral hepatitis rate rather than by uremia *per se*[28]. This does not violate our aforementioned assumption, despite appearing to do so, that PCS indirectly contributes to HCC occurrence through liver inflammation and cirrhosis. Further studies are warranted to clarify the link between PCS and HCC.

Cytokines constitute another topic worthy of discussion. Renal dysfunction is also known to increase the level of cytokines in the body, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α)[29-32]. Several studies have reported that IL-6, TNF-α, and other cytokines are associated with more severe problems in liver necrosis, tissue repair and regeneration, and the accumulation of mutations caused by aberrant cell proliferation, thus increasing the transformation potential of hepatocytes and the risk of HCC[33,34]. More studies focusing on the underlying pathophysiology are required to determine whether these cytokines lead to hepatic carcinogenesis, either directly or indirectly. In addition to proinflammatory properties, uremia has been recognized to compromise normal immune response by enhancing the apoptosis of activated immune cells[35-37]. The immune alterations associated with uremia possibly contribute to cancer occurrence. However, data on site-specific cancers such as HCC are lacking, and further studies are required to delineate the relationships

Table 1 Shared risk factors for hepatocellular carcinoma and chronic kidney disease

Risk factors	
Environmental toxins	Arsenic
	Cadmium
	Aflatoxin
	Aristolochic acid
Viral hepatitis	Hepatitis B virus
	Hepatitis C virus
Metabolic syndrome and related disorders	Non-alcoholic fatty liver disease
	Nonalcoholic steatohepatitis
	Diabetes mellitus
Vasoactive factors	Renin-angiotensin system activation

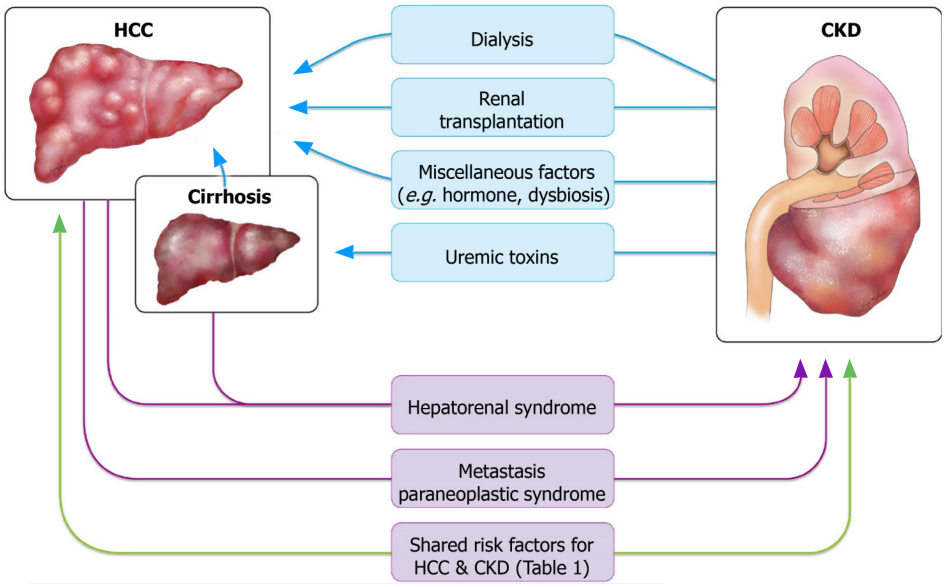

Figure 1 Association between hepatocellular carcinoma and chronic kidney disease. This figure summarizes the confirmed and presumptive links between hepatocellular carcinoma and renal dysfunction. HCC: Hepatocellular carcinoma; CKD: Chronic kidney disease.

between uremia, immune dysfunction, and HCC.

Effect of ESRD on chronic dialysis status: Studies have suggested that the overall cancer risk in chronic dialysis patients is significantly higher than that in the general population, for both HCC and cancers of other primary sites[10,12,13]. Whether such carcinogenic effects originate from the dialysis procedure itself or other ESRD-related factors remains to be determined[38]. Wong *et al*[39] found an increased dialysis time to be a significant risk factor for common solid organ cancers regardless of age. They demonstrated that a dose-dependent relationship exists between the duration of maintenance dialysis and overall cumulative cancer risk, and this relationship was independent of the dialysis modality. The findings were attributed to the immunodeficient and chronic inflammatory status in uremia and to the substances the patient was exposed to during dialysis, including nitrites, chloramines, and other unknown elements[39]. Another study on the pattern of excess cancer in dialysis and transplantation reported that dialysis was associated with a small increase in immune deficiency–related cancers, including liver cancer; however, the risk of liver cancer was not particularly high in patients on dialysis [standardized incidence ratio (SIR) 2.2, 95% confidence interval (CI): 1.2-3.7] relative to other types of immune deficiency–related cancers. Moreover, the overall findings for such cancers would be unchanged if liver cancer was excluded from the group[5]. Because both HBV and HCV are prevalent in patients on hemodialysis[40-42], studies have attributed HCC

incidence in part to exposure to oncogenic virus infection in dialysis populations through blood transfusion and contamination. Furthermore, a nationwide study in Taiwan revealed that liver cancer was the second most common cancer found in patients receiving dialysis and that the SIR of liver cancer in chronic dialysis patients was also higher than that of their healthy counterparts[9]. This result is inconsistent with the relatively lower frequency of liver cancer found in an international collaborative study[10]. A high frequency of liver cancer among the dialysis population might be explained by HBV and HCV infection being endemic to Taiwan[9]. In the aforementioned studies, except for the effects of infection-related factors, whether the dialysis procedure itself increases HCC risk remains inconclusive.

Kidney transplantation: Kidney transplantation is known to be associated with a marked increase in cancer risk at various sites[12]. In the late 1960s, the immunosuppressive agents administered to patients who underwent a transplant were discovered to increase the risk of cancer; compared with that in recipients of a cardiac or hepatic transplant, the aforementioned risk is a major outcome factor in recipients of a kidney transplant because of their longer survival owing to dialysis being widely available [43]. However, studies that have discussed HCC separately have reported mixed results on whether kidney transplant increases the incidence, reporting either no trend [5] or only a moderately increased risk[12,44]. Therefore, studies have provided limited support for the theory that kidney transplantation and the related application of immunosuppressive agents increases the risk of HCC.

Miscellaneous factors: HCC is more prevalent in men than in women. Both androgen and estrogen sex steroids can contribute to the gender disparity in HCC prevalence, where their effects are distinct to each sex[45]. Higher levels of androgen signaling are associated with an increased risk of HBV-related HCC[46,47], whereas higher estrogen pathway activity plays a protective role in female hepatocarcinogenesis. The estrogen axis is critical for maintaining a lower serum IL-6 level, thus reducing liver cancer risk in women[48,49]. A large cohort study[50] found CKD to increase liver cancer mortality in women and, to a lesser extent, in men; the gender disparity is likely explained by CKD-related hypogonadism, but this remains to be examined. The following sections discuss other possible risk factors shared by HCC and CKD, including environmental toxins, metabolic diseases, and genetic factors and their connections with CKD. However, the causal effects between these factors with CKD and HCC are still being debated.

Dysbiosis, which refers to the qualitative and quantitative alteration of gut microbiota, has been commonly observed in CKD patients. The imbalance of pathogenic flora and symbiotic flora was also implicated in the progression of CKD, increased cardiovascular risk, uremic toxicity, and inflammation[51]. An enhanced permeability of the intestinal barrier, allowing the passage of endotoxins and other bacterial products into the blood, was also reported in CKD[52]. Notably, dysbiosis is another possible risk factor for HCC that has been identified in recent years. Enterohepatic circulation is accompanied by low-grade exposure to gut microbiota–derived metabolites and products, often termed microbiota-associated molecular patterns (MAMPs)[53]. Changes in the intestinal barrier cause leakiness, leading to hepatic exposure to MAMPs. Accumulating evidence from the last decade, mostly from animal studies in rodents, suggests a key role of gut microbiota in the progression of chronic liver disease and in the development of HCC. The HCC risk induced by several types of carcinogens has been found to be profoundly reduced in gut-sterilized mice[54-56]. In a study exploring the differences between the gut microbiota of patients with nonalcoholic fatty liver disease (NAFLD)-related cirrhosis with and without HCC and in healthy controls, gut microbiota profile and systemic inflammation were significantly correlated and can occur together in the process of hepatocarcinogenesis[57]. It remains unclear whether chronic inflammation driven by the translocation of MAMPs from a leaky gut is the dominant contributor to HCC or whether the carcinogenic effect is limited to specific cases such as NAFLD, as does whether dysbiosis could serve as a causal link bridging CKD to HCC[58]. Further studies targeting dysbiosis may elucidate the association between CKD and HCC.

HCC and associated comorbidities causing renal dysfunction

A study found a significant prevalence of CKD in patients with cancer, particularly a higher rate of hematologic malignancy and liver cancer[59]. Hepatorenal syndrome (HRS), either with or without cirrhosis, is a major cause of CKD in patients with HCC. Direct invasion of the renal parenchyma by tumor cells is a rare cause but has been reported in the literature.

HRS: HRS is a unique type of kidney failure that usually occurs in advanced cirrhosis. HRS is characterized by functional impairment of the kidneys caused by vasoconstriction of the renal arteries in the absence of tubular dysfunction, proteinuria, or other histologic changes in the kidneys[54]. The exact mechanism of HRS is not completely understood, but its hallmark is severe vasodilation of the splanchnic arteries owing to portal hypertension, which compromises the effective arterial blood volume and arterial pressure[60]. HRS has two subtypes, which differ in terms of disease course and the presence of detectable precipitating factors[61]. Type 1 HRS is characterized by the rapid progression of renal failure, with the serum creatinine value increasing to greater than 2.5 mg/dL within 2 wk. It is often triggered by a precipitating event, such as bacterial infection, hypotension, or multiple organ failure. By definition, the renal dysfunction caused by type 1 HRS often falls into the category of acute kidney injury (AKI) or an acute deterioration of CKD termed acute-on-chronic kidney injury. By contrast, type 2 HRS is associated with gradual or insidious renal failure with a moderate rise in serum creatinine to 1.5-2.5 mg/dL. One of the major clinical manifestations of type 2 HRS is refractory ascites, for which a specific trigger is often lacking. With a median survival of 6 mo, Type 2 HRS has a superior prognosis compared with type 1 HRS, which has a median survival of less than 2 wk. The relatively moderate disease course is more consonant with the present article's focus on CKD.

Advanced cirrhosis, a critical precursor lesion of liver cancer, can cause portal hypertension, which may subsequently lead to HRS and result in kidney function deterioration[62]. In a 49-year-old man with HCC, ascites, and measured portal hypertension but no cirrhosis of the liver, the hypertension was secondary to microscopic invasion of the central and small portal veins[63]. Therefore, isolated liver cancer with high tumor burden has also been found to cause portal hypertension and HRS regardless of the presence of comorbid cirrhosis.

Direct tumor metastasis to the kidney: Renal metastasis of HCC is exceedingly rare. A literature search yielded only a few cases of renal metastasis from HCC[64]. Most renal metastases are small, bilateral, and multifocal; however, large and solitary metastatic tumors do occur. These tumors may cause difficulty in diagnosis because they often have no specific radiologic findings to distinguish them from primary renal neoplasms [65]. In some cases, metastatic tumors do not necessarily result in declined renal function. Nevertheless, in one case of HCC metastasis to the kidney mimicking renal cell carcinoma, a prolonged elevated serum creatinine level of 2.24 mg/dL was observed[66]. Therefore, direct invasion of the renal parenchyma by metastatic tumor cells still constitutes a differential diagnosis of renal dysfunction that should not be ignored in patients with HCC.

Paraneoplastic syndrome: Paraneoplastic syndromes arise from the tumor secretion of hormones, peptides, or cytokines or from immune cross-reactivity between malignant and normal tissues. These disorders may affect diverse organ systems, most notably the endocrine, nervous, dermatological, rheumatological, and hematological systems [67,68]. HCC may present with a wide range of paraneoplastic phenomena, which may precede local manifestations of the tumor, including hypercholesterolemia, erythrocytosis, hypoglycemia, and hypercalcemia. Hypercalcemia is a well-known paraneoplastic metabolic condition associated with numerous malignancies. In HCC, hypercalcemia accounts for 7.8% of paraneoplastic syndromes, and it mainly occurs as a terminal event[69]. Most malignancies associated with hypercalcemia have been verified to be caused by parathyroid hormone (PTH)-related peptide. The metastasis of malignancies to the bone can also cause osteolysis and lead to hypercalcemia. In rare cases, hypercalcemia may result from ectopic PTH production by tumors. Patients with hypercalcemia typically present with volume depletion, which might lead to a reduction in glomerular filtration rate (GFR) and calcium clearance[70]. Hypercalcemia may also provoke AKI or hypertension, or aggravate the tubular necrosis frequently found in cases of AKI[71]. Case reports on HCC-induced hypercalcemia have been published, but little information about renal function has been reported in these studies[72]. A case of combined HCC and neuroendocrine carcinoma with ectopic secretion of PTH was documented[73]; the authors observed impaired renal function (creatinine = 2.16 mg/dL), and continuous renal replacement therapy was applied to treat acute renal failure induced by hypercalcemia. However, the patient died during the study period. It is relatively certain that HCC may cause AKI through the paraneoplastic effect of hypercalcemia; nevertheless, more clinical observations and studies are warranted to determine whether HCC-related hypercalcemia causes sustained, even irreversible, renal dysfunction.

Risk factors shared by HCC and renal dysfunction

In the investigation of the relationship between CKD and HCC, some common risk factors have been found. The overlap of these risk factors leads to a high degree of comorbidities between HCC and renal dysfunction. These risk factors may cause the two diseases separately; however, little evidence exists for whether these factors serve as a causal link from HCC to CKD or vice versa. Hence, in this article we attempt to list these risk factors to provide clinicians and researchers with a useful summary. These risk factors can be further divided into several categories, including those of toxic, infectious, metabolic, and vascular origins (Table 1).

Environmental toxins: According to epidemiological and animal studies, several environmental toxins, including arsenic, cadmium, mycotoxins, and aristolochic acid (AA), are associated with both renal impairment and liver cancer[50].

In renal proximal tubules, arsenic or cadmium can cause the depletion of intracellular glutathione stores. This leads to the incremental production of free radicals and results in inflammation and apoptosis[74,75]. A high arsenic level in drinking water was discovered to be a cause for ESRD, independent of other documented risk factors[76]. Continual cadmium exposure can also progress to renal Fanconi syndrome and ultimately CKD[75]. By contrast, arsenic and cadmium carcinogenesis targets the liver[77]. Dimethylarsinic acid and trimethylarsine oxide, the organic metabolites of inorganic arsenic, have been found to cause oxidative DNA damage and enhance cell proliferation in rats[78,79]. In humans, arsenic exposure has also been potentially linked to HCC and other liver tumors or paraneoplastic lesions; for example, hepatomegaly, hepatoportal sclerosis, fibrosis, or cirrhosis often occurs after chronic arsenic exposure[80-82]. According to an *in vitro* experiment, cadmium is specifically internalized by Kupffer cells, which could lead to the release of various proinflammatory cytokines such as IL-6 and TNF-α[83,84]. Studies have also examined the potential effects of long-term cadmium exposure on the expression of cytochrome P450 (CYP) enzymes in the liver and its impact on the activation and clearance of therapeutic drugs, alcohol, and environmental substances. Under chronic cadmium exposure, DNA adducts associated with CYP-mediated metabolism are produced; they accumulate in liver cells and result in mutations, altered gene expression, and eventually carcinogenesis[85-87]. In epidemiological studies, elevated blood and urine cadmium levels have been found to play a role in HCC, although a direct effect has not been confirmed[77,88].

Aflatoxins (AFs) are highly toxic secondary metabolites that are synthesized by *Aspergillus flavus* and *Aspergillus parasiticu*[89]. Afs are the most toxic of all mycotoxins, causing considerable health problems and economic loss through the contamination of food and animal feed. Cereal crops, oil crops, and dairy products are frequently contaminated. Afs can be divided into AFB1 and AFB2, which emit blue fluorescence, and AFG1 and AFG2, which emit green fluorescence under chromatographic and fluorescence analysis[89]. Similar to cadmium, aflatoxin is metabolized by CYP enzymes into aflatoxin-8,9-exo-epoxide. The exo-epoxide can form derivatives with DNA, RNA, and proteins, including the p53 tumor suppressor gene. Moreover, the exo-epoxide can bind DNA to form the predominant promutagenic 8,9-dihydro-8-(N7 guanyl)-9-hydroxy AFB1 adduct (AFB1-N7-Gua), which may secondarily form the more mutagenic AFB1-formamidopyrimidine. These derivatives generate a risk of malignancy over time[89,90]. A review of the epidemiological evidence also indicated that AF is a critical contributor to the high incidence rates of HCC in Asia and Sub-Saharan Africa[91]. *In vitro* and *in vivo* studies have revealed that AFB1 and AFM1 cause kidney toxicity through oxidative stress by altering the expression of proline dehydrogenase and L-proline levels, leading to downstream apoptosis[89]. More population-based research is warranted to verify whether Afs are associated with renal dysfunction in humans. However, theoretically aflatoxin is likely to be a common risk factor shared by CKD and HCC.

Another factor worthy of discussion is AA. AA is traditionally known as the main culprit of Chinese herb nephropathy, a type of rapidly progressive renal failure characterized by severe anemia, glycosuria, leukocyturia, mild hypertension, and asymmetric kidneys[92]. Apart from being responsible for renal toxicity, AA has also been implicated in the genesis of urothelial carcinoma. AA-derived DNA adducts and TP53 mutations have been found in ureteric tissues, indicating the carcinogenic potential of AA on the urothelium[93,94]. AA can also result in significant DNA adduct formation and mutation in the liver, albeit at a lower level than in the kidneys [95]. Several epidemiological studies have implicated AA in the development of HCC in Asia, but more data are required to evaluate the impact of AA exposure on HCC occurrence worldwide[96,97].

Viral hepatitis: Chronic HBV and HCV infections are known to be dominant risk factors for HCC. HBV is the most frequent underlying cause of HCC. Case-control studies have demonstrated that chronic HBV carriers have a five- to fifteen-fold increased risk of HCC compared with the general population[98]. Approximately 70% to 90% of HBV-related HCCs develop in patients with cirrhosis, but HBV can also cause HCC in the absence of cirrhosis[99]. Generally, two processes are involved in the hepatocarcinogenesis of HBV infection. Direct mechanisms of hepatocyte transformation include a role for HBV DNA integration, virus mutations, transcriptional activation of growth regulatory genes by HBV-encoded proteins as well as effects on apoptosis, cellular signaling, and DNA repair. The progression of chronic hepatic disease and its associated inflammation, regenerative hyperplasia, and transcriptional deregulation to neoplasia contribute to the indirect pathogenesis of HCC[100,101]. By contrast, the mechanisms underlying HCV-associated carcinogenesis are mainly indirect effects of virus-deregulating host cellular processes, including virus-induced inflammation, oxidative stress, and host immune responses; the resulting genomic instability and mitochondrial damage; and the accompanying increased hepatocyte proliferation and steatosis[102].

In addition, HBV and HCV infection are also established risk factors for CKD. According to epidemiological studies, hepatitis B surface antigen positivity in serum is associated with higher risks of CKD and proteinuria[103]. HBV-related nephropathies include membranous glomerulonephritis, polyarteritis nodosa, and membranoproliferative glomerulonephritis (MPGN)[104]. Moreover, a clinical study reported that HBV causes apoptosis in renal tubular Fas upregulation[105]. HCV has been associated with the development of MPGN and cryoglobulinemia, and it has also been found to increase the risk of CKD[106,107]. Taken together, the aforementioned studies have indicated that both HBV and HCV can be considered critical shared factors in the high comorbidity of HCC and CKD. However, more research is required to verify whether these two infections are causally linked with HCC and CKD.

Metabolic diseases: Abundant epidemiological evidence suggests a correlation between noninsulin-dependent diabetes mellitus (NIDDM, or type 2 diabetes mellitus) and cancers, including HCC[108-110]. Several mechanisms likely explain such an association. Insulin or its precursors may stimulate mitogenesis or carcinogenesis in hepatocytes[111]. Augmented inflammation as measured by TNF-α and IL-6 levels has been found in diabetes[112,113]. Diabetes may also increase the risk of HCC through the development of NASH. Up to 40% of patients with type 1 or type 2 diabetes develop diabetic nephropathy, which is the leading cause of CKD in patients starting renal replacement therapy in developed countries[114]. Consequently, NIDDM is a non-negligible factor contributing to the high comorbidity of HCC and CKD. Likewise, fatty liver disease is a common risk factor for both HCC and CKD. The prevalence of NAFLD is 10%–30% in adults and tends to be higher in developed countries because of the prevalence of obesity and metabolic syndrome[115,116]. NASH belongs to the spectrum of NAFLD and is characterized by hepatic inflammation. In a study conducted to clarify the etiology of non-B, non-C HCC, a total 1374 patients with HCC were enrolled from 1995 to 2009. NASH was noted to be a critical risk factor for HCC, and cirrhosis was detected in 65% of NASH-HCC cases[117]. Studies have defined various factors involved in the necroinflammatory response of NASH, including cytokines, hormones, and neurotransmitters[118]. Rodent animal studies have demonstrated that NASH induced by a high-fat diet is associated with elevated TNF-α and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) with hepatocyte proliferation[113,119]. Hypoadiponectinemia possibly participates in NASH-HCC carcinogenesis, as verified by a adiponectin knock-out mice model[120]. Intriguingly, NAFLD was also found to be a driver of CKD[121]. The presence and severity of NAFLD were noted to be strongly and positively correlated with the prevalence and incidence of CKD, independent of obesity, hypertension, NIDDM, or other common risk factors[122]. Targher *et al*[123] found that higher levels of patatin-like phospholipase domain–containing protein 3 GG genotype are independently associated with a lower estimated GFR (eGFR) and increased 24-h proteinuria in patients with NAFLD. This single nucleotide polymorphism may be useful for identifying those patients with NAFLD who are also prone to developing CKD[123].

Vasoactive factors − activation of the renin–angiotensin system: The systemic renin–angiotensin system (RAS) regulates blood pressure and maintains normal kidney function. In addition to the traditionally known circulating RAS, scientists have uncovered the existence of a local angiotensin-generating system in several tissues, including the heart, liver, and kidney. The tissue RAS can act locally as a paracrine or

autocrine factor to meet the needs of individual tissues, independently of or in cooperation with the circulating counterpart[124]. The crucial role of the RAS in the pathogenesis of CKD has been well documented since the 1980s in experimental and clinical studies[125]. An activated RAS aggravates both systemic and glomerular capillary hypertension, causing hemodynamic injury to the vascular endothelium and glomerulus. Angiotensin II and the downstream product, aldosterone, also exert direct proinflammatory and profibrotic actions, which may promote kidney damage[126,127]. Angiotensin-converting-enzyme inhibition has exhibited considerable therapeutic efficacy in the control of systemic hypertension and the prevention of progressive kidney injury[128,129].

An increasing body of evidence has suggested that the RAS also contributes to liver fibrosis and hepatocarcinogenesis, although probably less so than it does for the kidneys[130]. The main source of the RAS comes from hepatocytes and Kupffer cells, but it has also been found in the bile duct epithelium of the liver[54]. In the liver, angiotensin II regulates cell growth, inflammation, and fibrosis. The expression of angiotensin receptors was found to increase activated hepatic satellite cells (HSCs) following injury. By acting through angiotensin receptors, angiotensin II can be mitogenic for human-activated HSCs, elicit a marked dose-dependent increase in intracellular calcium levels, and induce cell contraction. Angiotensin II also stimulates DNA synthesis and cell proliferation[131]. Angiotensin receptor blockers block the profibrotic and proinflammatory effects of angiotensin II on HSCs, including the expressions of inflammatory cytokines and growth factors (such as TGF-β1, IL-1β, NF-κB, and connective tissue growth factor) and the production of the extracellular matrix [132,133]. RAS might participate in the development of HCC because of the aforementioned proliferative and profibrotic effects. Moreover, angiotensin II was found to enhance vascular endothelial growth factor (VEGF), a potent angiogenic factor that plays an essential role in tumor growth and metastasis[134]. All these findings suggest that the RAS becomes involved in not only kidney injury but also HCC development.

EFFECT OF RENAL DYSFUNCTION ON THE OVERALL PROGNOSIS OF PATIENTS WITH HCC

Few studies have investigated the impact of comorbid renal dysfunction on the prognosis of patients with HCC. However, several studies have examined the influences of CKD or ESRD on specific treatment outcomes and prognosis, which are summarized in the next section. Before we discuss each of these topics in detail, we first provide a concise review of the literature on how renal dysfunction affects the prognosis of patients with HCC.

CKD increases the risk of death in cancer patients. A retrospective study investigating the association between CKD and mortality in cancer patients found an inverse relationship between eGFR and adjusted hazard ratios (HRs)[59]. A single-center study that recruited 440 patients with both CKD and HCC reported that survival from stage 4 and stage 5 CKD was inferior to that of stages 1 and 2. In a prospective population-based analysis, CKD was related to increased cancer-related mortality in liver, kidney, and urinary tract malignancies, with adjusted HRs of 1.74, 3.3, and 7.3, respectively[50]. However, in that study, the percentage of cancer-related mortality decreased, whereas the percentage of cardiovascular mortality markedly increased in patients in more advanced CKD stages. Taken together, we infer from these findings that CKD negatively affects both overall and cancer-related mortality in liver cancer, but some heterogeneity is possible in the etiology of mortality among different stages.

In terms of the prognosis of HCC patients with ESRD on long-term dialysis, studies have reported inconsistent results. In a single-centered observational study comparing the mortality rates of 1298 patients with HCC who were (n = 172) or were not (n = 1126) on long-term hemodialysis, those on hemodialysis had a 2.036-fold greater chance of death than did patients not on hemodialysis. However, cancer-related mortality was not reported and that study was limited by its retrospective nature and short follow-up duration[135]. In another single-center study including 2500 patients with HCC, with only a minority group (1.2%) having ESRD on maintenance dialysis, no significant overall survival difference between dialysis and nondialysis patients was found, although those receiving dialysis had a significantly higher serum bilirubin level, lower serum sodium level, more ascites, and worse performance status[136]. Because 63% of patients undergoing dialysis in that study had undergone nonpalliative management [resection, local ablation, or transarterial chemoembolization

(TACE)] for HCC, the authors attributed the unexpectedly good outcomes of the dialysis group to early and aggressive treatment. The authors further concluded that dialysis *per se* does not predict poor outcomes in patients with HCC and should not be considered a contraindication for active anticancer treatment. In summary, dialysis should not hamper the indicated group from receiving anticancer therapy according to currently available data, and whether dialysis affects the prognosis of HCC remains to be determined.

CHALLENGES OF TREATING HCC IN PATIENTS WITH RENAL DYSFUNCTION

Most patients with HCC have concomitant liver diseases such as chronic hepatitis or cirrhosis. Therefore, the benefits of treating the tumor must be weighed against the potential damage to liver function. This complexity in the management of HCC calls for a multidisciplinary approach, including expertise in hepatology, hepatobiliary surgery, pathology, oncology, radiology, and specialized nursing[137]. The BCLC algorithm classifies patients into one of five stages, taking not only the tumor burden but also the extent of liver dysfunction and the patients' performance status into consideration[138]. The tumor burden is quantified according to the number and size of nodules, along with the presence or absence of macrovascular tumor invasion or extrahepatic spread. The traditional Child–Turcotte–Pugh (CTP) score provides a subjective assessment of liver function but does not adequately capture the hepatic functional reserve. Alternatives include the Model for End-Stage Liver Disease (MELD) score and the albumin–bilirubin grade[139]. The algorithm then provides treatment recommendations for each stage. Ever since its release in 1999, the BCLC algorithm has been a widely used scoring strategy for HCC. In the very early (0) and early stage (A), patients with a solitary lesion or with up to three nodules less than 3 cm in diameter (without macrovascular invasion or extrahepatic spread) and with preserved liver function are suitable for radical therapies—namely resection, transplantation, or percutaneous treatment. Patients in the intermediate stage (B) do not exhibit symptoms but have large, multifocal tumors without vascular invasion or any spread beyond the liver. If liver function is preserved, these patients could be candidates for TACE. Patients at the advanced stage (C) have symptomatic tumors [grades 1 and 2 according to the Eastern Cooperative Oncology Group (ECOG) Performance Status] or an invasive tumoral pattern of vascular invasion/extrahepatic spread. This group of patients may benefit from systemic medical treatment, which can be categorized into targeted therapy and immunotherapy depending on which of the various pharmacological mechanisms are at work. Finally, patients with terminal disease (D) have poor liver function or marked cancer-related symptoms (ECOG Performance Status > 2). These patients have an extremely poor prognosis and require palliative care[140].

As mentioned in the previous section, CKD was reported to be an independent risk factor for the survival of cancer patients[59]. Treating HCC is difficult in patients with CKD because renal impairment may limit therapeutic options when effective therapy is sought[137]. Currently, perhaps because of the paucity of data regarding HCC outcomes in patients with renal dysfunction, no international treatment consensus exists for this specific population. In the following subsections, we use the BCLC algorithm as a template to discuss special concerns when treating HCC patients with different stages of renal dysfunction compared with the general population. Through reviewing the available literature, we hope to provide the necessary information for developing a modified BCLC for patients with CKD or ESRD (Table 1).

Liver resection

Liver resection is the treatment of choice in noncirrhotic patients and one of the main curative options for early HCC in selected patients with cirrhosis[141-144]. In the last decades, improved surgical techniques and perioperative management as well as improved patient selection have enabled the indications for liver resection to be expanded[145-148]. In a nationwide study using the National Surgical Quality Improvement Program database to investigate the impact of CKD and ESRD on outcomes following major abdominal surgery, 24572 patients were included, of whom only 149 (0.6%) were on hemodialysis preoperatively. In the dialysis group, 30-d postoperative mortality and the overall complication rate (pneumonia and sepsis particularly) were significantly higher than those in the nondialysis group. Furthermore, any degree of preoperative renal impairment, even mild or asympto-

matic disease, was associated with clinically significant increases in 30-d postoperative mortality and morbidity following major abdominal surgery[149]. Therefore, the safety and outcomes of liver resection in HCC patients with abnormal renal function deserve a detailed investigation.

Liver resection for HCC in patients with CKD: Few studies have reported on the efficacy and safety of hepatectomy for HCC patients with renal dysfunction. Toshima *et al*[150] retrospectively reviewed the clinical features of 722 patients with HCC undergoing curative hepatectomy between 1986 and 2009. Seventeen patients (2.4%) with preoperative serum creatinine levels > 2.0 mg/dL were defined as the renal dysfunction group. Clinicopathological characteristics and postoperative outcomes were compared between the renal dysfunction group (n = 17) and the nonrenal dysfunction group (n = 705). Overall survival (P = 0.177) and disease-free survival (P = 0.942) after hepatectomy did not differ significantly. The incidence rates of massive ascites (35.3% *vs* 14.3%; P = 0.034) and pleural effusion (52.9% *vs* 17.6%; P = 0.001), defined as massive effusion (ME), were significantly higher in the renal dysfunction group than in the nonrenal dysfunction group. Hypoalbuminemia (≤ 2.8 g/dL; P = 0.031), heavy blood loss (≥ 1000 mL; P = 0.012), and intraoperative blood transfusion (P = 0.007) were risk factors for ME. The authors concluded that preoperative improvement of anemia and reduction of blood loss by meticulous surgical techniques may prevent major complications in patients with renal dysfunction who require hepatectomy for HCC. In another study, data from 735 patients undergoing primary liver resection for HCC between 2002 and 2014 were analyzed[151]. Short- and long-term outcomes were compared between a renal dysfunction group, defined by a preoperative eGFR of < 45 mL/min/1.73 m^2, and a nonrenal dysfunction group. The incidence rates of postoperative pleural effusion (24% *vs* 11%; P = 0.007) and major complications (31% *vs* 15%; P = 0.003) were significantly higher in the 62 patients with renal dysfunction compared with the nonrenal dysfunction group. In patients with renal dysfunction with CTP score A, the 90-d mortality rate (1.9%) and median survival time (6.11 years) were comparable to those of patients without renal dysfunction. By contrast, patients with renal dysfunction with CTP score B had a very high 90-d mortality rate (22.2%), and a significantly shorter median survival time compared with patients without renal dysfunction (1.19 *vs* 4.84 years; P = 0.001). The authors concluded that liver resection is safe for CTP-A patients with renal dysfunction, who have comparable oncological outcomes to patients without renal dysfunction; however, liver resection for CTP-B patients with renal dysfunction should be subject to stricter consideration. These findings jointly indicate that CKD status may not necessarily affect overall survival but may lead to more surgical complications. The safety and efficacy of hepatectomy for HCC in patients with CKD could be acceptable if the appropriate patient group is carefully selected, along with judicious pre- and postoperative care.

Liver resection for HCC in patients with ESRD on dialysis: Compared with studies on the CKD population, studies on HCC patients with ESRD on dialysis undergoing hepatic resection are more abundant, probably because these patients' characteristics are well defined and more effectively targeted. To clarify the role of liver resection in treating HCC in patients with ESRD, Cheng *et al*[152] conducted a retrospective study to compare the clinicopathological characteristics and operative results of 12 patients with ESRD receiving resection for HCC with those of the other 456 patients without ESRD[152]. The 5-year disease-free survival rates for ESRD and non-ESRD groups were 35.0% and 34.2% (P = 0.31), whereas the 5-year overall survival rates were 67.8% and 53.3% (P = 0.54), respectively. The author commented that liver resection for HCC is justified in select patients with ESRD. In another retrospective study comparing the clinical features of 26 patients with ESRD and HCC with 1198 HCC patients without ESRD undergoing liver resection[153], elevated BUN and creatinine were the only two main independent factors differentiating patients with ESRD and HCC from their counterparts with HCC, and overall and disease-free survival rates were similar between the two groups. Lee *et al*[136] conducted a retrospective matched-control trial to compare long-term survival between patients with HCC (n = 2472) who were undergoing (n = 30) *vs* not undergoing dialysis[136]. The patients undergoing dialysis had dual HBV and HCV infection, lower serum α-fetoprotein level (AFP), worse performance status, and higher MELD scores than did the matched controls and patients not undergoing dialysis. No significant difference existed in long-term survival when patients undergoing dialysis were compared with patients who were not or with the matched controls (P = 0.684 and 0.373, respectively). Yeh *et al*[154] used Taiwan's National Health Institute Research Database to compare the disease-free

survival, overall survival, and perioperative complications between 596 nonuremic controls and 149 patients with uremia and HCC who were also undergoing liver resection. The survival outcomes were comparable between the uremia–HCC cohort and controls, regardless of the extent of hepatic resection. However, the aforementioned had a higher risk of postoperative infections requiring invasive interventions as well as an increased risk of life-threatening heart-associated complications relative to the controls. In summary, ESRD on dialysis does not seem to exert a particular influence on the survival outcomes of patients receiving liver resection for HCC. With careful operative techniques and perioperative care, comparable overall and disease-free survival can be achieved in select patients with ESRD and HCC undergoing liver resection. ESRD on dialysis is not expected to be an obstacle to hepatectomy in the indicated patient group.

Transplantation

Liver transplantation (LT) is considered the gold standard surgical therapy for early-stage HCC co-occurring with cirrhosis or chronic liver disease. The Milan criteria function as the most reliable border for transplantation feasibility both in Western and Asian HCC guidelines[155]. The expected 5-year survival rates of LT for HCC that meets the conventional Milan criteria (single tumor ≤ 5 cm or multiple tumors ≤ 3 nodules ≤ 3 cm in size, without vascular invasion) are 65%-80%, and patients meeting the Milan criteria have a significant survival advantage over patients who do not. LT is recommended as the first-line option for HCC within the Milan criteria but is unsuitable for resection. However, given the distinguished clinicopathological features of patients with renal dysfunction, whether the survival advantage of LT can be extended to this specific population is a more complicated matter. Can patients with renal dysfunction receive LT similar to the general population? How should one assess the feasibility of simultaneous liver kidney transplantation (SLKT)? The following paragraphs address these questions.

LT carries the risk of complications, which occur both immediately after transplantation and in the long term[156]. The main complications in the immediate postoperative period are related to graft dysfunction and rejection and to the surgical technique, infections, and dysfunction involved in the pulmonary, renal, or neurological systems. In the long term, complications are typically a consequence of prolonged immunosuppressive therapy, and they include diabetes mellitus, systemic arterial hypertension, de novo neoplasia, and organ toxicities[157]. AKI is a main complication of LT, especially in the early postoperative period. The reported incidence of AKI after transplantation varies widely because of the different diagnostic criteria used, ranging from 19.26% to 94%[158-161]. Hemodynamic changes during surgery, blood loss, and other stress may cause prerenal AKI or even acute tubular necrosis immediately after surgery[162,163]. Patients who developed AKI tended to have a markedly higher mortality rates[164,165]. It is unclear whether AKI after LT is the primary driver of poorer mortality outcomes or whether this is merely a correlation[166]. CKD is also a common complication after LT with an incidence ranging between 20% and 80%[167,168]. Numerous observations have implicated calcineurin-inhibitor (CNI) as a major risk factor of CKD in recipients of a transplant [169-171], and some studies have advocated the use of tacrolimus or mycophenolate mofetil instead of cyclosporin to reduce the incidence of chronic renal dysfunction after transplantation[170,172]. However, other studies have been unable to show that CNI fully explained post-transplant renal abnormalities[171,173]. Therefore, the hypothesis that CNI use is a major cause of renal dysfunction after LT remains unverified, and CNI's effect may be overestimated.

Patients with renal dysfunction have been reported to experience poor surgical outcomes following LT. An early study using the National Institute of Diabetes and Digestive and Kidney Diseases LT Database investigated the effect of renal insufficiency in patients with fulminant hepatic failure or chronic liver disease (cirrhosis); that study found that renal insufficiency in fulminant hepatic failure and renal insufficiency requiring dialysis or SLKT in cirrhosis predicts lower patient and graft survival rates after a transplant[174]. In another study reviewing the postoperative courses of 115 liver transplant recipients for liver cirrhosis, the population was divided into two groups based on the threshold of preoperative serum creatinine < 1.0 mg/dL[175]. Patients with preoperative serum creatinine > 1.0 mg/dL had significantly longer intensive care unit stays, higher rates of acute renal failure requiring dialysis, and a greatly increased mortality rate. In a study comparing the LT outcomes of patients with low and high MELD scores, renal function was the most crucial variable associated with morbidity and length of hospital stay[176]. The data not only called for

special attention during the perioperative period of renal dysfunction but also cast doubt on whether patients with renal dysfunction are ideal candidates for LT.

Several early studies have found that SLKT could be feasible in patients who have both advanced hepatic and renal dysfunction. In a study compared 16 patients with SLKT and 32 patients with LT matched by age, sex, date, and indication for transplantation; that study reported that both groups had similar levels of reoperation due to bleeding, bacterial infections, liver rejection, arterial hypertension, and median creatinine levels at the 1st and 3rd years[177]. However, early post-transplant dialysis was higher in SLKT than in LT. Survival rates at the 1st, 3rd, 5th, and 7th years were similar in both groups (87.5%, 74%, 74%, and 66% *vs* 81%, 75%, 75%, and 75% in LT and LKT, respectively). That author inferred that SLKT is an effective therapeutic option in patients with end-stage liver and kidney disease, with most early and late complications and long-term survival being similar to those observed in LT. In one study evaluating the success of SLKT, 20 patients (aged 14-64 years) received a total of 21 LT and 31 kidney transplantation procedures[178]. SLKT was performed in 14 patients, of whom five required further replacement of one or the other of the grafted organs. That study revealed that patients with liver cirrhosis had a very poor prognosis due to their poor overall clinical state at the time of terminal renal failure, whereas patients without liver cirrhosis were more appropriate candidates for SLKT. The author concluded that in general, the indication for SLKT ought to be considered earlier in this case than in the case of transplantation involving only one organ. Notably, a study found that pretransplantation renal dysfunction and exposure to dialysis might affect SLKT treatment outcomes[179]. Adult recipients receiving LT (n = 2700) or SLKT (n = 1361) with moderate renal insufficiency between 2003 and 2013 were included, and the study cohort was stratified into four groups based on serum creatinine level (Scr < 2 mg/dL *vs* Scr ≥ 2 mg/dL) and on dialysis status at both listing and transplant. SLKT administration led to a greater decrease in post-transplant mortality compared with LT administration across all four groups, but only reached statistical significance (HR 0.77; 95%CI: 0.62–0.96) in recipients not exposed to dialysis and with Scr ≥ 2 mg/dL at transplant. The study indicated the possible advantage of SLKT in patients with both severe liver disease and renal abnormalities. Some studies have indicated that the liver immunologically protects the kidneys after combined liver–kidney transplantation[180,181]. Therefore, patients with end-stage hepatic and renal anomalies may indeed benefit from SLKT. However, this technique faces limitations in being administered widely among HCC patients with renal dysfunction. For example, significant heterogeneity exists in the criteria for SLKT when it comes to noncirrhotic or compensated liver diseases and when it comes to liver transplant candidates with a moderate-to-severe reduction in GFR. To promote discussion and unify the criteria for the indication of SLKT by liver transplant groups, the Spanish LT Society (*La Sociedad Española de Trasplante Hepático*) held the 6th Consensus Document Meeting on October 20, 2016, in which experts from the 24 authorized Spanish LT programs participated[182]. According to the consensus, SLKT is recommended in patients with liver transplant criteria plus one of the following: (1) CKD in chronic dialysis or eGFR > 30 mL/min; or (2) CKD with eGFR between 30 and 40 mL/min and some signs of poor renal prognosis — such as proteinuria > 1 g/d (> 3 mo) and/or diabetic nephropathy — and/or histological findings of poor prognosis in renal biopsy (more than 30% glomerulosclerosis or more than 30% interstitial fibrosis). SLKT is also recommended in patients who are candidates for LT with acute kidney disease requiring dialysis for 6 consecutive weeks, either continuously or intermittently.

Several more recent studies have specifically focused on the outcomes of patients receiving SLKT for HCC. A study included 2606 patients (mean age: 53 years) receiving SLKT for primary biliary cirrhosis (PBC, n = 76), primary sclerosing cholangitis (n = 81), HBV (n = 98), HCV (n = 945), alcoholic liver disease (n = 495), alcohol and HCV (n = 152), cryptogenic cirrhosis (n = 289), NASH (n = 221), or HCC (n = 249); that study reported that HCV, NASH, and HCC had worse outcomes for liver graft (72%, 66%, and 72% *vs* 82%; HR: 2.5-3.1), kidney graft (71, 65%, and 71% *vs* 80%; HR: 2.3-2.8), and patient survival (74, 69, and 69% *vs* 82%; HR: 2.4-2.7) compared with PBC[183]. In another retrospective analysis of SLKT from the United Network for Organ Sharing registry[184], the authors compared the outcomes of HCC with other transplant indications. HCC was not associated with post-transplant survival among all patients (HR: 1.15; 95%CI: 0.84-1.58) or the propensity score-matched cohort (HR: 0.97; 95%CI: 0.64-1.47). SLKT-HCC patients had similar rates of acute rejection (13.3% *vs* 10.5%, P = 0.36) and liver graft failure requiring retransplantation (3.2% *vs* 2.3%, P = 0.44). The author commented that liver transplant candidates with advanced renal dysfunction and HCC may be considered for SLKT[184]. SLKT seems to be a treatment of choice for HCC patients with advanced renal dysfunction. However, more studies

specifically targeting patients with HCC as the main indication for SLKT are warranted to support the safety and efficacy of this treatment.

Radiofrequency ablation

Since the early 1990s, radiofrequency ablation (RFA) has been introduced to clinical practices and has rapidly become the first-choice local treatment for small (≤ 3 cm) HCC lesions. Based on the BCLC staging system, RFA is applied for the treatment of patients having very early (Stage 0) and early stage (Stage A) HCC (Figure 2)[138]. For most appropriate patients selected, this treatment is safe and efficient. However, reports of complications are common. Livraghi *et al*[185] and Takaki *et al*[186] reported mortality rates between 0.1% and 0.3%[185,186]. The major complication rate was estimated at 2.2% to 2.8%. The causes of death were bowel perforation, peritonitis, tumor rupture, and liver failure due to biliary stricture. The most frequent major complications were hemorrhage and tumor seeding, followed by liver abscess, bowel perforation, hemothorax, and liver failure. Minor complications included acute skin burn, self-limiting intraperitoneal bleeding, subcapsular or intrahepatic hematoma, arterioportal shunt, biliary portal shunt with hemobilia, transient liver decompensation, and direct renal tissue damage. In less common cases, the procedure may cause renal dysfunction or related side effects. Thermal injury could lead to hemolysis and rhabdomyolysis[187-189], and the extensive breakdown and transcellular shift of potassium may lead to varying (and even life-threatening) degrees of hyperkalemia, either in patients with normal baseline renal function or CKD[190]. This clinical implication is anticipated in case of prolonged ablation, and laboratory monitoring during extensive or prolonged RFA procedures is recommended to detect hemolysis early. Laboratory tests including hematocrit, serum potassium, urine hemoglobin, and serum creatine phosphokinase level should be considered[188]. Hemolysis and rhabdomyolysis could also result in AKI[188,189,191,192]. Most patients experience moderately impacted renal function and a slight increase in serum creatinine without deterioration. However, the hemoglobin-mediated obstruction of renal tubules might cause more severe AKI, oliguria, and sometimes even death. One case report even documented progression to CKD[193].

Few original studies or systematic reviews have discussed whether pre-existing renal dysfunction before RFA is related to treatment outcomes, although much more evidence indicating treatment outcomes in patients with ESRD on dialysis receiving RFA for HCC have been emerging. To examine the efficacy and safety of RFA in treating HCC in patients with HD, a study enrolled 108 HD patients with naïve HCC at 15 institutions between 1988 and 2014[194]. Fifty-eight patients with appropriate indications treated with either hepatectomy (n = 23) or RFA (n = 35) were compared with respect to their clinical features, complications, and prognosis. The two treatments did not significantly differ in their overall survival and disease-free survival rates. The author concluded that RFA had a therapeutic efficacy in HD patients with naïve HCC that is comparable to liver resection. Another study included 14 carefully selected HD patients with HCC (five naïve, nine recurrent) who underwent a total of 19 RFA treatments, and revealed no major complications, suggesting that the safety and effectiveness of RFA were not compromised in this specific population[195]. RFA seems to be a promising option for small HCC in patients undergoing regular HD. By contrast, a study using the Japanese Diagnosis Procedure Combination database compared the treatment outcomes in matched-pair samples of 437 dialyzed and 1345 nondialyzed patients[196]. In-hospital mortality and hemorrhagic complications were significantly higher in dialyzed patients with ESRD than in nondialyzed patients. In patients on HD for ESRD, mortality was significantly lower for those aged ≤ 70 years than for those aged older than that (P = 0.02). Patient age may be a useful indicator when considering RFA for HCC in patients with ESRD on HD. Hyperkalemia was also reported in a patient with ESRD on regular HD after RFA for HCC[197]. Therefore, the indications for RFA in dialysis-dependent patients should be considered carefully.

TACE

Transarterial therapy is a standard treatment for unresectable HCC and patients unfit for surgical resection due to compromised hepatic reserve or nonliver general comorbidities[138,198], following which regular contrast-enhanced imaging for residual disease is recommended. The chemotherapeutic agents used in TACE cause tumor necrosis through the combined effects of targeted chemotherapy and arterial embolization[199]. However, the use of a water-soluble iodinated contrast medium in TACE may induce renal failure, especially in high-risk patients with liver cirrhosis-associated nephropathy[200]. AKI is a common complication found after TACE in

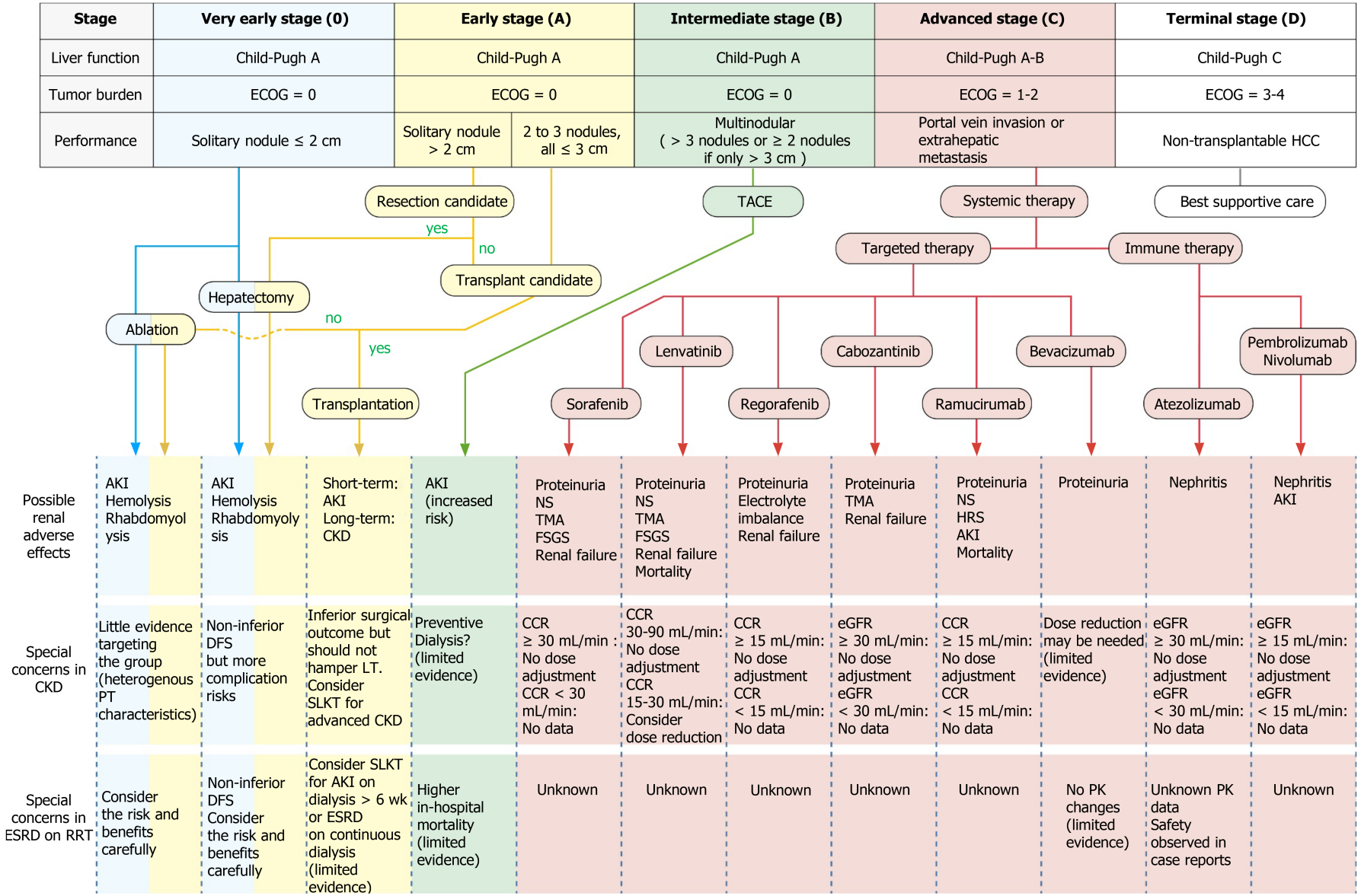

Figure 2 Current treatment algorithmsfor patients with hepatocellular carcinoma and chronic kidney disease. This figure is based on the Barcelona Clinic Liver Cancer algorithm, which classifies patients into five stages taking not

only the tumor burden but also the extent of liver dysfunction and the patients' performance status into consideration. Described in the table are the indications for each stage of hepatocellular carcinoma treatment, the treatment-related renal adverse effects, and special concerns for patients with pre-existing renal dysfunction in the applying aggressive treatments and the use of the systemic target and immunotherapy. The disease-free survival is compared to the general population without renal impairment. ECOG: The Eastern Cooperative Oncology Group performance status; HCC: Hepatocellular carcinoma; CKD: Chronic kidney disease; ESRD: End stage renal disease; RRT: Renal replacement therapy; AKI: Acute kidney injury; PT: Patient; LT: Liver transplantation; SLKT: Simultaneous liver kidney transplantation; DFS: Disease-free survival; NS: Nephrotic syndrome; TMA: Thrombotic microangiopathy; FSGS: Focal segmental glomerulosclerosis; CCR: Creatinine clearance (mL/min calculated *per* the Cockcroft-Gault formula); eGFR: Estimated glomerular filtration rate (mL/min/1.73 m^2); PK: Pharmacokinetics.

patients with HCC, and patients with post-TACE AKI have a higher risk of developing complications such as progression to CKD, ESRD, and death[200-204]. Preoperative CTP score, age, proteinuria, hemoglobin, serum total bilirubin, serum uric acid, aminotransferase level, post-TACE gastrointestinal bleeding, and previous post-TACE AKI history have been reported to be predictors of post-TACE AKI in HCC patients [201,205,206].

Given the nephrotoxicity inherent in the intervention, the application of TACE for HCC in patients with underlying renal dysfunction is challenging. According to a retrospective study that investigated the outcomes of TACE in patients with HCC and CKD, more post-therapy complications, including acute renal failure and sepsis, were found in the CKD group than in the non-CKD group[207]. Overall survival in the CKD group was significantly poor (10.9 ± 8.5 *vs* 23.5 ± 16.3 mo, $P < 0.01$). However, in another study conducted to clarify the benefits and risk of TACE in patients with HCC and CKD, 35 patients receiving TACE were enrolled and classified into a CKD group [including nondialysis CKD (NDCKD), $n = 10$ and ESRD, $n = 9$], and a non-CKD group ($n = 16$)[208]. The 2- and 5-year survival rates from initial diagnosis were comparable between the CKD and non-CKD groups. The 2- and 5-year survival rates were also similar in patients with NDCKD and those with ESRD. Of note is the strategy of "preventive HD" adopted in that study: All patients with CKD consulted a nephrologist, and HD was performed within 4 h after 20 of the 32 transarterial therapies in the 10 patients with NDCKD to prevent contrast-induced nephropathy in the CKD group. The authors concluded that TACE can be made feasible in patients with CKD by instituting periprocedural HD with survival rates that are similar to those of patients without CKD.

For patients already on regular hemodialysis for ESRD at the time of TACE, data are lacking because invasive treatment is rarely performed in this specific population. A Japanese pair-matched cohort using a nationwide database was recruited to evaluate the in-hospital mortality and complication rates following TACE in this population [209]. A total of 1551 dialyzed and 5585 nondialyzed patients with ESRD were enrolled. The complication rates did not differ between dialyzed and nondialyzed patients, but the in-hospital mortality rate was, at 2.2%, twice as high in dialyzed patients. Among the dialyzed patients, the mortality rate was not significantly associated with sex, age, or Charlson Comorbidity Index. The author concluded that indications for TACE in HD-dependent patients should be considered cautiously by weighing the benefits against the risks.

In summary, the available data regarding TACE in patients with pre-existing renal dysfunction are limited. More studies are warranted before we can definitely determine the safety and feasibility of TACE in patients with CKD or ESRD. Patients with advanced renal dysfunction may benefit from perioperative preventive HD, but further investigations are required to confirm the efficacy and safety of the measure. Because both the CKD and ESRD groups have reported worse prognoses after TACE compared with HCC patients without renal dysfunction, caution should be taken during the treatment planning process, and patients should be well-informed of the risks and complications involved.

Systemic therapy

If HCC is diagnosed at an early stage, a wide array of treatment options that increase overall survival and improve quality of life are available. However, because late diagnosis is common, 70% to 80% of advanced HCC cases will not benefit from tumor resection[3], and only one-third of patients are eligible for curative therapeutic approaches[210]. Current treatment options for patients with unresectable HCC include TACE and systemic medical treatments. Systemic treatments can generally be divided into two categories according to their mechanism of action: Targeted therapy [mainly tyrosine kinase inhibitor (TKI)] and immunotherapy. The following subsections concentrate on the mechanism of action, common adverse effects, and points of caution for people with renal dysfunction with respect to two groups of drugs.

Targeted therapy: The key signal transduction pathways participating in the pathogenesis of HCC include the Wnt-β catenin, EGFR-RAS-MAPK, and c-MET pathways as well as the insulin-like growth factor signaling, Akt/mTOR signaling, and VEGF and platelet-derived growth factor receptor signaling cascades[211]. TKIs are small molecules that inhibit the multiple receptor tyrosine kinases involved in tumor growth, angiogenesis, pathologic bone remodeling, drug resistance, and metastatic progression of cancer[204]. In 2007, a multi-kinase inhibitor (MKI) named sorafenib was approved as the first systemic agent for treating advanced unresectable HCC because a SHARP trial had suggested a survival benefit of approximately 3 mo [212,213]. Sorafenib is an oral MKI that blocks tyrosine kinase receptors (VEGFR-2/3, PDGFR-β, c-Kit, FLT-3, and RET) and other targets (c-Raf and B-Raf)[214]. In the kidneys, glomerular podocytes express VEGF and glomerular endothelial cells express VEGF receptors[215,216]. Podocyte-specific deletion of a single VEGF allele caused proteinuria and capillary endotheliosis in rodents, and disrupted glomerular VEGF signaling was strongly implicated in the pathogenesis of human preeclampsia[208]. Sorafenib's mechanism of action clearly indicates its ability to induce significant adverse effects on the kidneys, including proteinuria, nephrotic syndrome, and preeclampsia-like syndrome[217,218]. Cases of renal failure, thrombotic microangiopathy (TMA), and focal segmental glomerulosclerosis (FSGS) have also been documented[213,219]. In patients on sorafenib with pre-existing renal dysfunction, studies have found no trend in pharmacokinetic parameters for sorafenib or its metabolites among any renal function group[220,221]. Renal impairment appears to have no clinically relevant effect on the pharmacokinetics of sorafenib and its metabolites; therefore, no dose adjustment was indicated[221]. According to the Food and Drug Administration (FDA), the pharmacokinetics of sorafenib have not been thoroughly confirmed in patients on dialysis[222]. However, an Italian retrospective study investigating the safety and efficacy of sorafenib in patients with renal cell carcinoma and ESRD reported no unexpected major side effects, and the author concluded that sorafenib is not contraindicated in HD groups[223]. In a Japanese study, a 63-year-old man with ESRD on HD started sorafenib therapy (200 mg/d) 8 d after TACE[221]. The pharmacokinetic parameters of sorafenib and its active metabolite M-2 were within the reference levels of patients with normal renal function 8 and 9 d after the initiation of sorafenib. The authors concluded that sorafenib was well tolerated at an initial dose of 200 mg/d for a patient with HCC undergoing HD, thus indicating that renal failure is not necessarily a contraindication for sorafenib therapy.

After the success of sorafenib, various clinical trials were designed in the hope to outperform the efficacy of it. Nevertheless, not until in recent decade had some trials demonstrated the comparable efficacy with sorafenib or survival benefits after first-line treatment failure[224]. The notable novel agents include lenvatinib, regorafenib, cabozantinib, ramucirumab, and bevacizumab.

Lenvatinib was approved for first-line therapy in advanced HCC following the results of the REFLECT trial, a randomized phase III noninferiority trial by Kudo *et al* [225], which showed that lenvatinib was not inferior to sorafenib in overall survival in untreated advanced HCC[225]. Further multicenter findings have confirmed the efficacy of lenvatinib with or without previous TKI therapies[226,227]. Lenvatinib's nephrotoxic profile is similar to that sorafenib, including proteinuria, renal failure, TMA, and FSGS[225,228-231]. The enrollment criteria in the original REFLECT trial included adequate renal function, which was defined as creatinine clearance (CCR) > 30 mL/min as calculated using the Cockcroft–Gault formula[225]. In the FDA label, no dose adjustment is recommended for patients with mild (CCR 60-89 mL/min) or moderate (CCR 30-59 mL/min) renal impairment. Lenvatinib concentrations may increase in patients with differentiated thyroid cancer (DTC) or renal cell carcinoma (RCC) and severe (CCR 15-29 mL/min) renal impairment. It is recommended to reduce the dose for patients with DTC or RCC who also have severe renal impairment. However, there exists no recommended dose for lenvatinib in patients with HCC and severe renal impairment. Lenvatinib has not been studied in patients with ESRD[232].

Regorafenib was approved as the second-line therapy for advanced HCC following the results of the RESORCE trial. This randomized, double-blind, placebo-controlled phase III trial demonstrated the effectiveness of regorafenib in patients progressing after sorafenib treatment. The study confirmed the potential of second-line agents and ushered in the era of second-line therapy[233]. Further multicenter studies have verified the efficacy and safety indicated in the RESORCE trial[234,235]. The nephrotoxic effects include proteinuria and renal failure[236,237]. In regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT), an international, multicenter, randomized, placebo-controlled, and phase III trial reported diarrhea in 34% of patients, with 7% experiencing grade 3 or 4 diarrhea, leading to fluid and electrolyte depletion. The sequelae of fluid and electrolyte depletion may result in dehydration, renal failure, and potential cardiovascular compromise[238]. According to a pharmacokinetic modeling and simulation study, the pharmacokinetics of regorafenib are unlikely to be impacted by any stage of renal impairment[239]. The FDA label suggests that no dose adjustment is recommended for patients with renal impairment. The pharmacokinetics of regorafenib have not been studied in patients on dialysis and there exists no recommended dose for this patient population[240].

Cabozantinib is another TKI that blocks the receptors involved in oncogenesis and angiogenesis, including VEGFR 1, 2, and 3; hepatocyte growth factor receptor (MET); AXL; and the angiopoietin receptors TIE-2, RET, c-Kit, and FLT-3 *in vitro* and *in vivo*. Cabozantinib was also indicated to be a second-line treatment in the progression of HCC with acquired resistance to sorafenib[241]. In the CELESTIAL trial, cabozantinib achieved significantly superior overall survival compared with the placebo group and was thus approved by the FDA[242]. The nephrotoxic profile of cabozantinib is similar to those of sorafenib and lenvatinib, including renal failure, proteinuria, and TMA[243-245]. However, in the CELESTIAL trial, grade 5 adverse events considered to be related to the drug were reported in six patients in the cabozantinib group (one event each of hepatic failure, bronchoesophageal fistula, portal-vein thrombosis, upper gastrointestinal hemorrhage, pulmonary embolism, and HRS)[242]. The enrollment criteria for the CELESTIAL trial included serum creatinine ≤ 1.5 times the upper normal limit or calculated CCR ≥ 40 mL/min using the Cockcroft–Gault formula occurring in conjunction with either urine protein/creatinine ratio ≤ 1 mg/mg or 24-h urine protein < 1 g[242]. Two clinical pharmacology studies were conducted to characterize the single-dose pharmacokinetics of cabozantinib in individuals with renal and hepatic impairment, respectively[243]. Although mild-to-moderate renal impairment (eGFR ≥ 30 mL/min/1.73 m^2) did not result in a clinically relevant difference in the pharmacokinetics of cabozantinib, the author concluded that cabozantinib should be used cautiously in individuals with mild or moderate renal impairment. According to the FDA label, no dose adjustment is recommended in patients with mild or moderate renal impairment[246]. No experience of cabozantinib in patients with severe renal impairment or requiring dialysis has been documented.

Ramucirumab is a fully human recombinant immunoglobulin G (IgG) 1 monoclonal antibody targeting the VEGF2 receptor. A randomized, multicenter, double-blind, placebo-controlled, and phase III trial (REACH) was conducted to examine the safety and efficacy of ramucirumab as a second-line agent for HCC[247]. In the REACH trial, although the second-line treatment with ramucirumab did not significantly improve survival over placebo in patients with advanced HCC, a subgroup analysis revealed better survival in patients with AFP ≥ 400 ng/mL[248,249]. This was later verified in the REACH-2 trial, which was the first positive phase III trial conducted in a biomarker-selected patient population with HCC[250]. Therefore, ramucirumab was

approved by the FDA as a second-line treatment for advanced HCC. The renal toxicity profile of ramucirumab includes proteinuria and nephrotic syndrome[251,252]. Renal failure and TMA have also been reported[250,253]. Notably, several predictors of ramucirumab-induced proteinuria have been identified, including systemic blood pressure, the number of cycles, and calcium channel blocker use[248,254]. Notably, in the REACH-2 trial, three deaths in the ramucirumab group were judged to be related to study treatment: one each from AKI, HRS, and renal failure[250]. The FDA label reports no clinically meaningful effect on the pharmacokinetics of ramucirumab in patients with renal impairment (CCR calculated using Cockcroft–Gault, 15-89 mL/min), and thus, no dose adjustment is suggested[255]. The pharmacokinetics of ramucirumab in patients with ESRD are unknown.

Bevacizumab is a humanized anti-VEGF monoclonal antibody that was previously approved by the FDA as a first-line treatment for metastatic colorectal cancer in combination with chemotherapy. In a global, open-label, and phase III trial conducted in 2020, patients with unresectable HCC who had not previously received systemic treatment were randomly assigned at a 2:1 ratio to receive either atezolizumab (discussed later in the text) plus bevacizumab or sorafenib until unacceptable toxic effects or a loss of clinical benefit occurred[256]. The primary end points were overall survival and progression-free survival in the intention-to-treat population, as assessed at an independent review facility according to the Response Evaluation Criteria in Solid Tumors, version 1.1. The study revealed that in patients with unresectable HCC, atezolizumab combined with bevacizumab resulted in superior overall and progression-free survival outcomes than sorafenib did, which led to the combined therapy of atezolimumab plus bevacizumab being approved as the first-line treatment for unresectable HCC by the FDA. Similar to other anti-VEGF or VEGFR blocking agents, the renal toxicity profile of bevacizumab encompasses renal failure[257,258], proteinuria, and nephrotic syndrome[259,260]. Microvascular diseases such as TMA or hemolytic uremic syndrome are not particularly uncommon[261-263]. In addition, sporadic cases of interstitial nephritis have been documented, as verified by renal biopsy findings, improvement after steroid treatment, and cessation of the offending agents[264,265]. A case of minimal change disease was also reported[266]. *Per* the manufacturer's instructions, no studies have investigated the pharmacokinetics of bevacizumab in patients with CKD because the kidneys are not major organs for bevacizumab metabolism or excretion[267]. Only one report has been published about the pharmacokinetics of bevacizumab in a dialysis-dependent patient with metastatic renal cancer, who received 5 mg/kg every 2 wk[268]. The drug was not dialyzable, and its pharmacokinetic parameters were similar to the reference values of patients with normal renal function. The author concluded that the drug can be administered any time before or after hemodialysis. The FDA label does not provide information on dose adjustment in patients with renal dysfunction[267].

Immunotherapy: Immunotherapy has been proven to be effective and safe in treating various solid tumors, prolonging overall survival, and offering a tolerable toxicity profile[269]. Immunotherapy negates tumor-expressed extracellular ligands that suppress intrinsic immune response and can be achieved through three main approaches[270]. One approach is to target the inhibitory proteins that prevent T cells from recognizing and eliminating cancer cells and allow regulatory cells to avoid autoimmune destruction by downregulating T-cell activation[269]. Examples of these molecules are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein-1 (PD-1) in addition to its ligand PD-L1, and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3)[271,272]. Checkpoint inhibitors are antibodies that activate T-cell mediated antitumor responses by selectively blocking the checkpoint receptors PD-1, PD-L1, and CTLA-4[271]. Conversely, therapeutic cancer vaccines that use a tumor-associated antigen (TAA) originating either from whole-cell tumor lysates and recombinant tumor peptides or recombinant viruses encoding for TAAs bring new prospects in treating cancers. TAAs are transferred and presented by major histocompatibility complex class I molecules in atrial premature complexes to effectively induce the activation of cytotoxic T-lymphocytes[273,274]. Another strategy in immune-regulated antitumor response is that of adoptive cell transfer. Immune cells are extracted from patients' peripheral blood and undergo genetic engineering to express chimeric antigen receptors. These cell membrane proteins bind to specific cancer antigens and stimulate the immune destruction of tumor cells[275].

In HCC, two categories of immune checkpoint inhibitors have been thoroughly examined in clinical trials, namely PD-1/PD-L1 and CTLA-4. Other promising markers are being investigated in animal models, and new agents are being tested in

clinical trials[272]. Currently, the FDA has approved checkpoint inhibitors for advanced HCC, including atezolizumab, pembrolizumab, nivolumab, and ipilimumab. The following paragraphs discuss the mechanism of action, common adverse effects, and points of caution for people with renal dysfunction in the use of these agents. The anticipating checkpoint molecule TIM-3 blockade and the related clinical trials are mentioned as well.

Atezolizumab is an engineered IgG1 monoclonal antibody targeting PD-L1. Patients with unresectable HCC who had not previously received systemic treatment were randomly assigned at a 2:1 ratio to receive either atezolizumab plus bevacizumab or sorafenib until unacceptable toxic effects or a loss of clinical benefit occurred[256]. Atezolizumab combined with bevacizumab resulted in superior overall and progression-free survival outcomes than did sorafenib, which led to the FDA approving the combined therapy of atezolizumab plus bevacizumab as the first-line treatment for unresectable HCC (see the preceding paragraph on bevacizumab). In a study focusing on the use of atezolizumab in patients with renal insufficiency, the efficacy and safety of atezolizumab in these special subpopulations from an expanded access program was reported[276]. Objective responses occurred in 0/6 (0%), 4/19 (21%), 1/27 (3.7%), and 12/62 (19%) of evaluable patients with CCR < 30, 30-45, 45-60, and ≥ 60 mL/min, respectively, and stable disease course was observed in three patients with CCR < 30 mL/min. The author concluded that these findings verified the clinical benefit of atezolizumab in patients with compromised renal function. In one case report, a male patient with metastatic urothelial cell carcinoma and ESRD on dialysis was safely treated with atezolizumab[277]. The main kidney-related side effect caused by atezolizumab is acute tubulointerstitial nephritis, as reported in biopsy-proven cases[278,279]. Based on the FDA label, mild or moderate renal impairment (eGFR 30-89 mL/min/1.73 m^2) has no clinically significant effect on systemic exposure to atezolizumab; however, the effects of severe renal impairment (eGFR < 30 mL/min/1.73 m^2) or severe hepatic impairment on the pharmacokinetics of atezolizumab is unknown[280].

Pembrolizumab is an anti-PD-1 monoclonal antibody. The antitumor effects of pembrolizumab were examined in a phase II trial in patients who were previously treated with advanced HCC (KEYNOTE-224)[281]. Subsequently, a randomized, double-blind, and phase III study (KEYNOTE-240) was conducted to further verify the efficacy and safety of pembrolizumab in this population[282]. The study indicated a favorable risk-to-benefit ratio for pembrolizumab in this population, but the overall and progression-free survival did not reach statistical significance *per* the specified criteria. Based on the aforementioned trials, the FDA granted accelerated approval to pembrolizumab for patients with HCC who have been previously treated with sorafenib. Several adverse renal effects have been noted during the use of pembrolizumab, including acute tubular injury, acute interstitial nephritis, and minimal change disease; moreover, kidney biopsy was recommended for suspected pembrolizumab-related cases of AKI[283-285]. According to the FDA label, regarding the risk of immune-mediated nephritis, changes in renal function should be monitored during use. The FDA also advised withholding pembrolizumab and administering corticosteroids for grade 2 nephritis or higher, and they also advised permanently discontinuing the drug for severe (Grade 3) or life-threatening (Grade 4) nephritis. Renal impairment (eGFR ≥ 15 mL/min/1.73 m^2) has no clinically significant effect on the clearance of pembrolizumab. Insufficient information exists regarding whether clinically important differences exist in the clearance of pembrolizumab in patients with eGFR < 15 mL/min/1.73 m^2[286].

Nivolumab is another anti-PD-1 monoclonal antibody. An open-label, noncomparative, phase 1/2, and dose escalation and expansion trial (CheckMate-040) was conducted to assess the safety and efficacy of nivolumab in patients with advanced HCC with or without chronic viral hepatitis[287]. The FDA later granted accelerated approval to nivolumab for patients with HCC who have previously been treated with sorafenib. A randomized, multicenter phase III study (CheckMate-459) of nivolumab *vs* sorafenib in patients with advanced HCC is currently ongoing to examine the use of nivolumab as a first-line treatment[288]. The renal toxicity profile of nivolumab is similar to that of pembrolizumab, including AKI, acute tubular injury, and immune complex-mediated glomerulonephritis[284,289,290]. The FDA label contains special warnings, prescribes precaution for immune-mediated nephritis and renal dysfunction during use, and advises that patients should be monitored for changes in renal function. The drug should be withdrawn in cases of moderate or severe serum creatinine elevation and permanently discontinued in cases of life-threatening serum creatinine elevation. The effect of renal impairment on the clearance of nivolumab was evaluated through a population pharmacokinetics analysis in patients with mild

(eGFR: 60-89 mL/min/1.73 m^2), moderate (eGFR: 30-59 mL/min/1.73 m^2), or severe (eGFR: 15-29 mL/min/1.73 m^2) renal impairment. No clinically important differences in the clearance of nivolumab were found between patients with renal impairment and those with normal renal function. The FDA suggests no dose adjustment in patients with renal impairment[291].

Ipilimumab is a CTLA-4 immune checkpoint inhibitor. The anti-HCC effect of ipilimumab was demonstrated in the nivolumab plus ipilimumab cohort in CheckMate-040, a multicenter, open-label, and phase 1/2 study (described in the preceding paragraph on nivolumab)[292]. The FDA granted accelerated approval to the combination of nivolumab and ipilimumab for patients with HCC who have previously been treated with sorafenib. As with those for other immunotherapy agents, evidence of the adverse renal effects of ipilimumab has indicated their presence in the forms of acute interstitial nephritis and AKI, constituting a cause for alarm[293]. However, the onset of kidney injury as indicated by CTLA-4 antagonist-related renal injury occurs earlier (2–3 mo) than that indicated by PD-1 inhibitors (3–10 mo)[284,294]. Furthermore, a case of ipilimumab-induced lupus nephritis was also reported[295]. Notably, ipilimumab has also been associated with electrolyte disturbances. Ipilimumab-induced hyponatremia caused by pituitary hypophysitis has been documented in case reports[296,297]. The FDA label suggests that patients should be monitored for changes in renal function. Furthermore, the drug should be withdrawn in cases of moderate or severe serum creatinine elevation and permanently discontinued in cases of life-threatening serum creatinine elevation. The effect of renal impairment on the clearance of ipilimumab was evaluated in patients with mild (eGFR: 60-89 mL/min/1.73 m^2), moderate (eGFR: 30-59 mL/min/1.73 m^2), or severe (eGFR: 15-29 mL/min/1.73 m^2) renal impairment compared with patients with normal renal function (eGFR: ≥ 90 mL/min/1.73 m^2) in a population pharmacokinetics analysis. No clinically important differences in the clearance of ipilimumab were found between patients with renal impairment and patients with normal renal function. The FDA recommended no dose adjustment for patients with renal impairment[298].

In addition to antibodies against CTLA-4 and PD-1/PD-L1, checkpoint inhibitor targeting TIM-3 is another potential and promising candidate of immunotherapy for cancer treatment[272,299]. TIM-3, a type I surface glycoproteins encoded by the gene on chromosome 5q33.2, was first discovered in 2001 and identified as an immune checkpoint that specifically expressed on interferon-γ-secreting CD4(+) T helper 1 and CD8(+) T cytotoxic cells in both mice and humans[300,301]. TIM-3 acts as a negative regulator of T cell function by triggering cell death upon interaction with its ligand, galectin-9. TIM-3 overexpression has been implicated in the suppression of T-cell responses and T-cell dysfunction; a state referred to as T-cell exhaustion[302]. TIM-3 also has other ligands and is expressed on other cell types like dendritic cells[303], monocytes[304], and mast cells[305]. In chronic HBV infection, TIM-3 expression is elevated in T helper cells, cytotoxic T lymphocytes, dendritic cells, macrophages, and natural killer cells, accompanied by impaired function of these immunocytes[306]. The TIM-3/galectin-9 signaling pathway was found to mediate T-cell senescence in HBV-associated HCC[307]. In addition to the immunomodulation effect, the expression of TIM-3 on tumor cells has been found to regulate the function of tumor cells directly [308]. A mechanistic study showed that TIM-3 expressed by malignant hepatocytes served as a tumor cell-intrinsic receptor to promote tumor growth *via* triggering NF-κB/IL-6/STAT3 axis[309]. Therefore, TIM-3 is a drug target for treating both chronic viral infection and HCC.

Several clinical trials about the use of anti-TIM-3 monoclonal antibodies in different types of cancer have been registered on ClinicalTrials.gov. MBG453, an anti-TIM-3 monoclonal antibody, was tested for the safety and efficacy of a single agent or in combination with PDR001 (anti-PD-1 antibody) in adult patients with advanced malignancies in a phase I-Ib/II open-label multicenter study (NCT02608268). TSR-022 is another anti-TIM-3 monoclonal antibody and its safety and efficacy are assessed alone in patients with advanced solid tumors (NCT02817633) or in combination with TSR-042 (anti-PD-1 antibody) (NCT03307785). Notably, a phase II trial studying the effect of TSR-022 with TSR-042 in the treatment of patients with locally advanced or metastatic liver cancer is recruiting and results are pending in October 2023 (NCT03680508). There are also various TIM-3 inhibitors studied in the phase I trials, including Sym023 (NCT03489343), BMS986258 (NCT3446040), and RO7121661 (NCT03708328).

In the mice model of nephrotoxic serum nephritis, TIM-3 was found up-regulated in kidneys and exerted a protective role. Administration of the anti-TIM-3 antibody aggravated nephritis as shown by significantly increased albuminuria, respective

histological changes, and expression of the renal injury molecule lipocalin-2[310]. Paradoxically, in the other mice model of diabetic nephropathy, TIM-3 was found to worsen the disease *via* the NF-κB/TNF-α pathway, and its performance in macrophage worsened podocyte injury both *in vivo* and *in vitro* studies[311]. Given that limited data is available, its exact role in the development of renal diseases remains unclear. Currently, the renal side effects of anti-TIM-3 antibodies in humans are still unknown. The renal safety profile from the clinical trials is still awaited.

Antivirals use during HCC treatment

Concomitant antiviral therapy is common during HCC treatment, especially the use of anti-HBV nucleoside or nucleotide analogues (NUCs). NUCs therapy could suppress HBV viral replication, achieve biochemical remission, and ameliorate liver inflammation[312-315]. In addition, NUCs therapy could reduce the incidence of liver decompensation, particularly in HCC patients undergoing LT on immunosuppressant and TACE which are prone to cause HBV reactivation or flare[316-319]. Though there is emerging evidence shows NUCs could decrease HCC incidence and recurrence[315, 320-325], the extent to which NUCs therapy may reduce the risk for HCC has been debated[326-328]. Recent mainstay therapies for HBV include NUCs with high potency and high genetic barriers, such as entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF). However, when using in patients with renal dysfunction, there are special considerations that need to be watched.

ETV belongs to a nucleoside analogue with high potency. However, it has lower efficacy if lamivudine resistance presents previously[329]. Relatively safe renal safety profiles were reported both in rats and humans[330-333]. Since ETV is eliminated primarily from kidneys, renal dose adjustment is needed in patients with CCR less than 50 mL/min to avoid over-exposure to this drug[334,335].

TDF, a nucleotide analogue, is a prodrug of tenofovir that is absorbed from the intestine and cleaved to release tenofovir, which is then phosphorylated inside hepatocytes to form active tenofovir diphosphate targeting viral reverse transcriptase [336]. The adverse effects of long-term use include elevated creatinine, Fanconi syndrome, and osteoporosis[337,338]. TDF is also eliminated by the kidneys in the majority and is not suggested in patients with CCR less than 50 mL/min by some society guidelines because of its nephrotoxicity to proximal renal tubules[339]. Furthermore, when using in the scenario of HCC treatment, renal functions of patients receiving repeated computed tomography exams should be closely followed in case of deterioration[340].

TAF is the other novel prodrug of tenofovir. In an *in vitro* study, TAF resulted in high levels of the pharmacologically active metabolite tenofovir diphosphate than TDF [341]. A recent study showed comparable viral suppression and serologic response between TAF and TDF in non-cirrhotic HBV patients[342]. Besides, TAF has no proximal renal transporter-dependent cytotoxicity, which may lead to an improved renal safety profile[343]. Nevertheless, there was insufficient data in patients whose CCR below 15mL/min not receiving chronic hemodialysis and thus TAF is not recommended in this patient group[344].

CONCLUSION

The HCC patient population highly overlaps with those for CKD and ESRD. This article summarized the possible causes of the high comorbidity of HCC and renal dysfunction (Figure 1), including the possible mechanisms of CKD causing HCC, the pathophysiology of HCC affecting renal function, and the common risk factors shared by both HCC and CKD (Table 1). Both CKD and ESRD have been reported to negatively affect the prognosis of HCC. The article then adopted the well-known BCLC guidelines as a template (Figure 2) to discuss the indications for each stage of HCC treatment, the treatment-related adverse renal effects, and the concerns that are specific to patients with pre-existing renal dysfunction in the application of aggressive treatments such as liver resection, SLKT, RFA, and TACE, and in the use of the latest systemic target and immunotherapy approaches among the CKD and ESRD population. This article provides a comprehensive review of HCC patients with renal dysfunction from disease onset to treatment; the findings are expected to aid clinicians and scholars.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Ingrid Kuo and the Center for Big Data Analytics and Statistics at Chang Gung Memorial Hospital for creating the illustrations used herein.

REFERENCES

1 **Baecker A**, Liu X, La Vecchia C, Zhang ZF. Worldwide incidence of hepatocellular carcinoma cases attributable to major risk factors. *Eur J Cancer Prev* 2018; **27**: 205-212 [PMID: 29489473 DOI: 10.1097/CEJ.0000000000000428]
2 **Mak LY**, Cruz-Ramón V, Chinchilla-López P, Torres HA, LoConte NK, Rice JP, Foxhall LE, Sturgis EM, Merrill JK, Bailey HH, Méndez-Sánchez N, Yuen MF, Hwang JP. Global Epidemiology, Prevention, and Management of Hepatocellular Carcinoma. *Am Soc Clin Oncol Educ Book* 2018; **38**: 262-279 [PMID: 30231359 DOI: 10.1200/EDBK_200939]
3 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576 [PMID: 17570226 DOI: 10.1053/j.gastro.2007.04.061]
4 **El Nahas M**. The global challenge of chronic kidney disease. *Kidney Int* 2005; **68**: 2918-2929 [PMID: 16316385 DOI: 10.1111/j.1523-1755.2005.00774.x]
5 **Stewart JH**, Vajdic CM, van Leeuwen MT, Amin J, Webster AC, Chapman JR, McDonald SP, Grulich AE, McCredie MR. The pattern of excess cancer in dialysis and transplantation. *Nephrol Dial Transplant* 2009; **24**: 3225-3231 [PMID: 19589786 DOI: 10.1093/ndt/gfp331]
6 **Stengel B**. Chronic kidney disease and cancer: a troubling connection. *J Nephrol* 2010; **23**: 253-262 [PMID: 20349418]
7 **Zacks SL**, Fried MW. Hepatitis B and C and renal failure. *Infect Dis Clin North Am* 2001; **15**: 877-899 [PMID: 11570146 DOI: 10.1016/s0891-5520(05)70177-x]
8 **Chen YC**, Su YC, Li CY, Wu CP, Lee MS. A nationwide cohort study suggests chronic hepatitis B virus infection increases the risk of end-stage renal disease among patients in Taiwan. *Kidney Int* 2015; **87**: 1030-1038 [PMID: 25426815 DOI: 10.1038/ki.2014.363]
9 **Lin HF**, Li YH, Wang CH, Chou CL, Kuo DJ, Fang TC. Increased risk of cancer in chronic dialysis patients: a population-based cohort study in Taiwan. *Nephrol Dial Transplant* 2012; **27**: 1585-1590 [PMID: 21862456 DOI: 10.1093/ndt/gfr464]
10 **Maisonneuve P**, Agodoa L, Gellert R, Stewart JH, Buccianti G, Lowenfels AB, Wolfe RA, Jones E, Disney AP, Briggs D, McCredie M, Boyle P. Cancer in patients on dialysis for end-stage renal disease: an international collaborative study. *Lancet* 1999; **354**: 93-99 [PMID: 10408483]
11 **Chan-Yeung M**. A clinician's approach to determine the diagnosis, prognosis, and therapy of occupational asthma. *Med Clin North Am* 1990; **74**: 811-822 [PMID: 2186245 DOI: 10.1016/s0025-7125(16)30554-5]
12 **Vajdic CM**, McDonald SP, McCredie MR, van Leeuwen MT, Stewart JH, Law M, Chapman JR, Webster AC, Kaldor JM, Grulich AE. Cancer incidence before and after kidney transplantation. *JAMA* 2006; **296**: 2823-2831 [PMID: 17179459 DOI: 10.1001/jama.296.23.2823]
13 **Chapman JR**, Webster AC, Wong G. Cancer in the transplant recipient. *Cold Spring Harb Perspect Med* 2013; **3** [PMID: 23818517 DOI: 10.1101/cshperspect.a015677]
14 **Vamvakas S**, Bahner U, Heidland A. Cancer in end-stage renal disease: potential factors involved - editorial-. *Am J Nephrol* 1998; **18**: 89-95 [PMID: 9569948]
15 **Grulich AE**, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 2007; **370**: 59-67 [PMID: 17617273 DOI: 10.1016/S0140-6736(07)61050-2]
16 **Sun CY**, Chang SC, Wu MS. Uremic toxins induce kidney fibrosis by activating intrarenal renin-angiotensin-aldosterone system associated epithelial-to-mesenchymal transition. *PLoS One* 2012; **7**: e34026 [PMID: 22479508 DOI: 10.1371/journal.pone.0034026]
17 **Han H**, Chen Y, Zhu Z, Su X, Ni J, Du R, Zhang R, Jin W. p-Cresyl sulfate promotes the formation of atherosclerotic lesions and induces plaque instability by targeting vascular smooth muscle cells. *Front Med* 2016; **10**: 320-329 [PMID: 27527366 DOI: 10.1007/s11684-016-0463-x]
18 **Opdebeeck B**, Maudsley S, Azmi A, De Maré A, De Leger W, Meijers B, Verhulst A, Evenepoel P, D'Haese PC, Neven E. Indoxyl Sulfate and p-Cresyl Sulfate Promote Vascular Calcification and Associate with Glucose Intolerance. *J Am Soc Nephrol* 2019; **30**: 751-766 [PMID: 30940651 DOI: 10.1681/ASN.2018060609]
19 **Nitta T**, Kim JS, Mohuczy D, Behrns KE. Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling pathways. *Hepatology* 2008; **48**: 909-919 [PMID: 18712785 DOI: 10.1002/hep.22397]
20 **Dooley S**, Hamzavi J, Ciuclan L, Godoy P, Ilkavets I, Ehnert S, Ueberham E, Gebhardt R, Kanzler S, Geier A, Breitkopf K, Weng H, Mertens PR. Hepatocyte-specific Smad7 expression attenuates TGF-beta-mediated fibrogenesis and protects against liver damage. *Gastroenterology* 2008; **135**: 642-659 [PMID: 18602923 DOI: 10.1053/j.gastro.2008.04.038]
21 **Popov Y**, Schuppan D. Epithelial-to-mesenchymal transition in liver fibrosis: dead or alive? *Gastroenterology* 2010; **139**: 722-725 [PMID: 20682361 DOI: 10.1053/j.gastro.2010.07.015]

22 **Wells RG**. The epithelial-to-mesenchymal transition in liver fibrosis: here today, gone tomorrow? *Hepatology* 2010; **51**: 737-740 [PMID: 20198628 DOI: 10.1002/hep.23529]

23 **Lee WR**, Kim KH, An HJ, Kim JY, Lee SJ, Han SM, Pak SC, Park KK. Apamin inhibits hepatic fibrosis through suppression of transforming growth factor β1-induced hepatocyte epithelial-mesenchymal transition. *Biochem Biophys Res Commun* 2014; **450**: 195-201 [PMID: 24878534 DOI: 10.1016/j.bbrc.2014.05.089]

24 **Kong D**, Zhang F, Shao J, Wu L, Zhang X, Chen L, Lu Y, Zheng S. Curcumin inhibits cobalt chloride-induced epithelial-to-mesenchymal transition associated with interference with TGF-β/Smad signaling in hepatocytes. *Lab Invest* 2015; **95**: 1234-1245 [PMID: 26302188 DOI: 10.1038/labinvest.2015.107]

25 **Ramakrishna G**, Rastogi A, Trehanpati N, Sen B, Khosla R, Sarin SK. From cirrhosis to hepatocellular carcinoma: new molecular insights on inflammation and cellular senescence. *Liver Cancer* 2013; **2**: 367-383 [PMID: 24400224 DOI: 10.1159/000343852]

26 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]

27 **Tsai IT**, Wang CP, Yu TH, Lu YC, Lin CW, Lu LF, Wu CC, Chung FM, Lee YJ, Hung WC, Hsu CC. Circulating visfatin level is associated with hepatocellular carcinoma in chronic hepatitis B or C virus infection. *Cytokine* 2017; **90**: 54-59 [PMID: 27770715 DOI: 10.1016/j.cyto.2016.10.007]

28 **Hwang JC**, Weng SF, Weng RH. High incidence of hepatocellular carcinoma in ESRD patients: caused by high hepatitis rate or 'uremia'? *Jpn J Clin Oncol* 2012; **42**: 780-786 [PMID: 22782961 DOI: 10.1093/jjco/hys100]

29 **Cavaillon JM**, Poignet JL, Fitting C, Delons S. Serum interleukin-6 in long-term hemodialyzed patients. *Nephron* 1992; **60**: 307-313 [PMID: 1565183 DOI: 10.1159/000186770]

30 **Herbelin A**, Nguyen AT, Zingraff J, Ureña P, Descamps-Latscha B. Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor alpha. *Kidney Int* 1990; **37**: 116-125 [PMID: 2299797 DOI: 10.1038/ki.1990.16]

31 **Descamps-Latscha B**, Herbelin A, Nguyen AT, Roux-Lombard P, Zingraff J, Moynot A, Verger C, Dahmane D, de Groote D, Jungers P. Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. *J Immunol* 1995; **154**: 882-892 [PMID: 7814891]

32 **Rapa SF**, Di Iorio BR, Campiglia P, Heidland A, Marzocco S. Inflammation and Oxidative Stress in Chronic Kidney Disease-Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. *Int J Mol Sci* 2019; **21** [PMID: 31906008 DOI: 10.3390/ijms21010263]

33 **Rapicetta M**, Ferrari C, Levrero M. Viral determinants and host immune responses in the pathogenesis of HBV infection. *J Med Virol* 2002; **67**: 454-457 [PMID: 12116045 DOI: 10.1002/jmv.10096]

34 **Freeman AJ**, Marinos G, Ffrench RA, Lloyd AR. Immunopathogenesis of hepatitis C virus infection. *Immunol Cell Biol* 2001; **79**: 515-536 [PMID: 11903612 DOI: 10.1046/j.1440-1711.2001.01036.x]

35 **Fernández-Fresnedo G**, Ramos MA, González-Pardo MC, de Francisco AL, López-Hoyos M, Arias M. B lymphopenia in uremia is related to an accelerated in vitro apoptosis and dysregulation of Bcl-2. *Nephrol Dial Transplant* 2000; **15**: 502-510 [PMID: 10727545 DOI: 10.1093/ndt/15.4.502]

36 **Schmidt S**, Westhoff TH, Krauser P, Ignatius R, Jankowski J, Jankowski V, Zidek W, van der Giet M. The uraemic toxin phenylacetic acid impairs macrophage function. *Nephrol Dial Transplant* 2008; **23**: 3485-3493 [PMID: 18480077 DOI: 10.1093/ndt/gfn266]

37 **Meier P**, Dayer E, Blanc E, Wauters JP. Early T cell activation correlates with expression of apoptosis markers in patients with end-stage renal disease. *J Am Soc Nephrol* 2002; **13**: 204-212 [PMID: 11752039 DOI: 10.1681/ASN.V131204]

38 **Taborelli M**, Toffolutti F, Del Zotto S, Clagnan E, Furian L, Piselli P, Citterio F, Zanier L, Boscutti G, Serraino D; Italian Transplant & Cancer Cohort Study. Increased cancer risk in patients undergoing dialysis: a population-based cohort study in North-Eastern Italy. *BMC Nephrol* 2019; **20**: 107 [PMID: 30922296 DOI: 10.1186/s12882-019-1283-4]

39 **Wong G**, Turner RM, Chapman JR, Howell M, Lim WH, Webster AC, Craig JC. Time on dialysis and cancer risk after kidney transplantation. *Transplantation* 2013; **95**: 114-121 [PMID: 23222892 DOI: 10.1097/TP.0b013e31827743b4]

40 **Duong MC**, Nguyen VTT, Otsu S, McLaws ML. Prevalence of hepatitis B and C virus infections in hemodialysis patients in Vietnam: A systematic review and meta-analysis. *JGH Open* 2020; **4**: 29-38 [PMID: 32055694 DOI: 10.1002/jgh3.12199]

41 **Fabrizi F**, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. *Hepatology* 2002; **36**: 3-10 [PMID: 12085342 DOI: 10.1053/jhep.2002.34613]

42 **Fabrizi F**, Lunghi G, Martin P. Hepatitis B virus infection in hemodialysis: recent discoveries. *J Nephrol* 2002; **15**: 463-468 [PMID: 12455711]

43 **Vial T**, Descotes J. Immunosuppressive drugs and cancer. *Toxicology* 2003; **185**: 229-240 [PMID: 12581698 DOI: 10.1016/s0300-483x(02)00612-1]

44 **Kasiske BL**, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. *Am J Transplant* 2004; **4**: 905-913 [PMID: 15147424 DOI: 10.1111/j.1600-6143.2004.00450.x]

45 **Yeh SH**, Chen PJ. Gender disparity of hepatocellular carcinoma: the roles of sex hormones.

Oncology 2010; **78** Suppl 1: 172-179 [PMID: 20616601 DOI: 10.1159/000315247]
46 **Chiu CM**, Yeh SH, Chen PJ, Kuo TJ, Chang CJ, Yang WJ, Chen DS. Hepatitis B virus X protein enhances androgen receptor-responsive gene expression depending on androgen level. *Proc Natl Acad Sci U S A* 2007; **104**: 2571-2578 [PMID: 17259306 DOI: 10.1073/pnas.0609498104]
47 **Yu MW**, Cheng SW, Lin MW, Yang SY, Liaw YF, Chang HC, Hsiao TJ, Lin SM, Lee SD, Chen PJ, Liu CJ, Chen CJ. Androgen-receptor gene CAG repeats, plasma testosterone levels, and risk of hepatitis B-related hepatocellular carcinoma. *J Natl Cancer Inst* 2000; **92**: 2023-2028 [PMID: 11121465 DOI: 10.1093/jnci/92.24.2023]
48 **Naugler WE**, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 2007; **317**: 121-124 [PMID: 17615358 DOI: 10.1126/science.1140485]
49 **Prieto J**. Inflammation, HCC and sex: IL-6 in the centre of the triangle. *J Hepatol* 2008; **48**: 380-381 [PMID: 18093689 DOI: 10.1016/j.jhep.2007.11.007]
50 **Weng PH**, Hung KY, Huang HL, Chen JH, Sung PK, Huang KC. Cancer-specific mortality in chronic kidney disease: longitudinal follow-up of a large cohort. *Clin J Am Soc Nephrol* 2011; **6**: 1121-1128 [PMID: 21511834 DOI: 10.2215/CJN.09011010]
51 **Nallu A**, Sharma S, Ramezani A, Muralidharan J, Raj D. Gut microbiome in chronic kidney disease: challenges and opportunities. *Transl Res* 2017; **179**: 24-37 [PMID: 27187743 DOI: 10.1016/j.trsl.2016.04.007]
52 **Cigarran Guldris S**, González Parra E, Cases Amenós A. Gut microbiota in chronic kidney disease. *Nefrologia* 2017; **37**: 9-19 [PMID: 27553986 DOI: 10.1016/j.nefro.2016.05.008]
53 **Zitvogel L**, Galluzzi L, Viaud S, Vétizou M, Daillère R, Merad M, Kroemer G. Cancer and the gut microbiota: an unexpected link. *Sci Transl Med* 2015; **7**: 271ps1 [PMID: 25609166 DOI: 10.1126/scitranslmed.3010473]
54 composition of alcoholic beverages, additives and contaminants. *IARC Monogr Eval Carcinog Risks Hum* 1988; **44**: 71-99 [PMID: 3271892]
55 **Yu LX**, Yan HX, Liu Q, Yang W, Wu HP, Dong W, Tang L, Lin Y, He YQ, Zou SS, Wang C, Zhang HL, Cao GW, Wu MC, Wang HY. Endotoxin accumulation prevents carcinogen-induced apoptosis and promotes liver tumorigenesis in rodents. *Hepatology* 2010; **52**: 1322-1333 [PMID: 20803560 DOI: 10.1002/hep.23845]
56 **Yamada S**, Takashina Y, Watanabe M, Nagamine R, Saito Y, Kamada N, Saito H. Bile acid metabolism regulated by the gut microbiota promotes non-alcoholic steatohepatitis-associated hepatocellular carcinoma in mice. *Oncotarget* 2018; **9**: 9925-9939 [PMID: 29515780 DOI: 10.18632/oncotarget.24066]
57 **Ponziani FR**, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, Sanguinetti M, Morelli D, Paroni Sterbini F, Petito V, Reddel S, Calvani R, Camisaschi C, Picca A, Tuccitto A, Gasbarrini A, Pompili M, Mazzaferro V. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. *Hepatology* 2019; **69**: 107-120 [PMID: 29665135 DOI: 10.1002/hep.30036]
58 **Schwabe RF**, Greten TF. Gut microbiome in HCC - Mechanisms, diagnosis and therapy. *J Hepatol* 2020; **72**: 230-238 [PMID: 31954488 DOI: 10.1016/j.jhep.2019.08.016]
59 **Na SY**, Sung JY, Chang JH, Kim S, Lee HH, Park YH, Chung W, Oh KH, Jung JY. Chronic kidney disease in cancer patients: an independent predictor of cancer-specific mortality. *Am J Nephrol* 2011; **33**: 121-130 [PMID: 21242672 DOI: 10.1159/000323740]
60 **Erly B**, Carey WD, Kapoor B, McKinney JM, Tam M, Wang W. Hepatorenal Syndrome: A Review of Pathophysiology and Current Treatment Options. *Semin Intervent Radiol* 2015; **32**: 445-454 [PMID: 26622108 DOI: 10.1055/s-0035-1564794]
61 **Licata A**, Maida M, Bonaccorso A, Macaluso FS, Cappello M, Craxì A, Almasio PL. Clinical course and prognostic factors of hepatorenal syndrome: A retrospective single-center cohort study. *World J Hepatol* 2013; **5**: 685-691 [PMID: 24432185 DOI: 10.4254/wjh.v5.i12.685]
62 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]
63 **Cooney TG**, Bauer DC, Knauer CM. Portal hypertension associated with hepatocellular carcinoma with cirrhosis. *Am J Gastroenterol* 1980; **74**: 436-438 [PMID: 6263084]
64 **Aron M**, Nair M, Hemal AK. Renal metastasis from primary hepatocellular carcinoma. A case report and review of the literature. *Urol Int* 2004; **73**: 89-91 [PMID: 15263801 DOI: 10.1159/000078812]
65 **D'Antonio A**, Caleo A, Caleo O, Addesso M, Boscaino A. Hepatocellular carcinoma metastatic to the kidney mimicking renal oncocytoma. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 550-552 [PMID: 20943467]
66 **Jang TY**, Yeh ML. Hepatocellular carcinoma metastatic to kidney mimicking renal cell carcinoma. *Kaohsiung J Med Sci* 2017; **33**: 161-162 [PMID: 28254120 DOI: 10.1016/j.kjms.2016.10.009]
67 **Thomas DM**, Nasim MM, Gullick WJ, Alison MR. Immunoreactivity of transforming growth factor alpha in the normal adult gastrointestinal tract. *Gut* 1992; **33**: 628-631 [PMID: 1612477]
68 **Pelosof LC**, Gerber DE. Paraneoplastic syndromes: an approach to diagnosis and treatment. *Mayo Clin Proc* 2010; **85**: 838-854 [PMID: 20810794 DOI: 10.4065/mcp.2010.0099]
69 **Hyun HS**, Park PG, Kim JC, Hong KT, Kang HJ, Park KD, Shin HY, Kang HG, Ha IS, Cheong HI. A Case of Severe Hypercalcemia Causing Acute Kidney Injury: An Unusual Presentation of Acute

Lymphoblastic Leukemia. *Child Kidney Dis* 2017; **21**: 21-25 [DOI: 10.3339/jkspn.2017.21.1.21]
70 **Moysés-Neto M**, Guimarães FM, Ayoub FH, Vieira-Neto OM, Costa JA, Dantas M. Acute renal failure and hypercalcemia. *Ren Fail* 2006; **28**: 153-159 [PMID: 16538974 DOI: 10.1080/08860220500531005]
71 **Yen TC**, Hwang SJ, Wang CC, Lee SD, Yeh SH. Hypercalcemia and parathyroid hormone-related protein in hepatocellular carcinoma. *Liver* 1993; **13**: 311-315 [PMID: 8295494 DOI: 10.1111/j.1600-0676.1993.tb00651.x]
72 **Leone N**, Debernardi-Venon W, Marzano A, Massari M, Rizzetto M. Hypercalcaemia secondary to hepatocellular carcinoma. *Ital J Gastroenterol Hepatol* 1999; **31**: 604-606 [PMID: 10604102]
73 **Kwon HJ**, Kim JW, Kim H, Choi Y, Ahn S. Combined Hepatocellular Carcinoma and Neuroendocrine Carcinoma with Ectopic Secretion of Parathyroid Hormone: A Case Report and Review of the Literature. *J Pathol Transl Med* 2018; **52**: 232-237 [PMID: 29794961 DOI: 10.4132/jptm.2018.05.17]
74 **Jimi S**, Uchiyama M, Takaki A, Suzumiya J, Hara S. Mechanisms of cell death induced by cadmium and arsenic. *Ann N Y Acad Sci* 2004; **1011**: 325-331 [PMID: 15126309 DOI: 10.1007/978-3-662-41088-2_32]
75 **Johri N**, Jacquillet G, Unwin R. Heavy metal poisoning: the effects of cadmium on the kidney. *Biometals* 2010; **23**: 783-792 [PMID: 20354761 DOI: 10.1007/s10534-010-9328-y]
76 **Cheng YY**, Huang NC, Chang YT, Sung JM, Shen KH, Tsai CC, Guo HR. Associations between arsenic in drinking water and the progression of chronic kidney disease: A nationwide study in Taiwan. *J Hazard Mater* 2017; **321**: 432-439 [PMID: 27669384 DOI: 10.1016/j.jhazmat.2016.09.032]
77 **Satarug S**. Long-term exposure to cadmium in food and cigarette smoke, liver effects and hepatocellular carcinoma. *Curr Drug Metab* 2012; **13**: 257-271 [PMID: 22455552 DOI: 10.2174/138920012799320446]
78 **Shen J**, Wanibuchi H, Salim EI, Wei M, Kinoshita A, Yoshida K, Endo G, Fukushima S. Liver tumorigenicity of trimethylarsine oxide in male Fischer 344 rats--association with oxidative DNA damage and enhanced cell proliferation. *Carcinogenesis* 2003; **24**: 1827-1835 [PMID: 12919961 DOI: 10.1093/carcin/bgg143]
79 **Yamamoto S**, Konishi Y, Matsuda T, Murai T, Shibata MA, Matsui-Yuasa I, Otani S, Kuroda K, Endo G, Fukushima S. Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. *Cancer Res* 1995; **55**: 1271-1276 [PMID: 7882321]
80 **Chiu HF**, Ho SC, Wang LY, Wu TN, Yang CY. Does arsenic exposure increase the risk for liver cancer? *J Toxicol Environ Health A* 2004; **67**: 1491-1500 [PMID: 15371225 DOI: 10.1080/15287390490486806]
81 **Smith AH**, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood R, Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. *Environ Health Perspect* 1992; **97**: 259-267 [PMID: 1396465 DOI: 10.1289/ehp.9297259]
82 **Liu J**, Waalkes MP. Liver is a target of arsenic carcinogenesis. *Toxicol Sci* 2008; **105**: 24-32 [PMID: 18566022 DOI: 10.1093/toxsci/kfn120]
83 **Sabolić I**, Breljak D, Skarica M, Herak-Kramberger CM. Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. *Biometals* 2010; **23**: 897-926 [PMID: 20549307 DOI: 10.1007/s10534-010-9351-z]
84 **Souza V**, Escobar Md Mdel C, Gómez-Quiroz L, Bucio L, Hernández E, Cossio EC, Gutiérrez-Ruiz MC. Acute cadmium exposure enhances AP-1 DNA binding and induces cytokines expression and heat shock protein 70 in HepG2 cells. *Toxicology* 2004; **197**: 213-228 [PMID: 15033544 DOI: 10.1016/j.tox.2004.01.006]
85 **Hong F**, Si C, Gao P, Cederbaum AI, Xiong H, Lu Y. The role of CYP2A5 in liver injury and fibrosis: chemical-specific difference. *Naunyn Schmiedebergs Arch Pharmacol* 2016; **389**: 33-43 [PMID: 26363552 DOI: 10.1007/s00210-015-1172-8]
86 **Baker JR**, Edwards RJ, Lasker JM, Moore MR, Satarug S. Renal and hepatic accumulation of cadmium and lead in the expression of CYP4F2 and CYP2E1. *Toxicol Lett* 2005; **159**: 182-191 [PMID: 15994032 DOI: 10.1016/j.toxlet.2005.05.016]
87 **Baker JR**, Satarug S, Reilly PE, Edwards RJ, Ariyoshi N, Kamataki T, Moore MR, Williams DJ. Relationships between non-occupational cadmium exposure and expression of nine cytochrome P450 forms in human liver and kidney cortex samples. *Biochem Pharmacol* 2001; **62**: 713-721 [PMID: 11551516 DOI: 10.1016/s0006-2952(01)00716-x]
88 Carcinoma in Relation to Occupational and Environmental Heavy metals Exposure. *Eur J Community Med* 2015; **33**: 33-46 [DOI: 10.21608/ejcm.2015.714]
89 **Li H**, Xing L, Zhang M, Wang J, Zheng N. The Toxic Effects of Aflatoxin B1 and Aflatoxin M1 on Kidney through Regulating L-Proline and Downstream Apoptosis. *Biomed Res Int* 2018; **2018**: 9074861 [PMID: 30159329 DOI: 10.1155/2018/9074861]
90 **Kew MC**. Aflatoxins as a cause of hepatocellular carcinoma. *J Gastrointestin Liver Dis* 2013; **22**: 305-310 [PMID: 24078988]
91 **Wu HC**, Santella R. The role of aflatoxins in hepatocellular carcinoma. *Hepat Mon* 2012; **12**: e7238 [PMID: 23162603 DOI: 10.5812/hepatmon.7238]
92 **Reginster F**, Jadoul M, van Ypersele de Strihou C. Chinese herbs nephropathy presentation, natural history and fate after transplantation. *Nephrol Dial Transplant* 1997; **12**: 81-86 [PMID: 9027778

DOI: 10.1093/ndt/12.1.81]
93 **Lord GM**, Tagore R, Cook T, Gower P, Pusey CD. Nephropathy caused by Chinese herbs in the UK. *Lancet* 1999; **354**: 481-482 [PMID: 10465175 DOI: 10.1016/S0140-6736(99)03380-2]
94 **Lord GM**, Cook T, Arlt VM, Schmeiser HH, Williams G, Pusey CD. Urothelial malignant disease and Chinese herbal nephropathy. *Lancet* 2001; **358**: 1515-1516 [PMID: 11705569 DOI: 10.1016/s0140-6736(01)06576-x]
95 **Mei N**, Arlt VM, Phillips DH, Heflich RH, Chen T. DNA adduct formation and mutation induction by aristolochic acid in rat kidney and liver. *Mutat Res* 2006; **602**: 83-91 [PMID: 17010389 DOI: 10.1016/j.mrfmmm.2006.08.004]
96 **Ng AWT**, Poon SL, Huang MN, Lim JQ, Boot A, Yu W, Suzuki Y, Thangaraju S, Ng CCY, Tan P, Pang ST, Huang HY, Yu MC, Lee PH, Hsieh SY, Chang AY, Teh BT, Rozen SG. Aristolochic acids and their derivatives are widely implicated in liver cancers in Taiwan and throughout Asia. *Sci Transl Med* 2017; **9** [PMID: 29046434 DOI: 10.1126/scitranslmed.aan6446]
97 **Nault JC**, Letouzé E. Mutational Processes in Hepatocellular Carcinoma: The Story of Aristolochic Acid. *Semin Liver Dis* 2019; **39**: 334-340 [PMID: 31041788 DOI: 10.1055/s-0039-1685516]
98 **Shi J**, Zhu L, Liu S, Xie WF. A meta-analysis of case-control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. *Br J Cancer* 2005; **92**: 607-612 [PMID: 15685242 DOI: 10.1038/sj.bjc.6602333]
99 **Arbuthnot P**, Kew M. Hepatitis B virus and hepatocellular carcinoma. *Int J Exp Pathol* 2001; **82**: 77-100 [PMID: 11454100 DOI: 10.1111/j.1365-2613.2001.iep0082-0077-x]
100 **Beasley RP**, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981; **2**: 1129-1133 [PMID: 6118576 DOI: 10.1016/s0140-6736(81)90585-7]
101 **Chisari FV**, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S, Pinkert CA, Brinster RL, Palmiter RD. Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. *Cell* 1989; **59**: 1145-1156 [PMID: 2598264 DOI: 10.1016/0092-8674(89)90770-8]
102 **Ringelhan M**, McKeating JA, Protzer U. Viral hepatitis and liver cancer. *Philos Trans R Soc Lond B Biol Sci* 2017; **372** [PMID: 28893941 DOI: 10.1098/rstb.2016.0274]
103 **Hong YS**, Ryu S, Chang Y, Caínzos-Achirica M, Kwon MJ, Zhao D, Shafi T, Lazo M, Pastor-Barriuso R, Shin H, Cho J, Guallar E. Hepatitis B virus infection and development of chronic kidney disease: a cohort study. *BMC Nephrol* 2018; **19**: 353 [PMID: 30537940 DOI: 10.1186/s12882-018-1154-4]
104 **Bhimma R**, Coovadia HM. Hepatitis B virus-associated nephropathy. *Am J Nephrol* 2004; **24**: 198-211 [PMID: 14988643 DOI: 10.1159/000077065]
105 **Deng CL**, Song XW, Liang HJ, Feng C, Sheng YJ, Wang MY. Chronic hepatitis B serum promotes apoptotic damage in human renal tubular cells. *World J Gastroenterol* 2006; **12**: 1752-1756 [PMID: 16586546 DOI: 10.3748/wjg.v12.i11.1752]
106 **Si J**, Yu C, Guo Y, Bian Z, Qin C, Yang L, Chen Y, Yin L, Li H, Lan J, Chen J, Chen Z, Lv J, Li L; China Kadoorie Biobank Collaborative Group. Chronic hepatitis B virus infection and risk of chronic kidney disease: a population-based prospective cohort study of 0.5 million Chinese adults. *BMC Med* 2018; **16**: 93 [PMID: 29909773 DOI: 10.1186/s12916-018-1084-9]
107 **Chen YC**, Lin HY, Li CY, Lee MS, Su YC. A nationwide cohort study suggests that hepatitis C virus infection is associated with increased risk of chronic kidney disease. *Kidney Int* 2014; **85**: 1200-1207 [PMID: 24257691 DOI: 10.1038/ki.2013.455]
108 **Giovannucci E**, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. *Diabetes Care* 2010; **33**: 1674-1685 [PMID: 20587728 DOI: 10.2337/dc10-0666]
109 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468 [PMID: 14762783 DOI: 10.1053/j.gastro.2003.10.065]
110 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]
111 **Moore MA**, Park CB, Tsuda H. Implications of the hyperinsulinaemia-diabetes-cancer link for preventive efforts. *Eur J Cancer Prev* 1998; **7**: 89-107 [PMID: 9818771]
112 **Goyal R**, Faizy AF, Siddiqui SS, Singhai M. Evaluation of TNF-α and IL-6 Levels in Obese and Non-obese Diabetics: Pre- and Postinsulin Effects. *N Am J Med Sci* 2012; **4**: 180-184 [PMID: 22536561 DOI: 10.4103/1947-2714.94944]
113 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208 [PMID: 20141834 DOI: 10.1016/j.cell.2009.12.052]
114 **Gross JL**, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care* 2005; **28**: 164-176 [PMID: 15616252]
115 **Takuma Y**, Nouso K. Nonalcoholic steatohepatitis-associated hepatocellular carcinoma: our case series and literature review. *World J Gastroenterol* 2010; **16**: 1436-1441 [PMID: 20333782 DOI: 10.3748/wjg.v16.i12.1436]
116 **Dhamija E**, Paul SB, Kedia S. Non-alcoholic fatty liver disease associated with hepatocellular carcinoma: An increasing concern. *Indian J Med Res* 2019; **149**: 9-17 [PMID: 31115369 DOI:

10.4103/ijmr.IJMR_1456_17]
117 **Nagaoki Y**, Hyogo H, Aikata H, Tanaka M, Naeshiro N, Nakahara T, Honda Y, Miyaki D, Kawaoka T, Takaki S, Hiramatsu A, Waki K, Imamura M, Kawakami Y, Takahashi S, Chayama K. Recent trend of clinical features in patients with hepatocellular carcinoma. *Hepatol Res* 2012; **42**: 368-375 [PMID: 22151896 DOI: 10.1111/j.1872-034X.2011.00929.x]
118 **Diehl AM**, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005; **54**: 303-306 [PMID: 15647199 DOI: 10.1136/gut.2003.024935]
119 **Wang Y**, Ausman LM, Greenberg AS, Russell RM, Wang XD. Nonalcoholic steatohepatitis induced by a high-fat diet promotes diethylnitrosamine-initiated early hepatocarcinogenesis in rats. *Int J Cancer* 2009; **124**: 540-546 [PMID: 19004024 DOI: 10.1002/ijc.23995]
120 **Kamada Y**, Matsumoto H, Tamura S, Fukushima J, Kiso S, Fukui K, Igura T, Maeda N, Kihara S, Funahashi T, Matsuzawa Y, Shimomura I, Hayashi N. Hypoadiponectinemia accelerates hepatic tumor formation in a nonalcoholic steatohepatitis mouse model. *J Hepatol* 2007; **47**: 556-564 [PMID: 17459514 DOI: 10.1016/j.jhep.2007.03.020]
121 **Byrne CD**, Targher G. NAFLD as a driver of chronic kidney disease. *J Hepatol* 2020; **72**: 785-801 [PMID: 32059982 DOI: 10.1016/j.jhep.2020.01.013]
122 **Park H**, Dawwas GK, Liu X, Nguyen MH. Nonalcoholic fatty liver disease increases risk of incident advanced chronic kidney disease: a propensity-matched cohort study. *J Intern Med* 2019; **286**: 711-722 [PMID: 31359543 DOI: 10.1111/joim.12964]
123 **Targher G**, Mantovani A, Alisi A, Mosca A, Panera N, Byrne CD, Nobili V. Relationship Between PNPLA3 rs738409 Polymorphism and Decreased Kidney Function in Children With NAFLD. *Hepatology* 2019; **70**: 142-153 [PMID: 30912854 DOI: 10.1002/hep.30625]
124 **Leung PS**, Carlsson PO. Tissue renin-angiotensin system: its expression, localization, regulation and potential role in the pancreas. *J Mol Endocrinol* 2001; **26**: 155-164 [PMID: 11432370 DOI: 10.1677/jme.0.0260155]
125 **Siragy HM**, Carey RM. Role of the intrarenal renin-angiotensin-aldosterone system in chronic kidney disease. *Am J Nephrol* 2010; **31**: 541-550 [PMID: 20484892 DOI: 10.1159/000313363]
126 **Brewster UC**, Perazella MA. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. *Am J Med* 2004; **116**: 263-272 [PMID: 14969655 DOI: 10.1016/j.amjmed.2003.09.034]
127 **Remuzzi G**, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest* 2006; **116**: 288-296 [PMID: 16453013 DOI: 10.1172/JCI27699]
128 **Anderson S**, Rennke HG, Brenner BM. Therapeutic advantage of converting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *J Clin Invest* 1986; **77**: 1993-2000 [PMID: 3011863 DOI: 10.1172/JCI112528]
129 **Lewis EJ**, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N Engl J Med* 1993; **329**: 1456-1462 [PMID: 8413456 DOI: 10.1056/NEJM199311113292004]
130 **Simões E Silva AC**, Miranda AS, Rocha NP, Teixeira AL. Renin angiotensin system in liver diseases: Friend or foe? *World J Gastroenterol* 2017; **23**: 3396-3406 [PMID: 28596676 DOI: 10.3748/wjg.v23.i19.3396]
131 **Bataller R**, Ginès P, Nicolás JM, Görbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V, Rodés J. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149-1156 [PMID: 10833490 DOI: 10.1016/s0016-5085(00)70368-4]
132 **Tuncer I**, Ozbek H, Ugras S, Bayram I. Anti-fibrogenic effects of captopril and candesartan cilexetil on the hepatic fibrosis development in rat. The effect of AT1-R blocker on the hepatic fibrosis. *Exp Toxicol Pathol* 2003; **55**: 159-166 [PMID: 14620537 DOI: 10.1078/0940-2993-00309]
133 **Wei YH**, Jun L, Qiang CJ. Effect of losartan, an angiotensin II antagonist, on hepatic fibrosis induced by CCl4 in rats. *Dig Dis Sci* 2004; **49**: 1589-1594 [PMID: 15573910 DOI: 10.1023/b:ddas.0000043369.88701.5b]
134 **Yoshiji H**, Noguchi R, Kuriyama S, Yoshii J, Ikenaka Y, Yanase K, Namisaki T, Kitade M, Yamazaki M, Uemura M, Fukui H. Suppression of renin-angiotensin system attenuates hepatocarcinogenesis *via* angiogenesis inhibition in rats. *Anticancer Res* 2005; **25**: 3335-3340 [PMID: 16101147]
135 **Lee CH**, Hsieh SY, Chang CC, Wang IK, Huang WH, Weng CH, Hsu CW, Yen TH. Hepatocellular carcinoma in hemodialysis patients. *Oncotarget* 2017; **8**: 73154-73161 [PMID: 29069858 DOI: 10.18632/oncotarget.17127]
136 **Lee YH**, Hsu CY, Hsia CY, Huang YH, Su CW, Lin HC, Lee RC, Chiou YY, Huo TI. Hepatocellular carcinoma in uremic patients: is there evidence for an increased risk of mortality? *J Gastroenterol Hepatol* 2013; **28**: 348-356 [PMID: 23190248 DOI: 10.1111/jgh.12062]
137 **Sarno G**, Montalti R, Giglio MC, Rompianesi G, Tomassini F, Scarpellini E, De Simone G, De Palma GD, Troisi RI. Hepatocellular carcinoma in patients with chronic renal disease: Challenges of interventional treatment. *Surg Oncol* 2021; **36**: 42-50 [PMID: 33307490 DOI: 10.1016/j.suronc.2020.11.007]
138 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]
139 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]

140 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]

141 **Thelen A**, Benckert C, Tautenhahn HM, Hau HM, Bartels M, Linnemann J, Bertolini J, Moche M, Wittekind C, Jonas S. Liver resection for hepatocellular carcinoma in patients without cirrhosis. *Br J Surg* 2013; **100**: 130-137 [PMID: 23132620 DOI: 10.1002/bjs.8962]

142 **Smoot RL**, Nagorney DM, Chandan VS, Que FG, Schleck CD, Harmsen WS, Kendrick ML. Resection of hepatocellular carcinoma in patients without cirrhosis. *Br J Surg* 2011; **98**: 697-703 [PMID: 21280030 DOI: 10.1002/bjs.7401]

143 **Ercolani G**, Grazi GL, Ravaioli M, Del Gaudio M, Gardini A, Cescon M, Varotti G, Cetta F, Cavallari A. Liver resection for hepatocellular carcinoma on cirrhosis: univariate and multivariate analysis of risk factors for intrahepatic recurrence. *Ann Surg* 2003; **237**: 536-543 [PMID: 12677151 DOI: 10.1097/01.SLA.0000059988.22416.F2]

144 **Santambrogio R**, Kluger MD, Costa M, Belli A, Barabino M, Laurent A, Opocher E, Azoulay D, Cherqui D. Hepatic resection for hepatocellular carcinoma in patients with Child-Pugh's A cirrhosis: is clinical evidence of portal hypertension a contraindication? *HPB (Oxford)* 2013; **15**: 78-84 [PMID: 23216782 DOI: 10.1111/j.1477-2574.2012.00594.x]

145 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

146 **European Association For The Study Of The Liver**. European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

147 **Omata M**, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017; **11**: 317-370 [PMID: 28620797 DOI: 10.1007/s12072-017-9799-9]

148 **Allaire M**, Goumard C, Lim C, Le Cleach A, Wagner M, Scatton O. New frontiers in liver resection for hepatocellular carcinoma. *JHEP Rep* 2020; **2**: 100134 [PMID: 32695968 DOI: 10.1016/j.jhepr.2020.100134]

149 **Cloyd JM**, Ma Y, Morton JM, Kurella Tamura M, Poultsides GA, Visser BC. Does chronic kidney disease affect outcomes after major abdominal surgery? *J Gastrointest Surg* 2014; **18**: 605-612 [PMID: 24241964 DOI: 10.1007/s11605-013-2390-3]

150 **Toshima T**, Shirabe K, Yoshiya S, Muto J, Ikegami T, Yoshizumi T, Maehara Y. Outcome of hepatectomy for hepatocellular carcinoma in patients with renal dysfunction. *HPB (Oxford)* 2012; **14**: 317-324 [PMID: 22487069 DOI: 10.1111/j.1477-2574.2012.00452.x]

151 **Shirata C**, Hasegawa K, Kokudo T, Yamashita S, Yamamoto S, Arita J, Akamatsu N, Kaneko J, Sakamoto Y, Kokudo N. Liver Resection for Hepatocellular Carcinoma in Patients with Renal Dysfunction. *World J Surg* 2018; **42**: 4054-4062 [PMID: 29947980 DOI: 10.1007/s00268-018-4698-3]

152 **Cheng SB**, Wu CC, Shu KH, Ho WL, Chen JT, Yeh DC, Liu TJ, P'eng FK. Liver resection for hepatocellular carcinoma in patients with end-stage renal failure. *J Surg Oncol* 2001; **78**: 241-6; discussion 246 [PMID: 11745817]

153 **Yeh CN**, Lee WC, Chen MF. Hepatic resection for hepatocellular carcinoma in end-stage renal disease patients: two decades of experience at Chang Gung Memorial Hospital. *World J Gastroenterol* 2005; **11**: 2067-2071 [PMID: 15810070]

154 **Yeh CC**, Lin JT, Jeng LB, Charalampos I, Chen TT, Lee TY, Wu MS, Kuo KN, Liu YY, Wu CY. Hepatic resection for hepatocellular carcinoma patients on hemodialysis for uremia: a nationwide cohort study. *World J Surg* 2013; **37**: 2402-2409 [PMID: 23811792 DOI: 10.1007/s00268-013-2137-z]

155 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]

156 **Moreno R**, Berenguer M. Post-liver transplantation medical complications. *Ann Hepatol* 2006; **5**: 77-85 [PMID: 16807513]

157 **Liu LU**, Schiano TD. Long-term care of the liver transplant recipient. *Clin Liver Dis* 2007; **11**: 397-416 [PMID: 17606214 DOI: 10.1016/j.cld.2007.04.003]

158 **McCauley J**, Van Thiel DH, Starzl TE, Puschett JB. Acute and chronic renal failure in liver transplantation. *Nephron* 1990; **55**: 121-128 [PMID: 2362625 DOI: 10.1159/000185938]

159 **Karapanagiotou A**, Kydona C, Dimitriadis C, Sgourou K, Giasnetsova T, Fouzas I, Imvrios G, Gritsi-Gerogianni N. Acute kidney injury after orthotopic liver transplantation. *Transplant Proc* 2012; **44**: 2727-2729 [PMID: 23146506 DOI: 10.1016/j.transproceed.2012.09.096]

160 **Bilbao I**, Charco R, Balsells J, Lazaro JL, Hidalgo E, Llopart L, Murio E, Margarit C. Risk factors for acute renal failure requiring dialysis after liver transplantation. *Clin Transplant* 1998; **12**: 123-129 [PMID: 9575400]

161 **Klaus F**, Keitel da Silva C, Meinerz G, Carvalho LM, Goldani JC, Cantisani G, Zanotelli ML, Duro Garcia V, Keitel E. Acute kidney injury after liver transplantation: incidence and mortality. *Transplant Proc* 2014; **46**: 1819-1821 [PMID: 25131045 DOI: 10.1016/j.transproceed.2014.05.053]

162 **Chen X**, Ding X, Shen B, Teng J, Zou J, Wang T, Zhou J, Chen N, Zhang B. Incidence and outcomes of acute kidney injury in patients with hepatocellular carcinoma after liver transplantation. *J Cancer Res Clin Oncol* 2017; **143**: 1337-1346 [PMID: 28289896 DOI: 10.1007/s00432-017-2376-8]

163 **Fraley DS**, Burr R, Bernardini J, Angus D, Kramer DJ, Johnson JP. Impact of acute renal failure on mortality in end-stage liver disease with or without transplantation. *Kidney Int* 1998; **54**: 518-524 [PMID: 9690218 DOI: 10.1046/j.1523-1755.1998.00004.x]

164 **Zhu M**, Li Y, Xia Q, Wang S, Qiu Y, Che M, Dai H, Qian J, Ni Z, Axelsson J, Yan Y. Strong impact of acute kidney injury on survival after liver transplantation. *Transplant Proc* 2010; **42**: 3634-3638 [PMID: 21094830 DOI: 10.1016/j.transproceed.2010.08.059]

165 **Barri YM**, Sanchez EQ, Jennings LW, Melton LB, Hays S, Levy MF, Klintmalm GB. Acute kidney injury following liver transplantation: definition and outcome. *Liver Transpl* 2009; **15**: 475-483 [PMID: 19399734 DOI: 10.1002/lt.21682]

166 **Weber ML**, Ibrahim HN, Lake JR. Renal dysfunction in liver transplant recipients: evaluation of the critical issues. *Liver Transpl* 2012; **18**: 1290-1301 [PMID: 22847917 DOI: 10.1002/lt.23522]

167 **Herrero JI**, Cuervas-Mons V, Gómez-Bravo MÁ, Fabregat J, Otero A, Bilbao I, Salcedo MM, González-Diéguez ML, Fernández JR, Serrano MT, Jiménez M, Rodrigo JM, Narváez I, Sánchez G. Prevalence and progression of chronic kidney disease after liver transplant: a prospective, real-life, observational, two-year multicenter study. *Rev Esp Enferm Dig* 2018; **110**: 538-543 [PMID: 29893577 DOI: 10.17235/reed.2018.5431/2017]

168 **Kalisvaart M**, Schlegel A, Trivedi PJ, Roberts K, Mirza DF, Perera T, Isaac JI, Ferguson J, de Jonge J, Muiesan P. Chronic Kidney Disease After Liver Transplantation: Impact of Extended Criteria Grafts. *Liver Transpl* 2019; **25**: 922-933 [PMID: 30947384 DOI: 10.1002/lt.25468]

169 **Mason J**. The effect of cyclosporin on renal function. *J Autoimmun* 1992; **5** Suppl A: 349-354 [PMID: 1503631 DOI: 10.1016/0896-8411(92)90053-s]

170 **O'Riordan A**, Dutt N, Cairns H, Rela M, O'Grady JG, Heaton N, Hendry BM. Renal biopsy in liver transplant recipients. *Nephrol Dial Transplant* 2009; **24**: 2276-2282 [PMID: 19293134 DOI: 10.1093/ndt/gfp112]

171 **Ojo AO**, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndorfer J, Christensen L, Merion RM. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931-940 [PMID: 12954741 DOI: 10.1056/NEJMoa021744]

172 **Herrero JI**, Quiroga J, Sangro B, Girala M, Gómez-Manero N, Pardo F, Alvárez-Cienfuegos J, Prieto J. Conversion of liver transplant recipients on cyclosporine with renal impairment to mycophenolate mofetil. *Liver Transpl Surg* 1999; **5**: 414-420 [PMID: 10477843 DOI: 10.1002/lt.500050513]

173 **Fisher NC**, Nightingale PG, Gunson BK, Lipkin GW, Neuberger JM. Chronic renal failure following liver transplantation: a retrospective analysis. *Transplantation* 1998; **66**: 59-66 [PMID: 9679823 DOI: 10.1097/00007890-199807150-00010]

174 **Brown RS Jr**, Lombardero M, Lake JR. Outcome of patients with renal insufficiency undergoing liver or liver-kidney transplantation. *Transplantation* 1996; **62**: 1788-1793 [PMID: 8990364 DOI: 10.1097/00007890-199612270-00018]

175 **Lafayette RA**, Paré G, Schmid CH, King AJ, Rohrer RJ, Nasraway SA. Pretransplant renal dysfunction predicts poorer outcome in liver transplantation. *Clin Nephrol* 1997; **48**: 159-164 [PMID: 9342487]

176 **David AI**, Coelho MP, Paes AT, Leite AK, Della Guardia B, de Almeida MD, Meira SP, de Rezende MB, Afonso RC, Ferraz-Neto BH. Liver transplant outcome: a comparison between high and low MELD score recipients. *Einstein (Sao Paulo)* 2012; **10**: 57-61 [PMID: 23045827 DOI: 10.1590/s1679-45082012000100012]

177 **Aguilera V**, Ferrer I, Berenguer M, Rivera J, Rubín Á, Moya Á, Pareja E, Sánchez J, Prieto M, Mir J. Comparison of results of combined liver-kidney transplantation vs. isolated liver transplantation. *Ann Hepatol* 2013; **12**: 274-281 [PMID: 23396739]

178 **Kliem V**, Ringe B, Frei U, Pichlmayr R. Single-center experience of combined liver and kidney transplantation. *Clin Transplant* 1995; **9**: 39-44 [PMID: 7742582]

179 **Tanriover B**, MacConmara MP, Parekh J, Arce C, Zhang S, Gao A, Mufti A, Levea SL, Sandikci B, Ayvaci MU, Ariyamuthu VK, Hwang C, Mohan S, Mete M, Vazquez MA, Marrero JA. Simultaneous liver kidney transplantation in liver transplant candidates with renal dysfunction: Importance of creatinine levels, dialysis, and organ quality in survival. *Kidney Int Rep* 2016; **1**: 221-229 [PMID: 27942610 DOI: 10.1016/j.ekir.2016.07.008]

180 **Margreiter R**, Steurer W, Spechtenhauser B, Königsrainer A. Kidney transplantation together with another solid organ from the same donor--a single-center progress report. *Clin Nephrol* 2000; **53**: suppl 38-suppl 43 [PMID: 10809434]

181 **Zhu XF**, He XS, Chen GH, Chen LZ, Wang CX, Huang JF. Combined liver and kidney transplantation in Guangzhou, China. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 585-589 [PMID: 18086622]

182 **Pardo F**, Pons JA, Castells L, Colmenero J, Gómez MÁ, Lladó L, Pérez B, Prieto M, Briceño J. VI consensus document by the Spanish Liver Transplantation Society. *Gastroenterol Hepatol* 2018; **41**: 406-421 [PMID: 29866511 DOI: 10.1016/j.gastrohep.2018.05.012]

183 **Singal AK**, Salameh H, Kuo YF, Wiesner RH. Evolving frequency and outcomes of simultaneous liver kidney transplants based on liver disease etiology. *Transplantation* 2014; **98**: 216-221 [PMID:

24621538 DOI: 10.1097/TP.0000000000000048]

184 **Rich N**, Tanriover B, Singal AG, Marrero JA. Outcomes of Simultaneous Liver Kidney Transplantation in Patients With Hepatocellular Carcinoma. *Transplantation* 2017; **101**: e12-e19 [PMID: 28009759 DOI: 10.1097/TP.0000000000001515]

185 **Livraghi T**, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. *Radiology* 2003; **226**: 441-451 [PMID: 12563138 DOI: 10.1148/radiol.2262012198]

186 **Takaki H**, Yamakado K, Nakatsuka A, Yamada T, Shiraki K, Takei Y, Takeda K. Frequency of and risk factors for complications after liver radiofrequency ablation under CT fluoroscopic guidance in 1500 sessions: single-center experience. *AJR Am J Roentgenol* 2013; **200**: 658-664 [PMID: 23436859 DOI: 10.2214/AJR.12.8691]

187 **Li H**, Li B, Wei Y, Liu F. Hemolysis as a complication of radiofrequency ablation for hepatocellular carcinoma must be paid more attention. *Dig Dis Sci* 2011; **56**: 3391-3392 [PMID: 21567191 DOI: 10.1007/s10620-011-1737-4]

188 **Keltner JR**, Donegan E, Hynson JM, Shapiro WA. Acute renal failure after radiofrequency liver ablation of metastatic carcinoid tumor. *Anesth Analg* 2001; **93**: 587-589 [PMID: 11524322 DOI: 10.1097/00000539-200109000-00011]

189 **Tsui SL**, Lee AK, Lui SK, Poon RT, Fan ST. Acute intraoperative hemolysis and hemoglobinuria during radiofrequency ablation of hepatocellular carcinoma. *Hepatogastroenterology* 2003; **50**: 526-529 [PMID: 12749264]

190 **Metzner J**, Evans JL, Domino KB. Life-threatening hyperkalemia during radiofrequency ablation of hepatocellular carcinoma. *J Clin Anesth* 2010; **22**: 473-476 [PMID: 20868971 DOI: 10.1016/j.jclinane.2009.08.008]

191 **Jiao LR**, Hansen PD, Havlik R, Mitry RR, Pignatelli M, Habib N. Clinical short-term results of radiofrequency ablation in primary and secondary liver tumors. *Am J Surg* 1999; **177**: 303-306 [PMID: 10326848 DOI: 10.1016/s0002-9610(99)00043-4]

192 **Hargreaves GM**, Adam R, Bismuth H. Results after nonsurgical local treatment of primary liver malignancies. *Langenbecks Arch Surg* 2000; **385**: 185-193 [PMID: 10857489 DOI: 10.1007/s004230050263]

193 **Fukumori K**, Shakado S, Makihata T, Takemoto R, Fukuizumi K, Miyahara T, Yasumori K, Muranaka T, Watanabe J, Saitsu H, Sakai H, Sata M. [A case of chronic renal failure caused hyperkalemia following percutaneous radiofrequency ablation therapy for hepatocellular carcinoma]. *Nihon Shokakibyo Gakkai Zasshi* 2003; **100**: 702-706 [PMID: 12833866]

194 **Hiraoka A**, Kumada T, Michitaka K, Toyoda H, Tada T, Takaguchi K, Tsuji K, Itobayashi E, Takizawa D, Hirooka M, Koizumi Y, Ochi H, Joko K, Kisaka Y, Shimizu Y, Tajiri K, Tani J, Taniguchi T, Toshimori A, Fujioka S; Real-Life Practice Experts For Hepatocellular Carcinoma (HCC) (RELPEC) Study Group and The HCC 48 Group (HCC experts from 48 clinics). Clinical features of hemodialysis patients treated for hepatocellular carcinoma: Comparison between resection and radiofrequency ablation. *Mol Clin Oncol* 2017; **6**: 455-461 [PMID: 28413650 DOI: 10.3892/mco.2017.1192]

195 **Kondo Y**, Yoshida H, Tomizawa Y, Tateishi R, Shiina S, Tagawa K, Omata M. Percutaneous radiofrequency ablation of hepatocellular carcinoma in 14 patients undergoing regular hemodialysis for end-stage renal disease. *AJR Am J Roentgenol* 2009; **193**: 964-969 [PMID: 19770317 DOI: 10.2214/AJR.08.2236]

196 **Sato M**, Tateishi R, Yasunaga H, Matsui H, Horiguchi H, Fushimi K, Koike K. Mortality and hemorrhagic complications associated with radiofrequency ablation for treatment of hepatocellular carcinoma in patients on hemodialysis for end-stage renal disease: A nationwide survey. *J Gastroenterol Hepatol* 2017; **32**: 1873-1878 [PMID: 28266772 DOI: 10.1111/jgh.13780]

197 **Verhoeven BH**, Haagsma EB, Appeltans BM, Slooff MJ, de Jong KP. Hyperkalaemia after radiofrequency ablation of hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2002; **14**: 1023-1024 [PMID: 12352224 DOI: 10.1097/00042737-200209000-00015]

198 **Waghray A**, Murali AR, Menon KN. Hepatocellular carcinoma: From diagnosis to treatment. *World J Hepatol* 2015; **7**: 1020-1029 [PMID: 26052391 DOI: 10.4254/wjh.v7.i8.1020]

199 **Lee BC**, Liu KL, Lin CL, Kao CH. Risk of acute kidney injury after transarterial chemoembolisation in hepatocellular carcinoma patients: A nationwide population-based cohort study. *Eur Radiol* 2017; **27**: 4482-4489 [PMID: 28593432 DOI: 10.1007/s00330-017-4893-x]

200 **Hsu CY**, Huang YH, Su CW, Lin HC, Chiang JH, Lee PC, Lee FY, Huo TI, Lee SD. Renal failure in patients with hepatocellular carcinoma and ascites undergoing transarterial chemoembolization. *Liver Int* 2010; **30**: 77-84 [PMID: 19818004 DOI: 10.1111/j.1478-3231.2009.02128.x]

201 **Zhou C**, Wang R, Ding Y, Du L, Hou C, Lu D, Hao L, Lv W. Prognostic factors for acute kidney injury following transarterial chemoembolization in patients with hepatocellular carcinoma. *Int J Clin Exp Pathol* 2014; **7**: 2579-2586 [PMID: 24966972]

202 **Huo TI**, Wu JC, Lee PC, Chang FY, Lee SD. Incidence and risk factors for acute renal failure in patients with hepatocellular carcinoma undergoing transarterial chemoembolization: a prospective study. *Liver Int* 2004; **24**: 210-215 [PMID: 15189271 DOI: 10.1111/j.1478-3231.2004.00911.x]

203 **Aspelin P**, Aubry P, Fransson SG, Strasser R, Willenbrock R, Berg KJ; Nephrotoxicity in High-Risk Patients Study of Iso-Osmolar and Low-Osmolar Non-Ionic Contrast Media Study Investigators. Nephrotoxic effects in high-risk patients undergoing angiography. *N Engl J Med* 2003; **348**: 491-499 [PMID: 12571256 DOI: 10.1056/NEJMoa021833]

204 **Hong CX**, Lv LW, Hua LZ, Bo S, Sen CX, Xin NY, Wei YJ, Rui XJ, Qiang DX, Zhou ZJ. Epidemiology and Management of Acute Kidney Injury in Hepatocellular Carcinoma Patients Undergoing Transcatheter Arterial Chemoembolization. *Curr Protein Pept Sci* 2017; **18**: 1218-1223 [PMID: 27634445 DOI: 10.2174/1389203717666160909124305]

205 **Hao JF**, Zhang LW, Bai JX, Li YJ, Liu JN, Zhang XL, Han JM, Li X, Jiang H, Cao N. Incidence, risk factors, and prognosis of acute kidney injury following transarterial chemoembolization in patients with hepatocellular carcinoma: a prospective cohort study. *Indian J Cancer* 2015; **51** Suppl 2: e3-e8 [PMID: 25712839 DOI: 10.4103/0019-509X.151984]

206 **Hsu CY**, Huang YH, Su CW, Chiang JH, Lin HC, Lee PC, Lee FY, Huo TI, Lee SD. Transarterial chemoembolization in patients with hepatocellular carcinoma and renal insufficiency. *J Clin Gastroenterol* 2010; **44**: e171-e177 [PMID: 20048685 DOI: 10.1097/MCG.0b013e3181c88235]

207 **Lin WC**, Chang CW, Wang TE, Chen MJ, Wang HY. Challenges of transarterial therapy for hepatocellular carcinoma in patients with chronic kidney disease. *Medicine (Baltimore)* 2019; **98**: e17007 [PMID: 31464957 DOI: 10.1097/MD.0000000000017007]

208 **Watanabe M**, Shibuya A, Minamino T, Murano J, Matsunaga K, Fujii K, Ogasawara G, Irie T, Woodhams R, Koizumi W. Benefits and problems of transarterial therapy in patients with hepatocellular carcinoma and chronic kidney disease. *J Vasc Interv Radiol* 2014; **25**: 1947-55; quiz 1955 [PMID: 25306225 DOI: 10.1016/j.jvir.2014.08.024]

209 **Sato M**, Tateishi R, Yasunaga H, Matsui H, Fushimi K, Ikeda H, Yatomi Y, Koike K. In-hospital mortality associated with transcatheter arterial embolization for treatment of hepatocellular carcinoma in patients on hemodialysis for end stage renal disease: a matched-pair cohort study using a nationwide database. *BJR Open* 2019; **1**: 20190004 [PMID: 33178938 DOI: 10.1259/bjro.20190004]

210 **Llovet JM**, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. *Nat Rev Clin Oncol* 2015; **12**: 408-424 [PMID: 26054909 DOI: 10.1038/nrclinonc.2015.103]

211 **Roberts LR**, Gores GJ. Hepatocellular carcinoma: molecular pathways and new therapeutic targets. *Semin Liver Dis* 2005; **25**: 212-225 [PMID: 15918149 DOI: 10.1055/s-2005-871200]

212 **Chen S**, Cao Q, Wen W, Wang H. Targeted therapy for hepatocellular carcinoma: Challenges and opportunities. *Cancer Lett* 2019; **460**: 1-9 [PMID: 31207320 DOI: 10.1016/j.canlet.2019.114428]

213 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

214 **Wilhelm S**, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 2006; **5**: 835-844 [PMID: 17016424 DOI: 10.1038/nrd2130]

215 **Eremina V**, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 2003; **111**: 707-716 [PMID: 12618525 DOI: 10.1172/JCI17423]

216 **Maynard S**, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. *Annu Rev Med* 2008; **59**: 61-78 [PMID: 17937587 DOI: 10.1146/annurev.med.59.110106.214058]

217 **Patel TV**, Morgan JA, Demetri GD, George S, Maki RG, Quigley M, Humphreys BD. A preeclampsia-like syndrome characterized by reversible hypertension and proteinuria induced by the multitargeted kinase inhibitors sunitinib and sorafenib. *J Natl Cancer Inst* 2008; **100**: 282-284 [PMID: 18270341 DOI: 10.1093/jnci/djm311]

218 **Okuno Y**, Kume H, Hosoda C, Homma Y. Development of nephrotic syndrome after administration of sorafenib in a case of metastatic renal cell carcinoma. *Case Rep Med* 2011; **2011**: 710216 [PMID: 22007236 DOI: 10.1155/2011/710216]

219 **Hanna RM**, Selamet U, Hasnain H, El-Masry M, Saab S, Wallace WD, Yanny B, Wilson J. Development of Focal Segmental Glomerulosclerosis and Thrombotic Microangiopathy in a Liver Transplant Patient on Sorafenib for Hepatocellular Carcinoma: A Case Report. *Transplant Proc* 2018; **50**: 4033-4037 [PMID: 30577309 DOI: 10.1016/j.transproceed.2018.07.020]

220 **Hilger RA**, Richly H, Grubert M, Kredtke S, Thyssen D, Eberhardt W, Hense J, Schuler M, Scheulen ME. Pharmacokinetics of sorafenib in patients with renal impairment undergoing hemodialysis. *Int J Clin Pharmacol Ther* 2009; **47**: 61-64 [PMID: 19203541 DOI: 10.5414/cpp47061]

221 **Ishii T**, Hatano E, Taura K, Mizuno T, Kawai T, Fukudo M, Katsura T, Uemoto S. Sorafenib in a hepatocellular carcinoma patient with end-stage renal failure: A pharmacokinetic study. *Hepatol Res* 2014; **44**: 685-688 [PMID: 23639221 DOI: 10.1111/hepr.12156]

222 **Bayer HealthCare Pharmaceuticals Inc**. NEXAVAR (sorafenib) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/021923s024lblrpl.pdf

223 **Masini C**, Sabbatini R, Porta C, Procopio G, Di Lorenzo G, Onofri A, Buti S, Iacovelli R, Invernizzi R, Moscetti L, Aste MG, Pagano M, Grosso F, Lucia Manenti A, Ortega C, Cosmai L, Del Giovane C, Conte PF. Use of tyrosine kinase inhibitors in patients with metastatic kidney cancer receiving haemodialysis: a retrospective Italian survey. *BJU Int* 2012; **110**: 692-698 [PMID: 22364110 DOI:

10.1111/j.1464-410X.2012.10946.x]

224 **Huang A**, Yang XR, Chung WY, Dennison AR, Zhou J. Targeted therapy for hepatocellular carcinoma. *Signal Transduct Target Ther* 2020; **5**: 146 [PMID: 32782275 DOI: 10.1038/s41392-020-00264-x]

225 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

226 **Hiraoka A**, Kumada T, Kariyama K, Takaguchi K, Itobayashi E, Shimada N, Tajiri K, Tsuji K, Ishikawa T, Ochi H, Hirooka M, Tsutsui A, Shibata H, Tada T, Toyoda H, Nouso K, Joko K, Hiasa Y, Michitaka K; Real-life Practice Experts for HCC (RELPEC) Study Group and the HCC 48 Group (hepatocellular carcinoma experts from 48 clinics in Japan). Therapeutic potential of lenvatinib for unresectable hepatocellular carcinoma in clinical practice: Multicenter analysis. *Hepatol Res* 2019; **49**: 111-117 [PMID: 30144256 DOI: 10.1111/hepr.13243]

227 **Takeda H**, Nishijima N, Nasu A, Komekado H, Kita R, Kimura T, Kudo M, Osaki Y. Long-term antitumor effect of lenvatinib on unresectable hepatocellular carcinoma with portal vein invasion. *Hepatol Res* 2019; **49**: 594-599 [PMID: 30499247 DOI: 10.1111/hepr.13294]

228 **Hiraoka A**, Kumada T, Kariyama K, Takaguchi K, Atsukawa M, Itobayashi E, Tsuji K, Tajiri K, Hirooka M, Shimada N, Shibata H, Ishikawa T, Ochi H, Tada T, Toyoda H, Nouso K, Tsutsui A, Itokawa N, Imai M, Joko K, Hiasa Y, Michitaka K; Real-life Practice Experts for HCC (RELPEC) Study Group; HCC 48 Group (hepatocellular carcinoma experts from 48 clinics in Japan). Clinical features of lenvatinib for unresectable hepatocellular carcinoma in real-world conditions: Multicenter analysis. *Cancer Med* 2019; **8**: 137-146 [PMID: 30575325 DOI: 10.1002/cam4.1909]

229 **Iwasaki H**, Yamazaki H, Takasaki H, Suganuma N, Sakai R, Nakayama H, Toda S, Masudo K. Renal dysfunction in patients with radioactive iodine-refractory thyroid cancer treated with tyrosine kinase inhibitors: A retrospective study. *Medicine (Baltimore)* 2019; **98**: e17588 [PMID: 31626129 DOI: 10.1097/MD.0000000000017588]

230 **Hyogo Y**, Kiyota N, Otsuki N, Goto S, Imamura Y, Chayahara N, Toyoda M, Nibu KI, Hyodo T, Hara S, Masuoka H, Kasahara T, Ito Y, Miya A, Hirokawa M, Miyauchi A, Minami H. Thrombotic Microangiopathy with Severe Proteinuria Induced by Lenvatinib for Radioactive Iodine-Refractory Papillary Thyroid Carcinoma. *Case Rep Oncol* 2018; **11**: 735-741 [PMID: 30519176 DOI: 10.1159/000494080]

231 **Furuto Y**, Hashimoto H, Namikawa A, Outi H, Takahashi H, Horiuti H, Honda K, Shibuya Y. Focal segmental glomerulosclerosis lesion associated with inhibition of tyrosine kinases by lenvatinib: a case report. *BMC Nephrol* 2018; **19**: 273 [PMID: 30340546 DOI: 10.1186/s12882-018-1074-3]

232 **Eisai Inc**. LENVIMA (lenvatinib) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/206947s018lbl.pdf

233 **Bruix J**, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G; RESORCE Investigators. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **389**: 56-66 [PMID: 27932229 DOI: 10.1016/S0140-6736(16)32453-9]

234 **Yoo C**, Park JW, Kim YJ, Kim DY, Yu SJ, Lim TS, Lee SJ, Ryoo BY, Lim HY. Multicenter retrospective analysis of the safety and efficacy of regorafenib after progression on sorafenib in Korean patients with hepatocellular carcinoma. *Invest New Drugs* 2019; **37**: 567-572 [PMID: 30523474 DOI: 10.1007/s10637-018-0707-5]

235 **Finn RS**, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Gerolami R, Caparello C, Cabrera R, Chang C, Sun W, LeBerre MA, Baumhauer A, Meinhardt G, Bruix J. Outcomes of sequential treatment with sorafenib followed by regorafenib for HCC: Additional analyses from the phase III RESORCE trial. *J Hepatol* 2018; **69**: 353-358 [PMID: 29704513 DOI: 10.1016/j.jhep.2018.04.010]

236 **Eisen T**, Joensuu H, Nathan PD, Harper PG, Wojtukiewicz MZ, Nicholson S, Bahl A, Tomczak P, Pyrhonen S, Fife K, Bono P, Boxall J, Wagner A, Jeffers M, Lin T, Quinn DI. Regorafenib for patients with previously untreated metastatic or unresectable renal-cell carcinoma: a single-group phase 2 trial. *Lancet Oncol* 2012; **13**: 1055-1062 [PMID: 22959186 DOI: 10.1016/S1470-2045(12)70364-9]

237 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D; CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/S0140-6736(12)61900-X]

238 **Krishnamoorthy SK**, Relias V, Sebastian S, Jayaraman V, Saif MW. Management of regorafenib-related toxicities: a review. *Therap Adv Gastroenterol* 2015; **8**: 285-297 [PMID: 26327919 DOI: 10.1177/1756283X15580743]

239 **Sturm A**. Cleton MBBPJGKSMGF-THSRFHZJTI. Evaluation of exposure of regorafenib and its

metabolites in cancer patients with renal impairment by modelling, simulation, and clinical study. *Ann Oncol* 2016; **27**: vi526-vi544 [DOI: 10.1093/annonc/mdw392.37]

240 **Bayer HealthCare Pharmaceuticals Inc**. STIVARGA (regorafenib) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/203085s013lbl.pdf

241 **Cochin V**, Gross-Goupil M, Ravaud A, Godbert Y, Le Moulec S. [Cabozantinib: Mechanism of action, efficacy and indications]. *Bull Cancer* 2017; **104**: 393-401 [PMID: 28477875 DOI: 10.1016/j.bulcan.2017.03.013]

242 **Abou-Alfa GK**, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE, Schwab G, Kelley RK. Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. *N Engl J Med* 2018; **379**: 54-63 [PMID: 29972759 DOI: 10.1056/NEJMoa1717002]

243 **Nguyen L**, Holland J, Ramies D, Mamelok R, Benrimoh N, Ciric S, Marbury T, Preston RA, Heuman DM, Gavis E, Lacy S. Effect of Renal and Hepatic Impairment on the Pharmacokinetics of Cabozantinib. *J Clin Pharmacol* 2016; **56**: 1130-1140 [PMID: 26865195 DOI: 10.1002/jcph.714]

244 **Cappagli V**, Moriconi D, Bonadio AG, Giannese D, La Manna G, Egidi MF, Comai G, Vischini G, Bottici V, Elisei R, Viola D. Proteinuria is a late-onset adverse event in patients treated with cabozantinib. *J Endocrinol Invest* 2021; **44**: 95-103 [PMID: 32363491 DOI: 10.1007/s40618-020-01272-y]

245 **Paschke L**, Lincke T, Mühlberg KS, Jabs WJ, Lindner TH, Paschke R. Anti VEGF-TKI Treatment and New Renal Adverse Events Not Reported in Phase III Trials. *Eur Thyroid J* 2018; **7**: 308-312 [PMID: 30574461 DOI: 10.1159/000491387]

246 **Exelixis Inc**. COMETRIQ (cabozantinib) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/203756s009lbl.pdf

247 **Zhu AX**, Park JO, Ryoo BY, Yen CJ, Poon R, Pastorelli D, Blanc JF, Chung HC, Baron AD, Pfiffer TE, Okusaka T, Kubackova K, Trojan J, Sastre J, Chau I, Chang SC, Abada PB, Yang L, Schwartz JD, Kudo M; REACH Trial Investigators. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol* 2015; **16**: 859-870 [PMID: 26095784 DOI: 10.1016/S1470-2045(15)00050-9]

248 **Park JO**, Ryoo BY, Yen CJ, Kudo M, Yang L, Abada PB, Cheng R, Orlando M, Zhu AX, Okusaka T. Second-line ramucirumab therapy for advanced hepatocellular carcinoma (REACH): an East Asian and non-East Asian subgroup analysis. *Oncotarget* 2016; **7**: 75482-75491 [PMID: 27776351 DOI: 10.18632/oncotarget.12780]

249 **Zhu AX**, Baron AD, Malfertheiner P, Kudo M, Kawazoe S, Pezet D, Weissinger F, Brandi G, Barone CA, Okusaka T, Wada Y, Park JO, Ryoo BY, Cho JY, Chung HC, Li CP, Yen CJ, Lee KD, Chang SC, Yang L, Abada PB, Chau I. Ramucirumab as Second-Line Treatment in Patients With Advanced Hepatocellular Carcinoma: Analysis of REACH Trial Results by Child-Pugh Score. *JAMA Oncol* 2017; **3**: 235-243 [PMID: 27657674 DOI: 10.1001/jamaoncol.2016.4115]

250 **Zhu AX**, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, Assenat E, Brandi G, Pracht M, Lim HY, Rau KM, Motomura K, Ohno I, Merle P, Daniele B, Shin DB, Gerken G, Borg C, Hiriart JB, Okusaka T, Morimoto M, Hsu Y, Abada PB, Kudo M; REACH-2 study investigators. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2019; **20**: 282-296 [PMID: 30665869 DOI: 10.1016/S1470-2045(18)30937-9]

251 **Fujii T**, Kawasoe K, Tonooka A, Ohta A, Nitta K. Nephrotic syndrome associated with ramucirumab therapy: A single-center case series and literature review. *Medicine (Baltimore)* 2019; **98**: e16236 [PMID: 31277139 DOI: 10.1097/MD.0000000000016236]

252 **Abdel-Rahman O**, ElHalawani H. Proteinuria in Patients with Solid Tumors Treated with Ramucirumab: A Systematic Review and Meta-Analysis. *Chemotherapy* 2014; **60**: 325-333 [PMID: 26302785 DOI: 10.1159/000437253]

253 **Yamada R**, Okawa T, Matsuo K, Suzuki M, Mori N, Mori K. Renal-limited thrombotic microangiopathy after switching from bevacizumab to ramucirumab: a case report. *BMC Nephrol* 2019; **20**: 14 [PMID: 30634936 DOI: 10.1186/s12882-018-1194-9]

254 **Hirai T**, Shuji Y, Takiyama M, Hanada K, Itoh T. Renin-angiotensin system inhibitors for countering proteinuria induced by angiogenesis inhibitors: a retrospective observational analysis. *Cancer Chemother Pharmacol* 2019; **84**: 195-202 [PMID: 31119376 DOI: 10.1007/s00280-019-03876-5]

255 **Eli Lilly and Company**. CYRAMZA (ramucirumab) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125477s037lbl.pdf

256 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020; **382**: 1894-1905 [PMID: 32402160 DOI: 10.1056/NEJMoa1915745]

257 **Sassier M**, Dugué AE, Clarisse B, Lesueur P, Avrillon V, Bizieux-Thaminy A, Auliac JB,

Kaluzinski L, Tillon J, Robinet G, Le Caer H, Monnet I, Madroszyk A, Boza G, Falchero L, Fournel P, Egenod T, Toffart AC, Leiber N, Do P, Gervais R. Renal insufficiency is the leading cause of double maintenance (bevacizumab and pemetrexed) discontinuation for toxicity to advanced non-small cell lung cancer in real world setting. *Lung Cancer* 2015; **89**: 161-166 [PMID: 26037036 DOI: 10.1016/j.lungcan.2015.05.005]

258 **Zhao J**, Li H, Wang M. Acute renal failure in a patient receiving anti-VEGF therapy for advanced non-small cell lung cancer. *J Thorac Oncol* 2009; **4**: 1185-1187 [PMID: 19704343 DOI: 10.1097/JTO.0b013e3181b2362f]

259 **Wu S**, Kim C, Baer L, Zhu X. Bevacizumab increases risk for severe proteinuria in cancer patients. *J Am Soc Nephrol* 2010; **21**: 1381-1389 [PMID: 20538785 DOI: 10.1681/ASN.2010020167]

260 **Zhao T**, Wang X, Xu T, Xu X, Liu Z. Bevacizumab significantly increases the risks of hypertension and proteinuria in cancer patients: A systematic review and comprehensive meta-analysis. *Oncotarget* 2017; **8**: 51492-51506 [PMID: 28881662 DOI: 10.18632/oncotarget.18190]

261 **Usui J**, Glezerman IG, Salvatore SP, Chandran CB, Flombaum CD, Seshan SV. Clinicopathological spectrum of kidney diseases in cancer patients treated with vascular endothelial growth factor inhibitors: a report of 5 cases and review of literature. *Hum Pathol* 2014; **45**: 1918-1927 [PMID: 25087655 DOI: 10.1016/j.humpath.2014.05.015]

262 **Uy AL**, Simper NB, Champeaux AL, Perkins RM. Progressive bevacizumab-associated renal thrombotic microangiopathy. *NDT Plus* 2009; **2**: 36-39 [PMID: 25949282 DOI: 10.1093/ndtplus/sfn168]

263 **Vakiti A**, Singh D, Pilla R, Alhaj-Moustafa M, Fitzpatrick KW. Bevacizumab-induced atypical hemolytic uremic syndrome and treatment with eculizumab. *J Oncol Pharm Pract* 2019; **25**: 1011-1015 [PMID: 29768958 DOI: 10.1177/1078155218774895]

264 **Lomax AJ**, Hill PA, Ashley DM. Case report of interstitial nephritis induced by bevacizumab therapy for glioblastoma multiforme. *J Oncol Pharm Pract* 2013; **19**: 365-368 [PMID: 23235917 DOI: 10.1177/1078155212466421]

265 **Barakat RK**, Singh N, Lal R, Verani RR, Finkel KW, Foringer JR. Interstitial nephritis secondary to bevacizumab treatment in metastatic leiomyosarcoma. *Ann Pharmacother* 2007; **41**: 707-710 [PMID: 17374619 DOI: 10.1345/aph.1H635]

266 **Hanna RM**, Lopez E, Wilson J, Barathan S, Cohen AH. Minimal change disease onset observed after bevacizumab administration. *Clin Kidney J* 2016; **9**: 239-244 [PMID: 26985375 DOI: 10.1093/ckj/sfv139]

267 **Genentech Inc**. AVASTIN (bevacizumab) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125085s337lbl.pdf

268 **Garnier-Viougeat N**, Rixe O, Paintaud G, Ternant D, Degenne D, Mouawad R, Deray G, Izzedine H. Pharmacokinetics of bevacizumab in haemodialysis. *Nephrol Dial Transplant* 2007; **22**: 975 [PMID: 17093010 DOI: 10.1093/ndt/gfl664]

269 **Pardoll DM**. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252-264 [PMID: 22437870 DOI: 10.1038/nrc3239]

270 **Kole C**, Charalampakis N, Tsakatikas S, Vailas M, Moris D, Gkotsis E, Kykalos S, Karamouzis MV, Schizas D. Immunotherapy for Hepatocellular Carcinoma: A 2021 Update. *Cancers (Basel)* 2020; **12** [PMID: 33020428 DOI: 10.3390/cancers12102859]

271 **Rosenberg SA**. Decade in review-cancer immunotherapy: entering the mainstream of cancer treatment. *Nat Rev Clin Oncol* 2014; **11**: 630-632 [PMID: 25311350 DOI: 10.1038/nrclinonc.2014.174]

272 **Greten TF**, Sangro B. Targets for immunotherapy of liver cancer. *J Hepatol* 2017 [PMID: 28923358 DOI: 10.1016/j.jhep.2017.09.007]

273 **Cerezo D**, Peña MJ, Mijares M, Martínez G, Blanca I, De Sanctis JB. Peptide vaccines for cancer therapy. *Recent Pat Inflamm Allergy Drug Discov* 2015; **9**: 38-45 [PMID: 25642777 DOI: 10.2174/1872213x09666150131141953]

274 **Parmiani G**, Russo V, Maccalli C, Parolini D, Rizzo N, Maio M. Peptide-based vaccines for cancer therapy. *Hum Vaccin Immunother* 2014; **10**: 3175-3178 [PMID: 25483658 DOI: 10.4161/hv.29418]

275 **Akce M**, Zaidi MY, Waller EK, El-Rayes BF, Lesinski GB. The Potential of CAR T Cell Therapy in Pancreatic Cancer. *Front Immunol* 2018; **9**: 2166 [PMID: 30319627 DOI: 10.3389/fimmu.2018.02166]

276 **Hoffman-Censits J**, Pal S, Kaiser C, Ding B, Bellmunt J. Atezolizumab in patients with renal insufficiency and mixed variant histology: analyses from an expanded access program in platinum-treated locally advanced or metastatic urothelial carcinoma. *J Immunother Cancer* 2020; **8** [PMID: 32641319 DOI: 10.1136/jitc-2019-000419]

277 **Parisi A**, Cortellini A, Cannita K, Bersanelli M, Ficorella C. Safe Administration of anti-PD-L1 Atezolizumab in a Patient with Metastatic Urothelial Cell Carcinoma and End-Stage Renal Disease on Dialysis. *Case Rep Oncol Med* 2019; **2019**: 3452762 [PMID: 30881713 DOI: 10.1155/2019/3452762]

278 **McDermott DF**, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, Fong L, Joseph RW, Pal SK, Reeves JA, Sznol M, Hainsworth J, Rathmell WK, Stadler WM, Hutson T, Gore ME, Ravaud A, Bracarda S, Suárez C, Danielli R, Gruenwald V, Choueiri TK, Nickles D, Jhunjhunwala S, Piault-Louis E, Thobhani A, Qiu J, Chen DS, Hegde PS, Schiff C, Fine GD, Powles T. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus

sunitinib in renal cell carcinoma. *Nat Med* 2018; **24**: 749-757 [PMID: 29867230 DOI: 10.1038/s41591-018-0053-3]

279 **McDermott DF**, Sosman JA, Sznol M, Massard C, Gordon MS, Hamid O, Powderly JD, Infante JR, Fassò M, Wang YV, Zou W, Hegde PS, Fine GD, Powles T. Atezolizumab, an Anti-Programmed Death-Ligand 1 Antibody, in Metastatic Renal Cell Carcinoma: Long-Term Safety, Clinical Activity, and Immune Correlates From a Phase Ia Study. *J Clin Oncol* 2016; **34**: 833-842 [PMID: 26755520 DOI: 10.1200/JCO.2015.63.7421]

280 **Genentech Inc**. TECENTRIQ (atezolizumab) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/761034s020lbl.pdf

281 **Zhu AX**, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, Sarker D, Verset G, Chan SL, Knox J, Daniele B, Webber AL, Ebbinghaus SW, Ma J, Siegel AB, Cheng AL, Kudo M; KEYNOTE-224 investigators. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018; **19**: 940-952 [PMID: 29875066 DOI: 10.1016/S1470-2045(18)30351-6]

282 **Finn RS**, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL; KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 2020; **38**: 193-202 [PMID: 31790344 DOI: 10.1200/JCO.19.01307]

283 **Izzedine H**, Mathian A, Champiat S, Picard C, Mateus C, Routier E, Varga A, Malka D, Leary A, Michels J, Michot JM, Marabelle A, Lambotte O, Amoura Z, Soria JC, Kaaki S, Quellard N, Goujon JM, Brocheriou I. Renal toxicities associated with pembrolizumab. *Clin Kidney J* 2019; **12**: 81-88 [PMID: 30746132 DOI: 10.1093/ckj/sfy100]

284 **Wanchoo R**, Karam S, Uppal NN, Barta VS, Deray G, Devoe C, Launay-Vacher V, Jhaveri KD; Cancer and Kidney International Network Workgroup on Immune Checkpoint Inhibitors. Adverse Renal Effects of Immune Checkpoint Inhibitors: A Narrative Review. *Am J Nephrol* 2017; **45**: 160-169 [PMID: 28076863 DOI: 10.1159/000455014]

285 **Mamlouk O**, Selamet U, Machado S, Abdelrahim M, Glass WF, Tchakarov A, Gaber L, Lahoti A, Workeneh B, Chen S, Lin J, Abdel-Wahab N, Tayar J, Lu H, Suarez-Almazor M, Tannir N, Yee C, Diab A, Abudayyeh A. Nephrotoxicity of immune checkpoint inhibitors beyond tubulointerstitial nephritis: single-center experience. *J Immunother Cancer* 2019; **7**: 2 [PMID: 30612580 DOI: 10.1186/s40425-018-0478-8]

286 **Merck Sharp & Dohme Corp**. KEYTRUDA (pembrolizumab) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125514s088lbl.pdf

287 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]

288 **Sangro B**, Park J-W, Cruz CMD, Anderson J, Lang L, Neely J, Shaw JW, Cheng AL. A randomized, multicenter, phase 3 study of nivolumab vs sorafenib as first-line treatment in patients (pts) with advanced hepatocellular carcinoma (HCC): CheckMate-459. *J Clin Oncol* 2016; **34**: TPS4147-TPS4147 [DOI: 10.1200/JCO.2016.34.15_suppl.TPS4147]

289 **Jung K**, Zeng X, Bilusic M. Nivolumab-associated acute glomerulonephritis: a case report and literature review. *BMC Nephrol* 2016; **17**: 188 [PMID: 27876011 DOI: 10.1186/s12882-016-0408-2]

290 **Uchida N**, Tsuji S, Fujita K, Koizumi M, Moriyoshi K, Mio T. Nivolumab-induced severe acute kidney injury with a long latent phase in a patient with non-small-cell lung cancer: A case report. *Clin Case Rep* 2018; **6**: 2185-2188 [PMID: 30455917 DOI: 10.1002/ccr3.1848]

291 **Bristol-Myers Squibb Company**. OPDIVO (nivolumab) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125527s000lbl.pdf

292 **Yau T**, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, Melero I, Kudo M, Hou MM, Matilla A, Tovoli F, Knox JJ, Ruth He A, El-Rayes BF, Acosta-Rivera M, Lim HY, Neely J, Shen Y, Wisniewski T, Anderson J, Hsu C. Efficacy and Safety of Nivolumab Plus Ipilimumab in Patients With Advanced Hepatocellular Carcinoma Previously Treated With Sorafenib: The CheckMate 040 Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: e204564 [PMID: 33001135 DOI: 10.1001/jamaoncol.2020.4564]

293 **Cortazar FB**, Marrone KA, Troxell ML, Ralto KM, Hoenig MP, Brahmer JR, Le DT, Lipson EJ, Glezerman IG, Wolchok J, Cornell LD, Feldman P, Stokes MB, Zapata SA, Hodi FS, Ott PA, Yamashita M, Leaf DE. Clinicopathological features of acute kidney injury associated with immune checkpoint inhibitors. *Kidney Int* 2016; **90**: 638-647 [PMID: 27282937 DOI: 10.1016/j.kint.2016.04.008]

294 **Iannello A**, Thompson TW, Ardolino M, Marcus A, Raulet DH. Immunosurveillance and immunotherapy of tumors by innate immune cells. *Curr Opin Immunol* 2016; **38**: 52-58 [PMID: 26686774 DOI: 10.1016/j.coi.2015.11.001]

295 **Fadel F**, El Karoui K, Knebelmann B. Anti-CTLA4 antibody-induced lupus nephritis. *N Engl J Med* 2009; **361**: 211-212 [PMID: 19587352 DOI: 10.1056/NEJMc0904283]

296 **Barnard ZR**, Walcott BP, Kahle KT, Nahed BV, Coumans JV. Hyponatremia associated with Ipilimumab-induced hypophysitis. *Med Oncol* 2012; **29**: 374-377 [PMID: 21264545 DOI: 10.1007/s12032-010-9794-7]

297 **Chodakiewitz Y**, Brown S, Boxerman JL, Brody JM, Rogg JM. Ipilimumab treatment associated pituitary hypophysitis: clinical presentation and imaging diagnosis. *Clin Neurol Neurosurg* 2014; **125**: 125-130 [PMID: 25127260 DOI: 10.1016/j.clineuro.2014.06.011]

298 **Bristol-Myers Squibb Company**. YERVOY (ipilimumab) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125377s119lbl.pdf

299 **Wang Z**, Yin N, Zhang Z, Zhang Y, Zhang G, Chen W. Upregulation of T-cell Immunoglobulin and Mucin-Domain Containing-3 (Tim-3) in Monocytes/Macrophages Associates with Gastric Cancer Progression. *Immunol Invest* 2017; **46**: 134-148 [PMID: 27911104 DOI: 10.1080/08820139.2016.1229790]

300 **Ganjalikhani Hakemi M**, Jafarinia M, Azizi M, Rezaeepoor M, Isayev O, Bazhin AV. The Role of TIM-3 in Hepatocellular Carcinoma: A Promising Target for Immunotherapy? *Front Oncol* 2020; **10**: 601661 [PMID: 33425759 DOI: 10.3389/fonc.2020.601661]

301 **Zhu C**, Anderson AC, Kuchroo VK. TIM-3 and its regulatory role in immune responses. *Curr Top Microbiol Immunol* 2011; **350**: 1-15 [PMID: 20700701 DOI: 10.1007/82_2010_84]

302 **Sakuishi K**, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010; **207**: 2187-2194 [PMID: 20819927 DOI: 10.1084/jem.20100643]

303 **Anderson AC**, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, Chandwaskar R, Karman J, Su EW, Hirashima M, Bruce JN, Kane LP, Kuchroo VK, Hafler DA. Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. *Science* 2007; **318**: 1141-1143 [PMID: 18006747 DOI: 10.1126/science.1148536]

304 **Monney L**, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA, Freeman GJ, Kuchroo VK. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 2002; **415**: 536-541 [PMID: 11823861 DOI: 10.1038/415536a]

305 **Wiener Z**, Kohalmi B, Pocza P, Jeager J, Tolgyesi G, Toth S, Gorbe E, Papp Z, Falus A. TIM-3 is expressed in melanoma cells and is upregulated in TGF-beta stimulated mast cells. *J Invest Dermatol* 2007; **127**: 906-914 [PMID: 17096021 DOI: 10.1038/sj.jid.5700616]

306 **Pfeffer CR**. Assessment of suicidal children and adolescents. *Psychiatr Clin North Am* 1989; **12**: 861-872 [PMID: 2690029]

307 **Li H**, Wu K, Tao K, Chen L, Zheng Q, Lu X, Liu J, Shi L, Liu C, Wang G, Zou W. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. *Hepatology* 2012; **56**: 1342-1351 [PMID: 22505239 DOI: 10.1002/hep.25777]

308 **Liu F**, Liu Y, Chen Z. Tim-3 expression and its role in hepatocellular carcinoma. *J Hematol Oncol* 2018; **11**: 126 [PMID: 30309387 DOI: 10.1186/s13045-018-0667-4]

309 **Zhang H**, Song Y, Yang H, Liu Z, Gao L, Liang X, Ma C. Tumor cell-intrinsic Tim-3 promotes liver cancer *via* NF-κB/IL-6/STAT3 axis. *Oncogene* 2018; **37**: 2456-2468 [PMID: 29449693 DOI: 10.1038/s41388-018-0140-4]

310 **Schroll A**, Eller K, Huber JM, Theurl IM, Wolf AM, Weiss G, Rosenkranz AR. Tim3 is upregulated and protective in nephrotoxic serum nephritis. *Am J Pathol* 2010; **176**: 1716-1724 [PMID: 20167865 DOI: 10.2353/ajpath.2010.090859]

311 **Yang H**, Xie T, Li D, Du X, Wang T, Li C, Song X, Xu L, Yi F, Liang X, Gao L, Yang X, Ma C. Tim-3 aggravates podocyte injury in diabetic nephropathy by promoting macrophage activation *via* the NF-κB/TNF-α pathway. *Mol Metab* 2019; **23**: 24-36 [PMID: 30862474 DOI: 10.1016/j.molmet.2019.02.007]

312 **Berg T**, Zoulim F, Moeller B, Trinh H, Marcellin P, Chan S, Kitrinos KM, Dinh P, Flaherty JF Jr, McHutchison JG, Manns M. Long-term efficacy and safety of emtricitabine plus tenofovir DF vs. tenofovir DF monotherapy in adefovir-experienced chronic hepatitis B patients. *J Hepatol* 2014; **60**: 715-722 [PMID: 24295873 DOI: 10.1016/j.jhep.2013.11.024]

313 **Buti M**, Tsai N, Petersen J, Flisiak R, Gurel S, Krastev Z, Aguilar Schall R, Flaherty JF, Martins EB, Charuworn P, Kitrinos KM, Subramanian GM, Gane E, Marcellin P. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. *Dig Dis Sci* 2015; **60**: 1457-1464 [PMID: 25532501 DOI: 10.1007/s10620-014-3486-7]

314 **Ahn J**, Lee HM, Lim JK, Pan CQ, Nguyen MH, Ray Kim W, Mannalithara A, Trinh H, Chu D, Tran T, Min A, Do S, Te H, Reddy KR, Lok AS. Entecavir safety and effectiveness in a national cohort of treatment-naïve chronic hepatitis B patients in the US - the ENUMERATE study. *Aliment Pharmacol Ther* 2016; **43**: 134-144 [PMID: 26510638 DOI: 10.1111/apt.13440]

315 **Watanabe T**, Tokumoto Y, Joko K, Michitaka K, Mashiba T, Hiraoka A, Ochi H, Koizumi Y, Tada F, Hirooka M, Yoshida O, Imai Y, Abe M, Hiasa Y. Effects of long-term entecavir treatment on the incidence of hepatocellular carcinoma in chronic hepatitis B patients. *Hepatol Int* 2016; **10**: 320-327 [PMID: 26198757 DOI: 10.1007/s12072-015-9647-8]

316 **Loomba R**, Liang TJ. Hepatitis B Reactivation Associated With Immune Suppressive and

Biological Modifier Therapies: Current Concepts, Management Strategies, and Future Directions. *Gastroenterology* 2017; **152**: 1297-1309 [PMID: 28219691 DOI: 10.1053/j.gastro.2017.02.009]

317 **Lok AS**, Ward JW, Perrillo RP, McMahon BJ, Liang TJ. Reactivation of hepatitis B during immunosuppressive therapy: potentially fatal yet preventable. *Ann Intern Med* 2012; **156**: 743-745 [PMID: 22586011 DOI: 10.7326/0003-4819-156-10-201205150-00013]

318 **Nagington J**. Reactivation of hepatitis b after transplantation operations. *Lancet* 1977; **1**: 558-560 [PMID: 65656 DOI: 10.1016/s0140-6736(77)91995-x]

319 **Reddy KR**, Beavers KL, Hammond SP, Lim JK, Falck-Ytter YT; American Gastroenterological Association Institute. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology* 2015; **148**: 215-9; quiz e16 [PMID: 25447850 DOI: 10.1053/j.gastro.2014.10.039]

320 **Choi J**, Jo C, Lim YS. Tenofovir Versus Entecavir on Recurrence of Hepatitis B Virus-Related Hepatocellular Carcinoma After Surgical Resection. *Hepatology* 2021; **73**: 661-673 [PMID: 32324905 DOI: 10.1002/hep.31289]

321 **Hosaka T**, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kumada H. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 2013; **58**: 98-107 [PMID: 23213040 DOI: 10.1002/hep.26180]

322 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J; Cirrhosis Asian Lamivudine Multicentre Study Group. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531 [PMID: 15470215 DOI: 10.1056/NEJMoa033364]

323 **Tseng CH**, Hsu YC, Chen TH, Ji F, Chen IS, Tsai YN, Hai H, Thuy LTT, Hosaka T, Sezaki H, Borghi JA, Cheung R, Enomoto M, Nguyen MH. Hepatocellular carcinoma incidence with tenofovir versus entecavir in chronic hepatitis B: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 1039-1052 [PMID: 33007228 DOI: 10.1016/S2468-1253(20)30249-1]

324 **Yip TC**, Wong VW, Chan HL, Tse YK, Lui GC, Wong GL. Tenofovir Is Associated With Lower Risk of Hepatocellular Carcinoma Than Entecavir in Patients With Chronic HBV Infection in China. *Gastroenterology* 2020; **158**: 215-225. e6 [PMID: 31574268 DOI: 10.1053/j.gastro.2019.09.025]

325 **Papatheodoridis GV**, Idilman R, Dalekos GN, Buti M, Chi H, van Boemmel F, Calleja JL, Sypsa V, Goulis J, Manolakopoulos S, Loglio A, Siakavellas S, Keskın O, Gatselis N, Hansen BE, Lehretz M, de la Revilla J, Savvidou S, Kourikou A, Vlachogiannakos I, Galanis K, Yurdaydin C, Berg T, Colombo M, Esteban R, Janssen HLA, Lampertico P. The risk of hepatocellular carcinoma decreases after the first 5 years of entecavir or tenofovir in Caucasians with chronic hepatitis B. *Hepatology* 2017; **66**: 1444-1453 [PMID: 28622419 DOI: 10.1002/hep.29320]

326 **Pellicelli AM**, Vignally P, Messina V, Izzi A, Mazzoni E, Barlattani A, Bacca D, Romano M, Mecenate F, Stroffolini T, Furlan C, Picardi A, Gentilucci UV, Gulminetti R, Bonaventura ME, Villani R, D'Ambrosio C, Paffetti A, Mastropietro C, Marignani M, Fondacaro L, Cerasari G, Andreoli A, Barbarini G. Long term nucleotide and nucleoside analogs treatment in chronic hepatitis B HBeAg negative genotype D patients and risk for hepatocellular carcinoma. *Ann Hepatol* 2014; **13**: 376-385 [PMID: 24927608]

327 **Kim SS**, Hwang JC, Lim SG, Ahn SJ, Cheong JY, Cho SW. Effect of virological response to entecavir on the development of hepatocellular carcinoma in hepatitis B viral cirrhotic patients: comparison between compensated and decompensated cirrhosis. *Am J Gastroenterol* 2014; **109**: 1223-1233 [PMID: 24890440 DOI: 10.1038/ajg.2014.145]

328 **Cho JY**, Paik YH, Sohn W, Cho HC, Gwak GY, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC. Patients with chronic hepatitis B treated with oral antiviral therapy retain a higher risk for HCC compared with patients with inactive stage disease. *Gut* 2014; **63**: 1943-1950 [PMID: 24615378 DOI: 10.1136/gutjnl-2013-306409]

329 **Tenney DJ**, Rose RE, Baldick CJ, Levine SM, Pokornowski KA, Walsh AW, Fang J, Yu CF, Zhang S, Mazzucco CE, Eggers B, Hsu M, Plym MJ, Poundstone P, Yang J, Colonno RJ. Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother* 2007; **51**: 902-911 [PMID: 17178796 DOI: 10.1128/AAC.00833-06]

330 **Tsai MC**, Chen CH, Tseng PL, Hung CH, Chiu KW, Wang JH, Lu SN, Lee CM, Chang KC, Yen YH, Lin MT, Chou YP, Hu TH. Comparison of renal safety and efficacy of telbivudine, entecavir and tenofovir treatment in chronic hepatitis B patients: real world experience. *Clin Microbiol Infect* 2016; **22**: 95.e1-95. e7 [PMID: 26055419 DOI: 10.1016/j.cmi.2015.05.035]

331 **Bryant ML**, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, Gosselin G, Imbach JL, Hernandez B, Juodawlkis A, Tennant B, Korba B, Cote P, Marion P, Cretton-Scott E, Schinazi RF, Sommadossi JP. Antiviral L-nucleosides specific for hepatitis B virus infection. *Antimicrob Agents Chemother* 2001; **45**: 229-235 [PMID: 11120971 DOI: 10.1128/AAC.45.1.229-235.2001]

332 **Genovesi EV**, Lamb L, Medina I, Taylor D, Seifer M, Innaimo S, Colonno RJ, Standring DN, Clark JM. Efficacy of the carbocyclic 2'-deoxyguanosine nucleoside BMS-200475 in the woodchuck model of hepatitis B virus infection. *Antimicrob Agents Chemother* 1998; **42**: 3209-3217 [PMID: 9835516 DOI: 10.1128/AAC.42.12.3209]

333 **Suzuki K**, Suda G, Yamamoto Y, Furuya K, Baba M, Kimura M, Maehara O, Shimazaki T, Yamamoto K, Shigesawa T, Nakamura A, Ohara M, Kawagishi N, Nakai M, Sho T, Natsuizaka M, Morikawa K, Ogawa K, Sakamoto N; NORTE Study Group. Entecavir treatment of hepatitis B

virus-infected patients with severe renal impairment and those on hemodialysis. *Hepatol Res* 2019; **49**: 1294-1304 [PMID: 31260579 DOI: 10.1111/hepr.13399]

334 **Matthews SJ**. Entecavir for the treatment of chronic hepatitis B virus infection. *Clin Ther* 2006; **28**: 184-203 [PMID: 16678641 DOI: 10.1016/j.clinthera.2006.02.012]

335 **Bristol-Myers Squibb Company**. BARACLUDE (entecavir) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/021797s023, 021798s024lbl.pdf

336 **Gilead Sciences, Inc**. VIREAD (tenofovir disoproxil fumarate) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/021356s058,022577s014lbl.pdf

337 **Ustianowski A**, Arends JE. Tenofovir: What We Have Learnt After 7.5 Million Person-Years of Use. *Infect Dis Ther* 2015; **4**: 145-157 [PMID: 26032649 DOI: 10.1007/s40121-015-0070-1]

338 **Morlat P**, Vivot A, Vandenhende MA, Dauchy FA, Asselineau J, Déti E, Gerard Y, Lazaro E, Duffau P, Neau D, Bonnet F, Chêne G; Groupe D'epidémiologie Clinique du Sida en Aquitaine (Gecsa). Role of traditional risk factors and antiretroviral drugs in the incidence of chronic kidney disease, ANRS CO3 Aquitaine cohort, France, 2004-2012. *PLoS One* 2013; **8**: e66223 [PMID: 23776637 DOI: 10.1371/journal.pone.0066223]

339 **Lucas GM**, Ross MJ, Stock PG, Shlipak MG, Wyatt CM, Gupta SK, Atta MG, Wools-Kaloustian KK, Pham PA, Bruggeman LA, Lennox JL, Ray PE, Kalayjian RC; HIV Medicine Association of the Infectious Diseases Society of America. Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2014; **59**: e96-138 [PMID: 25234519 DOI: 10.1093/cid/ciu617]

340 **Jeon MY**, Lee JS, Lee HW, Kim BK, Park JY, Kim DY, Han KH, Ahn SH, Kim SU. Entecavir and tenofovir on renal function in patients with hepatitis B virus-related hepatocellular carcinoma. *J Viral Hepat* 2020; **27**: 932-940 [PMID: 32365240 DOI: 10.1111/jvh.13313]

341 **Murakami E**, Wang T, Park Y, Hao J, Lepist EI, Babusis D, Ray AS. Implications of efficient hepatic delivery by tenofovir alafenamide (GS-7340) for hepatitis B virus therapy. *Antimicrob Agents Chemother* 2015; **59**: 3563-3569 [PMID: 25870059 DOI: 10.1128/AAC.00128-15]

342 **Agarwal K**, Brunetto M, Seto WK, Lim YS, Fung S, Marcellin P, Ahn SH, Izumi N, Chuang WL, Bae H, Sharma M, Janssen HLA, Pan CQ, Çelen MK, Furusyo N, Shalimar D, Yoon KT, Trinh H, Flaherty JF, Gaggar A, Lau AH, Cathcart AL, Lin L, Bhardwaj N, Suri V, Mani Subramanian G, Gane EJ, Buti M, Chan HLY; GS-US-320-0110; GS-US-320-0108 Investigators. 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. *J Hepatol* 2018; **68**: 672-681 [PMID: 29756595 DOI: 10.1016/j.jhep.2017.11.039]

343 **Bam RA**, Yant SR, Cihlar T. Tenofovir alafenamide is not a substrate for renal organic anion transporters (OATs) and does not exhibit OAT-dependent cytotoxicity. *Antivir Ther* 2014; **19**: 687-692 [PMID: 24699134 DOI: 10.3851/IMP2770]

344 **Gilead Sciences, Inc**. VEMLIDY (tenofovir alafenamide) [package insert]. U.S. Food and Drug Administration website. [cited 12 March 2021]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/Label/2021/208464s012 Lbl.pdf

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4143-4159

DOI: 10.3748/wjg.v27.i26.4143

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Abdominal and gastrointestinal manifestations in COVID-19 patients: Is imaging useful?

Piero Boraschi, Luigi Giugliano, Giuseppe Mercogliano, Francescamaria Donati, Stefania Romano, Emanuele Neri

ORCID number: Piero Boraschi 0000-0001-5655-3921; Luigi Giugliano 0000-0003-0441-0499; Giuseppe Mercogliano 0000-0002-2235-952X; Francescamaria Donati 0000-0002-0608-8360; Stefania Romano 0000-0002-6240-7266; Emanuele Neri 0000-0001-7950-4559.

Author contributions: Boraschi P, Giugliano L, Mercogliano G and Donati F searched PubMed for relevant articles and contributed to writing, revising and submitting the manuscript; Romano S and Neri E co-authored and critically revised the manuscript; all authors approved the final version of the article.

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

Piero Boraschi, Francescamaria Donati, Department of Diagnostic Imaging, Second Division of Radiology, Azienda Ospedaliero-Universitaria Pisana-University of Pisa, Pisa 56124, Italy

Luigi Giugliano, Giuseppe Mercogliano, Department of Radiology, University of Naples "Federico II", Naples 80131, Italy

Stefania Romano, Department of Diagnostic Imaging, Santa Maria delle Grazie Hospital, Naples 80078, Italy

Emanuele Neri, Diagnostic and Interventional Radiology, Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa 56126, Italy

Corresponding author: Piero Boraschi, MD, Doctor, Department of Diagnostic Imaging, Second Division of Radiology, Azienda Ospedaliero-Universitaria Pisana-University of Pisa, *via* Paradisa 2, Pisa 56124, Italy. p.boraschi@gmail.com

Abstract

Coronavirus disease 2019 (COVID-19) can be considered a systemic disease with a specific tropism for the vascular system, in which the alterations of the microcirculation have an important pathogenetic role. The lungs are the main organ involved in COVID-19, and severe progressive respiratory failure is the leading cause of death in the affected patients; however, many other organs can be involved with variable clinical manifestations. Concerning abdominal manifestations, the gastrointestinal tract and the hepatobiliary system are mainly affected, although the pancreas, urinary tract and spleen may also be involved. The most common gastrointestinal symptoms are loss of appetite, followed by nausea and vomiting, diarrhea and abdominal pain. Gastrointestinal imaging findings include bowel wall thickening, sometimes associated with hyperemia and mesenteric thickening, fluid-filled segments of the large bowel and rarely intestinal pneumatosis and ischemia. Hepatic involvement manifests as an increase in the enzymatic levels of alanine aminotransferase, aspartate aminotransferase, serum bilirubin and γ-glutamyl transferase with clinical manifestations in most cases mild and transient. The most frequent radiological features are hepatic steatosis, biliary sludge and gallstones. Edematous acute pancreatitis, kidney infarct and acute kidney injury from acute tubular necrosis have been described more rarely in COVID-19. Lastly, splenic involvement is characterized by splenomegaly and by the development of solitary or multifocal splenic infarcts with classic wedge-

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Italy

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

Received: January 26, 2021
Peer-review started: January 26, 2021
First decision: March 29, 2021
Revised: April 8, 2021
Accepted: April 21, 2021
Article in press: April 21, 2021
Published online: July 14, 2021

P-Reviewer: Şehirli AÖ
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Wang LL

shaped or even rounded morphology, with irregular or smooth profiles. In summary, the abdominal radiological findings of COVID-19 are nonspecific and with poor pathological correlation reported in the literature. Ultrasound and particularly computed tomography with multiphasic acquisition are the diagnostic methods mainly utilized in COVID-19 patients with abdominal clinical symptoms and signs. Although radiological signs are not specific of abdominal and gastrointestinal involvement, the diagnostic imaging modalities and in particular computed tomography are helpful for the clinician in the management, evaluation of the severity and evolution of the COVID-19 patients.

Key Words: COVID-19; SARS-CoV-2; Abdominal findings; Gastrointestinal findings; Computed tomography; Ultrasound

Core Tip: Coronavirus disease 2019 (COVID-19) pulmonary involvement has been extensively reported in the literature. Nowadays, a series of published data highlight how COVID-19 is a systemic disease affecting many other organs. Abdominal and gastrointestinal clinical manifestations have been more recently investigated with imaging modalities such as ultrasound and computed tomography. The aim of this review is to report the most common imaging features of abdominal and gastrointestinal involvement and the possible role of ultrasound and particularly computed tomography in the management, evaluation of the severity and evolution of COVID-19 patients.

Citation: Boraschi P, Giugliano L, Mercogliano G, Donati F, Romano S, Neri E. Abdominal and gastrointestinal manifestations in COVID-19 patients: Is imaging useful? *World J Gastroenterol* 2021; 27(26): 4143-4159
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4143.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4143

INTRODUCTION

Severe acute respiratory syndrome by severe acute respiratory syndrome corona-virus 2 (SARS-CoV-2) was first recognized in December 2019 in Wuhan (Hubei province, China), but its origin is still unknown and debated. Since then, the disease spread worldwide, leading the World Health Organization to declare a global pandemic on March 11, 2020[1]. More than 21 million confirmed cases of coronavirus disease 2019 (COVID-19) have been reported on all continents except Antarctica, and the incidence is steadily increasing[2]. In all territories affected by the COVID-19 pandemic, the diagnostic tests, the workload of the intensive care units and the initiation of mitigation strategies, such as the restriction of interpersonal contacts, have been established and increased over time[3]. Men seem to be disproportionately more commonly affected by a SARS-CoV-2 infection, and the hospital mortality rate among males is significantly higher than female patients[4]. Main vehicles of transmission of SARS-CoV-2 disease are respiratory droplets being released during coughing, sneezing or conversation between subjects and accumulate on surfaces causing an indirect contamination[5].

SARS-CoV-2 pathogenic agent of this pandemic disease belongs to the Coronaviridae family. Coronaviruses are nonsegmented enveloped RNA viruses with a single-strand linear positive-sense RNA[6]. They are routinely present among animals as well as humans. They are the most common cause of colds, particularly in cats and dogs[7]. Although the origin of the new mutant strain SARS-CoV-2 remains uncertain, it is likely that it originated in a wet market in Wuhan, where animals of all kinds are slaughtered in poor sanitary conditions and their meats are sometimes eaten raw[8].

Six types of coronaviruses causing human disease have been identified: Four of them cause mild respiratory symptoms, whereas the other two, Middle East respiratory syndrome coronavirus and SARS-CoV-1, have previously resulted in epidemics with high mortality rates[9]. SARS-CoV-2 has 80% genomic compatibility

with SARS-CoV-1 and uses the same angiotensin-converting enzyme 2 (ACE2) receptor to enter cells. In fact, ACE2 is an integral membrane protein that seems to be the host cell receptor for SARS-CoV-2, which appears significantly increased in COVID-19 positive patients[10]. ACE2 positive endothelial cells from patients with COVID-19 show significant changes in their morphology, disruption of the intercellular junctions, cell swelling and loss of contact with the basement membrane [11]. The presence of the SARS-CoV-2 virus within endothelial cells suggests that direct viral effect as well as perivascular inflammation may contribute to endothelial damage[11]. This underlines the importance of microcirculation alterations in the pathogenesis and subsequent manifestations at the systemic level[11,12].

Pulmonary involvement in the COVID-19 pandemic is the most known and largely studied because progressive severe respiratory failure represents the leading cause of death in affected patients. Pathological samples of peripheral lung of patients who died from COVID-19 showed a histological pattern of diffuse alveolar damage with perivascular infiltration of T cells[13]. Pulmonary parenchymal tissue also showed typical vascular findings, represented by severe endothelial lesions with the presence of intracellular viruses and interrupted cell membranes[14].

Histological analysis of pulmonary vessels in affected patients showed diffuse thrombosis with microangiopathy; alveolar capillary microthrombi seem to be nine times more frequent as well as the amount of new vessel growth was reported to be 2.7 times higher in patients with COVID-19 in respect to those affected by influenza virus disease[15]. Therefore, three distinctive angiocentric characteristics of COVID-19 were found in the lung: (1) severe endothelial injury associated with the intracellular SARS-CoV-2 virus and rupture of endothelial cell membranes; (2) diffuse vascular thrombosis with microangiopathy and occlusion of the alveolar capillaries; and (3) significant growth of new vessels through an intussusceptive angiogenesis mechanism [13]. Although COVID-19 typically manifests as a respiratory illness and most of the literature described pulmonary signs and symptoms, this disease can affect other anatomical districts and structures, whose clinical manifestations are relatively poorly known. A whole series of signs and symptoms related to systemic manifestations are known to have a certain relevance. In particular, it has been reported that 57% of patients with low severity COVID-19 disease could have reported abdominal discomfort alone or in combination with pulmonary symptomatology[16]. The abdominal clinical manifestations have been related to the gastrointestinal tract and the hepato-biliary-pancreatic system, whereas urinary tract and spleen involvement have been less frequently reported[17].

Our review was aimed to report the current abdominal and gastrointestinal imaging features in COVID-19 patients as well as to define the role of the diagnostic imaging modalities in the abdominal manifestations.

GASTROINTESTINAL TRACT

Abdominal and gastrointestinal signs and symptoms in COVID-19 are being increasingly reported[18]. Indeed, on January 19, 2020, the first known patient of COVID-19 in Washington, United States reported a history of nausea and vomiting in addition to respiratory symptoms[19]. In the literature, it has been reported that series of COVID-19 patients presenting to the emergency room with abdominal pain but without the typical respiratory symptoms of SARS-CoV-2; therefore, the abdominal radiologist was the first to suggest COVID-19 infection because of the typical findings in the lung lower lobes on computed tomography (CT) scans of the abdomen, such as peripheral and subpleural ground-glass opacities[18,20,21] (Figure 1). In this context, some authors have proposed additional CT of the whole chest as part of a CT imaging pathway of acute abdominal pain during the COVID-19 pandemic; this did not get approval because in these patients it was enough just to review the pulmonary bases on abdominal CT scans[22].

Patients with primarily mild gastrointestinal symptoms may not be identified as COVID-19 patients, with serious consequences for themselves and their contacts[18]; in this setting the most common gastrointestinal symptom is loss of appetite, followed by nausea and vomiting, while diarrhea and abdominal pain are the presenting symptoms in only a small percentage of cases[18].

In a retrospective study by Han *et al*[16], patients with gastrointestinal symptoms compared with patients with only respiratory symptoms, tended to have a longer course between symptom onset and viral clearance and took longer to report for medical care, a finding observed in other studies[23]; this suggests that in these

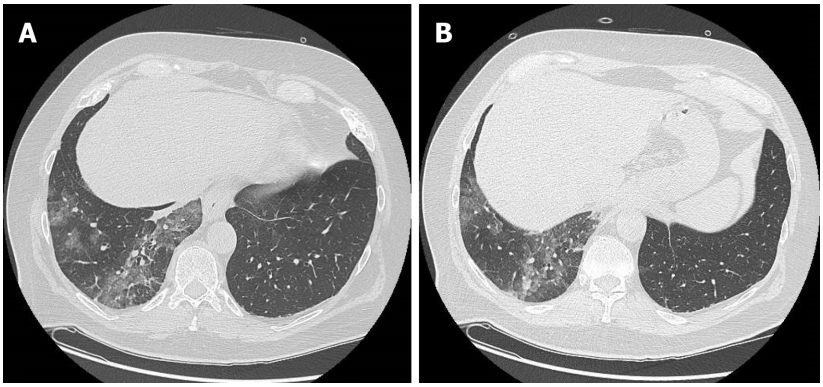

Figure 1 A 63-year-old man who presented to the emergency room for abdominal pain and no significant alterations on abdominal computed tomography. A: Computed tomography scan with pulmonary window at the level of lung inferior lobes (included in the volume acquisition) shows the presence of ground-glass opacities and crazy-paving pattern in the right side; B: Computed tomography scan with pulmonary window at lower levels of lung inferior lobes always exhibited ground-glass opacities and crazy-paving pattern in the right side.

patients the COVID-19 involvement was not initially recognized leading to delayed diagnosis[24]. Moreover, SARS-CoV-2 has been identified in stool samples of a proportion of infected patients[16]; viral replication in both small and large intestine was confirmed by the result of electron microscopy of autopsy biopsy specimen[24, 25]. In particular, an autoptic study on the small intestine of two COVID-19 patients showed endothelitis of the submucosa vessels with mononuclear cell infiltrates within the intima along the lumen of many vessels, besides the evidence of direct viral infection of endothelial cells[11].

The inflammatory response in the gut due to active viral replication is also supported by the evidence of elevated fecal calprotectin concentrations in COVID-19 patients with diarrhea when compared with COVID-19 patients without diarrhea[26].

The radiological alterations of the gastrointestinal system in COVID-19 patients are represented by nonspecific thickening of various regions of the small and large bowel wall[27,28] (Figures 2 and 3), sometimes associated with hyperemia and mesenteric thickening[28] (Figure 4). Goldberg-Stein *et al*[29] retrospectively reported that the most common gastrointestinal symptom in 141 COVID-19 patients was the abdominal pain, present in 73.8% of patients with negative abdominal CT findings and in 53.8% of patients with positive abdominal CT findings; in this series the most commonly reported CT finding was represented by segmental wall thickening of the gastrointestinal tract[29]. In addition, 64% of patients with no positive CT abdominal findings but gastrointestinal symptoms showed suggestive features for COVID-19 pneumonia at the lung bases[29]. This suggests that abdominal symptoms may be present in COVID-19 patients without correlative CT abdominal findings; in fact, in patients with pneumonia it was hypothesized that the abdominal and back pain may be secondary to pleural irritation[30,31].

Tirumani *et al*[32], instead, in a retrospective study identified the incidence of abdominal findings in COVID-19 patients with and without abdominal symptoms and concluded that bowel abnormalities are the most common finding in the abdomen in patients with COVID-19, often without abdominal symptoms and especially regardless of the severity of lung involvement[32]. The most common CT abdominal findings are a fluid-filled colon with no wall thickening (Figures 5 and 6), severe colitis (characterized by a thickened and edematous wall with fat stranding), gastritis and small bowel pneumatosis with portal venous gas[32].

Other minor manifestations are also reported. In a case report, Noda *et al*[33] described a COVID-19 healthy teenager who initially presented with abdominal discomfort. The patient underwent CT scan that demonstrated only isolated mesenteric adenopathy and adjacent fat stranding associated to ground-glass opacities and solid consolidation as well as interlobular septal thickening at lung bases[33]. This case highlights how abdominal findings in COVID-19 patients are not specific and should be suspected given the continued emergence of new manifestations of the disease.

Bhayana *et al*[34] retrospectively analyzed 42 abdominal CT scans performed in COVID-19 patients for abdominal pain or septic status. Colorectal and small bowel wall thickening (defined as single-wall thickness greater than 3 mm in distended intestine and greater than 5 mm in collapsed intestine) were found in 12 out of 42

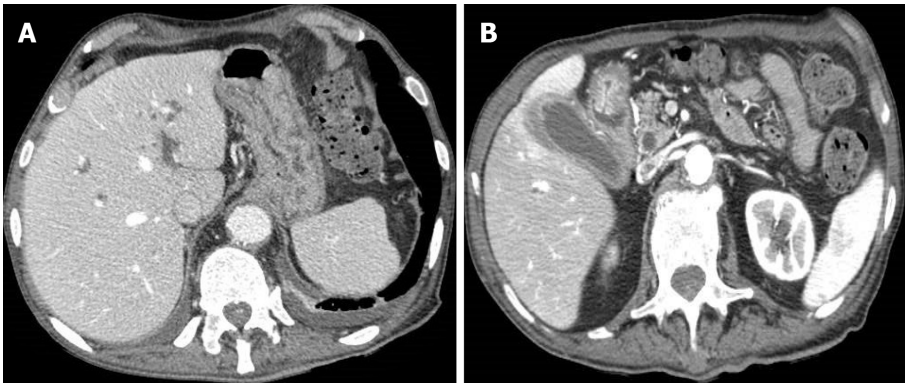

Figure 2 An 83-year-old woman with epigastric pain. A: Abdominal contrast-enhanced portal-venous phase computed tomography showed diffuse thickening of the submucosa of the gastric walls and intrahepatic biliary dilatation; B: Abdominal contrast-enhanced portal-venous phase computed tomography also depicts thickening of the submucosa of the pyloric region and signs of cholecystitis.

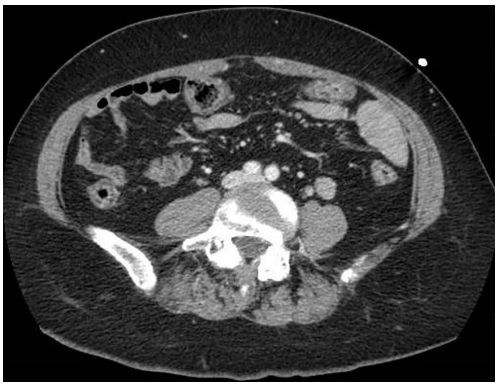

Figure 3 A 68-year-old woman with abdominal pain. Abdominal contrast-enhanced portal-venous phase computed tomography image showed circumferential thickening of the submucosa of the right colon that appeared hypodense, in the absence of both significant contrast-enhancement and perivisceral fat stranding.

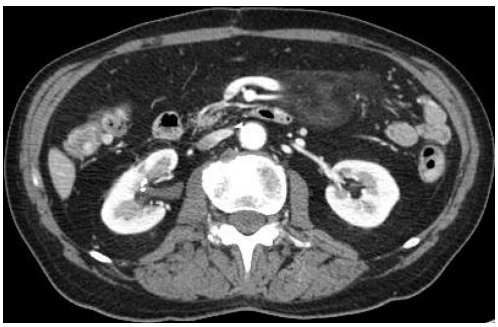

Figure 4 A 74-year-old man with abdominal pain. Abdominal contrast-enhanced portal-venous phase computed tomography image showed well-circumscribed hyperattenuation of the fat surrounding the mesenteric vessels.

abdominal CT, whereas pneumatosis associated to gas in the portal vein and fluid-filled colon (defined as homogeneous, low-attenuation colonic content) were identified in 4 and 18 out of 42 cases, respectively. The remaining 8 patients did not exhibit CT alterations of the gastrointestinal tract[34]. The 4 cases of pneumatosis and portal vein gas underwent exploratory laparotomy; 2 cases had necrotic bowel at surgery with a yellow discoloration of the small bowel in contrast with the usual black or purple color of a necrotic bowel. One patient also underwent a bowel resection demonstrating ischemic enteritis with patchy necrosis and submucosal arterioles containing fibrin thrombi[34].

Bowel ischemia has also been described in COVID-19 patients, particularly in those admitted to the intensive care unit[35], constituting a life-threatening clinical

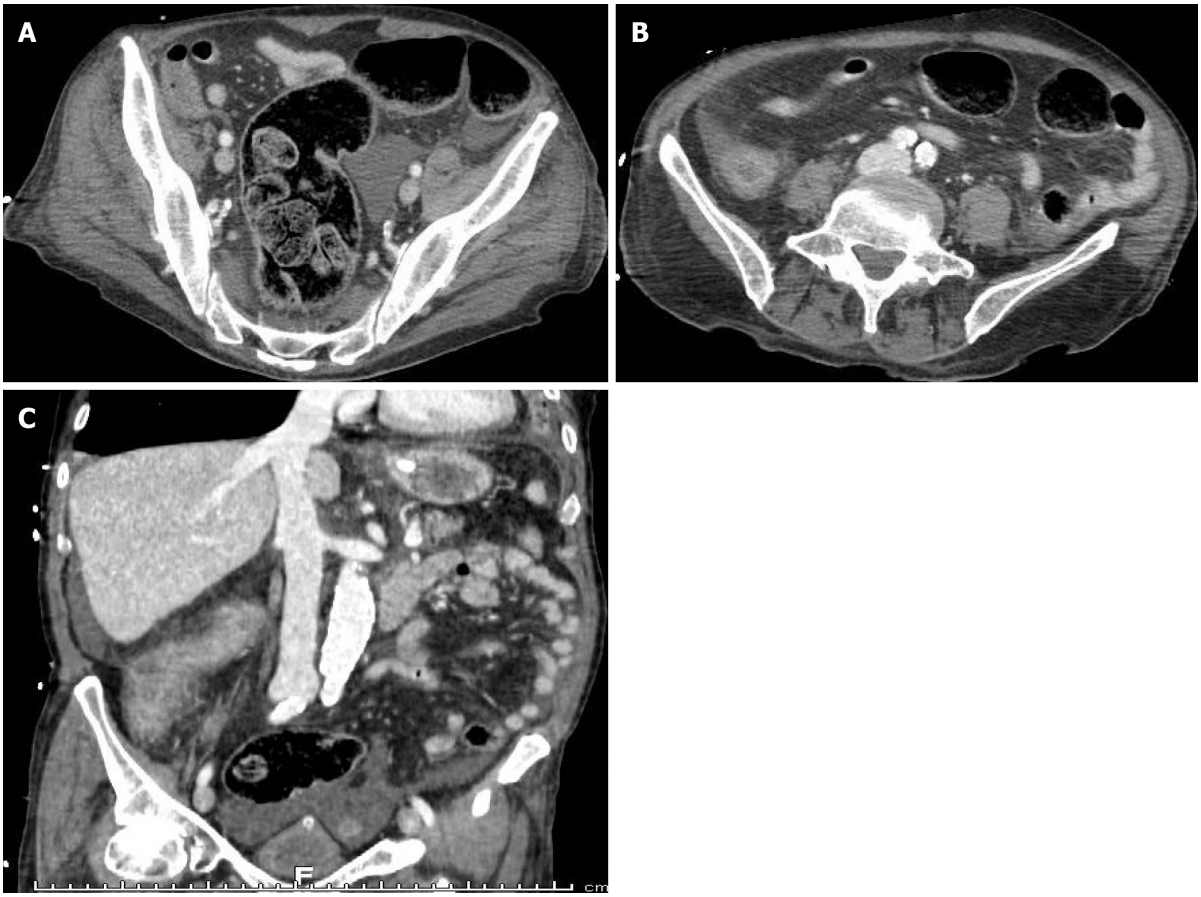

Figure 5 A 73-year-old woman with abdominal pain. A: Contrast-enhanced portal-venous phase computed tomography image of the lower abdomen demonstrated gas distension of the sigma-rectum with evidence of multiple stools inside the lumen; free effusion was also appreciable around the colon; B: Contrast-enhanced portal-venous phase computed tomography image of the abdomen showed distention of the left bowel and hyperemic thickened walls (particularly affecting the mucosa) of the right colon with perivisceral fat suffusion; C: Coronal multiplanar reconstruction from contrast-enhanced computed tomography well demonstrated these findings.

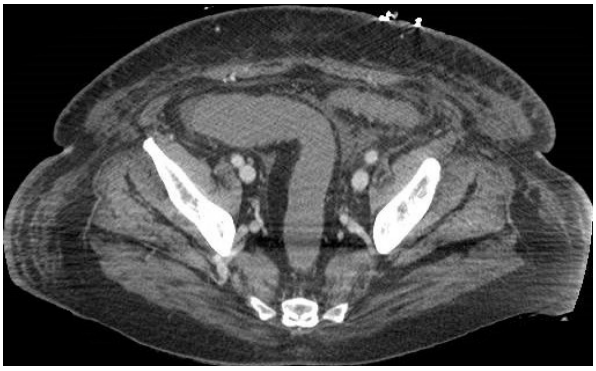

Figure 6 A 69-year-old woman with diarrhea. Contrast-enhanced portal-venous phase computed tomography image of the abdomen showed evidence of fluid-filled distension of the large bowel, particularly of the sigma and rectum, without evidence of parietal thickening. Free effusion was also present in the abdomen and between the intestinal loops with associated diffuse imbibition of the subcutaneous soft tissues.

emergency[9]. It is well known that the hypercoagulable state induced by COVID-19 results in micro- and macrovascular complications[36]. The microvascular complications are detected in the early stages of the disease, while the macrovascular ones are more typically observed in severely ill patients[37]. Anyway, thromboembolic disease within the mesenteric vascular system is not frequently identified on CT imaging[35].

Revzin *et al*[38] divided the COVID-19 related bowel ischemia into early, intermediate and late presentations[38]. On CT images the early phase shows contracted gasless bowel that may transform into dilated gas-filled bowel with a paper-thin bowel wall in the intermediate phase. The CT findings of the late phase

include intestinal wall pneumatosis, absence of mucosal enhancement and luminal dilatation. It is important to note that these phases reflect those of a classic intestinal ischemia regardless of etiology[39] and that the presence of pneumatosis intestinalis suggests bowel ischemia[36], but its presence must be interpreted with caution because it may be secondary to mechanical ventilation in patients with severe COVID-19[40] (Figure 7).

Actually, the exact pathological mechanism of intestinal ischemic disorders is not clearly known. In a letter to the editor, Parry *et al*[41] reported four possible mechanisms that work alone or in varying combination: coagulation disorder, elevated levels of von Willebrand Factor, expression of ACE2 on enterocytes of the small bowel and shock/hemodynamic compromise associated with COVID-19 pneumonia[41].

COVID-19 patients may be in a state of hypercoagulability induced by a systemic inflammatory state, endothelial activation, hypoxia and immobilization. These could lead to mesenteric microvascular thrombosis without involvement of the large mesenteric vessels, resulting in a condition of thrombosis in situ rather than an embolic event[41].

Elevated levels of von Willebrand factor have been reported in severe COVID-19 patients[42]. von Willebrand factor is released from endothelium in response to damage caused by SARS-CoV-2 and consequent endothelium dysfunction and vascular thrombosis[43].

Regarding the last two points, the enterocytes express ACE2, the target receptor for SARS-CoV-2 with intestinal tropism and direct bowel damage. Lastly, shock or hemodynamic compromise, which is commonly associated with severe COVID-19 pneumonia, may lead to a non-occlusive mesenteric ischemia[42].

LIVER, BILIARY TRACT AND PANCREAS

The liver is the second most frequently injured organ after the lung in COVID-19[44]. The mechanism of liver damage is probably due to a series of events that can occur simultaneously. Direct cytopathic effect of SARS-CoV-2, indirect damage from systemic inflammation, drug hepatotoxicity[44] and hypoxic alterations related to ventilation have been mainly described[45]. SARS-CoV-2 causes direct liver damage because the ACE2 receptor is widely expressed in the liver, more on cholangiocytes than on hepatocytes[46]. The virus alters the barrier and bile acid transport functions of cholangiocytes through the dysregulation of genes involved in tight junction formation and bile acid transport[47]. Drugs commonly used during SARS-CoV-2 infection causing liver toxicity include remdesivir, tocilizumab, chloroquine, hydroxychloroquine and azithromycin[48]. The immune-mediated cytokine storm also participates in the damage; in fact, we have a marked activation of inflammatory markers, including abnormal levels of C-reactive protein, lymphocytes, neutrophils and cytokines, in particular interleukin-6. The control of cytokine dysregulation at an early stage could be useful to slow down the progression of the disease[45]. In addition, respiratory-induced hypoxia can cause elevated serum aminotransferase concentrations, a laboratory marker of liver injury[49]. The combination of these events determines a generalized coagulopathy state that determines an alteration of the microcirculation with microthrombosis within the hepatic sinusoids[20]. As a support to this etiopathogenetic theory, liver autopsy results by Medeiros *et al*[50] show periportal necrosis, lymphocytic infiltration of the sinusoids, dense infiltration of the gate by abnormally small lymphocytes, central venous thrombosis and cirrhotic alterations with fibrosis in a retrospective series of 316 patients[50].

Liver injury usually manifests as an increase in enzyme levels. Current literature data show that 14.8%-53.0% of COVID-19 patients have abnormal levels of alanine aminotransferase and aspartate aminotransferase and a slight increase in serum bilirubin levels during the course of the disease[16]. Phipps *et al*[51] found that an alanine aminotransferase spike was significantly associated with clinical outcome[51]. In respect to mild to moderate or no liver injury, patients with severe liver injury have a higher rate of intubation and renal replacement therapy. Additionally, approximately 50% of COVID-19 patients had increased levels of γ-glutamyl transferase[52].

To the best of our knowledge, hepatic manifestations of COVID-19 were in most cases mild and transient. Despite this, there is an ever increasing number of subjects with severe hepatic manifestations that are very often associated with the pulmonary ones[53].

For the management of COVID-19 patients with liver injury, the American Association for the Study of Liver Diseases provides recommendations, highlighting

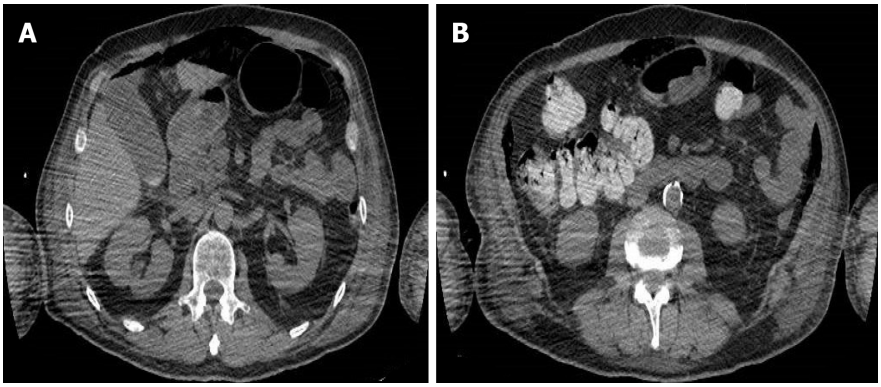

Figure 7 A 65-year-old man with abdominal pain and severe pulmonary involvement. A: Unenhanced computed tomography scan of the upper abdomen showed signs of pneumoperitoneum secondary to mechanical ventilation and cholelithiasis; B: The presence of gas within the peritoneal cavity was also appreciable at lower levels of the abdomen on unenhanced computed tomography scan.

that there are no contraindications to the use of drugs such as remdesivir, tocilizumab, chloroquine, hydroxychloroquine and azithromycin unless alanine aminotransferase or aspartate aminotransferase are no more than five times the upper normal limit[48].

A systematic review and meta-analysis of international data on the hepatic manifestations of COVID-19 was performed by The American Gastroenterological Association Institute[54]. Among COVID-19 patients with liver injury, more than 60% of patients had mild hepatic injury (1 time upper normal limit to 5 times upper normal limit)[51]. It should be noted that previous liver disease and underlying liver function may have influenced the results. In fact, some patients may have abnormal liver function prior to SARS-CoV-2 infection, such as nonalcoholic fatty liver disease or chronic hepatitis B[55]. Moreover, there is a higher prevalence of hepatic steatosis that is probably due to the known association between infection and obesity[50].

Hepatic steatosis is a very frequent and nonspecific finding of COVID-19 patients, which has been identified with both ultrasound (US) and CT. The findings of hepatic steatosis are the same as those found in non-COVID-19 patients. On US we can observe the typical "bright liver" that is characterized by an increase in echogenicity compared to the renal cortex or spleen (in the case of renal pathology), loss of physiological hyperechogenicity of the portal branches walls and posterior attenuation of the ultrasonic beam with failure to visualize the diaphragm (Figure 8). On the other hand, diffuse liver hypodensity is the typical sign on CT scans. In the presence of a slight steatosis a hepatic attenuation of less than 10 HU compared to the density of the spleen is observed, whereas in moderate/severe forms hepatic attenuation is less than 40 HU compared to the spleen[56] (Figure 9).

Ji *et al*[57] found that COVID-19 patients with nonalcoholic fatty liver disease were more likely to have liver damage and disease progression than patients without nonalcoholic fatty liver disease[57]. Singh *et al*[58] studied the impact of pre-existing liver disease on outcomes in a large cohort of COVID-19 patients and found that the risk of hospitalization and death in patients with pre-existing liver disease was significantly higher[58].

Further, in 54% of patients with COVID-19, biliary sludge and gallstones were found and were closely related to an increase in cholestasis indexes[59], values that appear to be markedly increased in comparison with the incidence in the general population (about 10%-20%)[38]. US is considered the gold standard for detecting gallstones. It allows the evaluation of macro- and microlithiasis, sludge and cholesterol deposits as well as the structural evaluation of the gallbladder[60]. The cholesterol deposition along the gallbladder walls appears as hyperechogenic spots with the typical "comet sign" whereas the biliary sludge as sediment in the declivous portion of the gallbladder lumen[60] (Figure 10). Although the role of CT in the evaluation of biliary lithiasis is marginal compared to US in the normal patient, this technique easily allows the visualization of hyperdense calcium stones and hypodense cholesterol stones in COVID-19 patients because US is not easily performed in the most compromised patients[61].

Pancreatic involvement has also been described in COVID-19 patients. As for other districts, the pathogenetic mechanism is not yet clear and could be the result of direct (cytopathic effect of the virus) or indirect (immune-mediated storm) mechanisms[62].

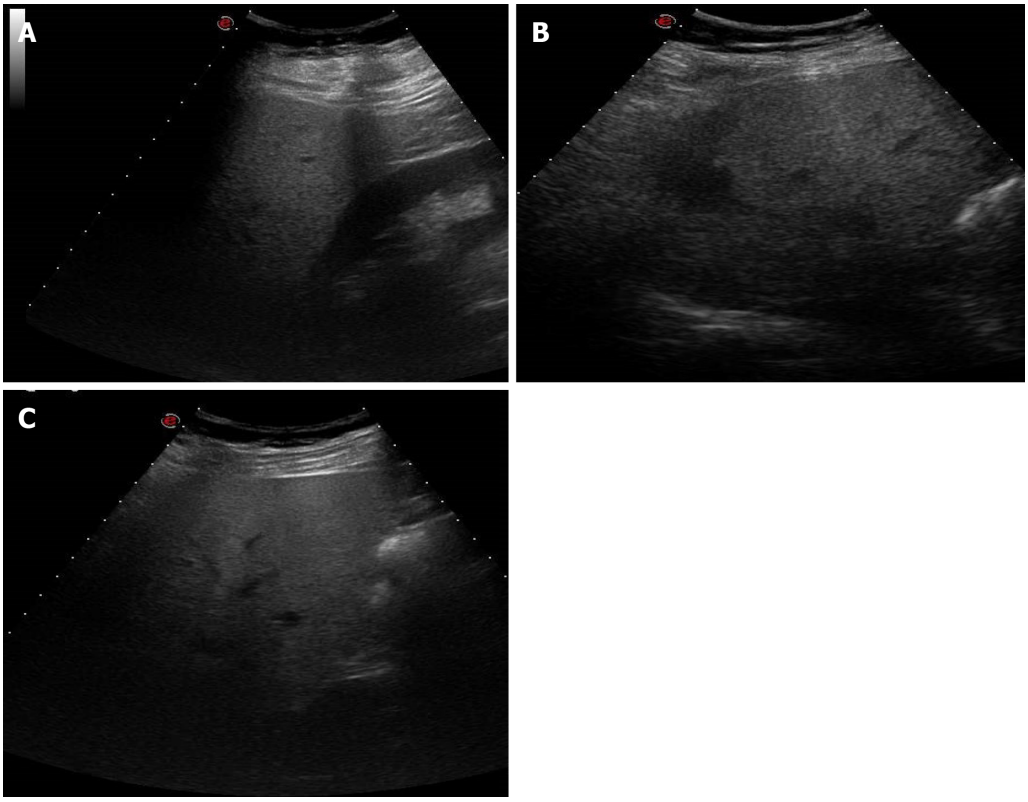

Figure 8 A 38-year-old man with abdominal discomfort. A: Abdominal ultrasound image demonstrated hepatic steatosis as an increase in echogenicity compared to the renal cortex; B: Abdominal ultrasound image demonstrated hepatic steatosis as loss of physiological hyperechogenicity of the wall of the portal branches; C: Abdominal ultrasound image demonstrated hepatic steatosis as posterior attenuation of the ultrasonic beam with failure to visualize the diaphragm.

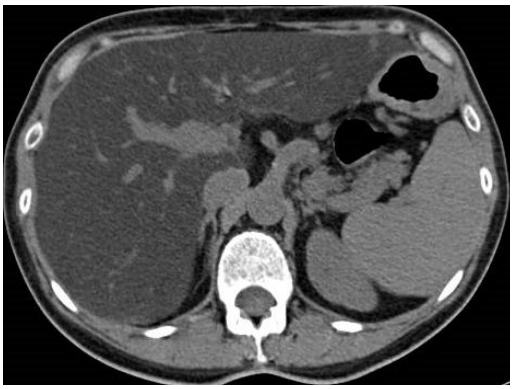

Figure 9 A 46-year-old woman with abdominal discomfort. Unenhanced computed tomography image showed increased liver hypodensity compared to the spleen, with attenuation value less than 40 HU.

The presence of the ACE2 receptor on the cells of the pancreatic islets and exocrine glands allows the penetration of the virus thus determining the manifestations. In a series of 52 patients with COVID-19 pneumonia by Wang *et al*[63], 17% of them had pancreatic injury with elevated blood glucose levels. These results show potential mild pancreatic injury patterns in patients with COVID-19 pneumonia with no severe pancreatitis as a common manifestation[63]. However, some cases of pancreatitis have been reported in the literature[64]; in 64 patients with severe COVID-19 Liu *et al*[62] reported that 17.9% and 16.4% had increased amylase and lipase levels, respectively [62]. Thirteen out of 64 patients were examined with CT scans; of these only 5 patients showed radiological pancreatic alterations. It is worth underlining that most of these cases have been reported in moderate or severe disease. This seemingly suggests that the pathophysiology of pancreatitis could be based on the systemic inflammatory response rather than a direct histopathological effect[62].

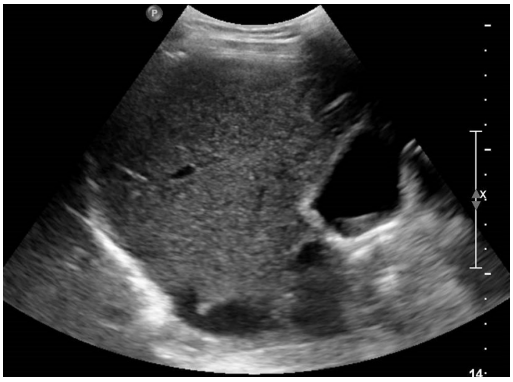

Figure 10 A 46-year-old woman with right hypochondrium pain. Abdominal ultrasound showed an enlarged gallbladder containing deposit of biliary sludge in the infundibular region.

The radiological signs of pancreatitis in COVID-19 patients were the same as we find in classic acute pancreatitis: Focal or diffuse parenchymal enlargement and density changes due to edema with indistinct pancreatic margins and stranding of retroperitoneal fat[65] (Figure 11). More rarely, necrotic-hemorrhagic forms of pancreatitis have been described[65]. The presence of gas (emphysematous pancreatitis) and the presence of calcifications as a sign of acute on chronic pancreatitis have also been reported[66].

URINARY TRACT

There is increasing evidence that acute kidney injury (AKI) develops commonly in COVID-19 patients[67] because it affects approximately 20%-40% of patients admitted to the hospital and particularly to the intensive care unit in Europe and in the United States[68,69]. COVID-19 patients developing AKI, in conjunction with respiratory symptoms, have a poor prognosis, with a 35% reported mortality[70].

Possible causes of COVID-19-related AKI include dehydration, hypoperfusion from myocardial dysfunction, immune response dysregulation (cytokine storm) or direct kidney endothelial damage by SARS-CoV-2[69], which manifests clinically and pathologically by the development of acute tubular necrosis, interstitial inflammation, podocytopathy, microangiopathy and collapsing glomerulopathy[69].

In a postmortem renal histopathological analysis of 26 COVID-19 patients, Su *et al* [71] reported a histopathological finding of acute tubular necrosis due to endothelial damage causing microvascular lumen occlusion. The authors hypothesized that their results had been dependent on the possible kidney cells' infections with SARS-CoV-2 [71].

In this setting the imaging modality of choice is US. It is a bedside examination that can show increased cortical echogenicity or heterogeneity and loss of corticomedullary differentiation in patients with COVID-19 and AKI[72]. In cases of renal infarction, heterogeneity and hypoperfusion of the renal parenchyma and wedge-shaped areas of decreased perfusion and/or enhancement may be visualized on US and contrast-enhanced CT and may be multifocal, involving both kidneys[72] (Figure 12). It is important to note that if renal function is impaired, the use of iodinated contrast material is not recommended, thus making US the imaging modality of choice in the evaluation of COVID-19 patients with suspected renal vascular injury.

In a retrospective study, Hectors *et al*[73] found that cortex-to-aorta enhancement index (*i.e.* the ratio of renal cortical density to aorta density on contrast-enhanced CT) at the time of COVID-19 diagnosis was significantly reduced in patients who ultimately developed AKI. The authors suggest that reduced renal perfusion in COVID-19 precedes full-fledged AKI[73].

In another retrospective study, Huang *et al*[74] highlighted the usefulness of non-contrast CT on the assessment of renal impairment associated with COVID-19, featuring as perinephric fat stranding (PFS) and decreased renal parenchymal density. PFS corresponds to the thickening of perinephric bridging septa, which are fibrous lamellae that divide the perinephric space into multiple compartments, limiting the distribution of fluids, such as urine, pus and blood[75]. In this study, patients with PFS

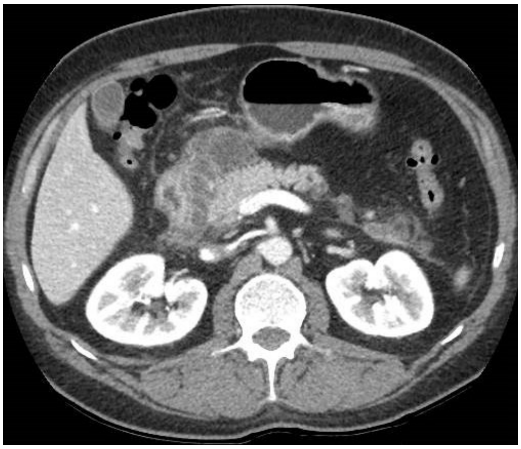

Figure 11 A 60-year-old man with abdominal pain and increased amylase and lipase levels. Abdominal contrast-enhanced portal-venous phase computed tomography image showed fluid collections at the level of the pancreatic head and isthmic region and thickening of the left anterior pararenal fascia and perivisceral fat.

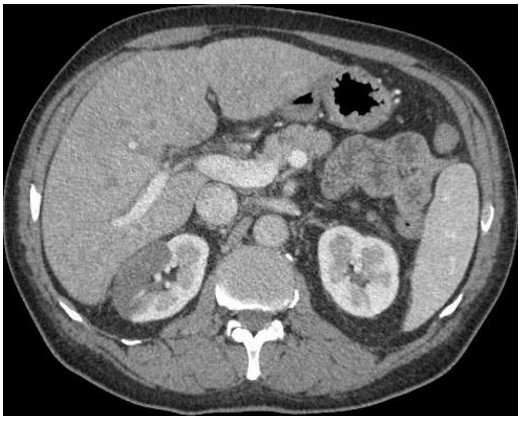

Figure 12 A 69-year-old man with hematuria and right abdominal pain. Abdominal contrast-enhanced portal-venous phase computed tomography image depicted wedge-shaped parenchymal defects that involved both the renal cortex and medulla with extension to the capsular surface, suggesting a renal infarct.

showed elevated serum creatinine levels, higher than that of the group of COVID-19 patients without PFS. Always in this study renal parenchymal attenuation in COVID-19 patients decreased. Moreover, patients with PFS showed a greater attenuation decrease, whereas patients without PFS presented a smaller decrease[75]. Therefore, the authors propose the use of PFS and renal parenchymal attenuation on non-contrast CT as a qualitative indicator for detecting renal damage associated with COVID-19.

Currently, no report has demonstrated the association between COVID-19 and urolithiasis[76], despite the nonsteroidal anti-inflammatory drugs (commonly used in stone-related colic pain) increase ACE2. This might increase the risk of developing severe and fatal COVID-19[77]. Nevertheless, the United States Food and Drug Administration recently announced that there was not enough scientific evidence connecting the use nonsteroidal anti-inflammatory drugs with worsening COVID-19 symptoms[78].

SPLEEN

Splenic injury is commonly encountered in COVID-19 patients. Red pulp cells and endothelial cells of blood vessels show an abundance of ACE2 receptors on their surfaces, so COVID-19 can directly target macrophages and dendritic cells in the spleen[79]. Autopsies in patients who died from COVID-19 exhibited splenic parenchymal congestion, hemorrhage and lack of lymphoid follicles with splenic parenchymal atrophy[79].

Splenic injuries are characterized by splenomegaly and by the development of either solitary or multifocal splenic infarcts[79]. On US examination splenic infarcts are hypoechoic compared to the remaining splenic parenchyma, although in the acute phases they may appear isoechoic and therefore difficult to identify. The morphology of the infarcts can vary as they may be classically wedge-shaped but also rounded, with irregular or smooth profiles. Over time, phenomena of contraction and scarring can develop, and in this case, they will appear as a hyperechoic region with retraction of the splenic capsule. In case of liquefaction, the area may be rounded and anechoic (splenic pseudocyst)[80]. CT is often considered the imaging method of choice, with the best visualization of infarcts during the portal-venous phase, to avoid confusing the heterogeneous improvement normally seen during the arterial phase. The CT imaging characteristics may vary with the stage of the infarct. In the hyperacute phase, there are areas of greater mottled attenuation, which represent areas of a hemorrhagic infarction typically with a wedge-shaped morphology and peripheral localization[81]. In the chronic phase, splenic infarcts may no longer be seen, or more commonly they may undergo fibrotic contraction with consequent loss of volume of the splenic parenchyma (Figure 13). As mentioned for the US, if the infarct liquefies, a cystic lesion can be left with central fluid density[81].

RADIOLOGICAL COMMENTS

As pointed out by the data of the most recent literature, COVID-19 is a systemic disease and not just a pulmonary disease, with a specific tropism for the vascular system. In fact, many other organs besides the lung can be involved by SARS-CoV-2 and thus the clinical manifestations can be variable. The gastrointestinal tract and hepato-biliary system are mainly affected, although the pancreas, urinary tract and spleen may also be involved.

The abdominal radiological findings are nonspecific and with poor pathological correlation reported in the literature. The correct use of imaging modalities in the management of these patients can be extremely helpful for the clinicians. US and particularly CT with multiphasic acquisition are the diagnostic methods mainly utilized in the COVID-19 patients with abdominal clinical symptoms and signs.

Although US can be quickly performed at the bedside and in intensive care units, this diagnostic modality is not always able to provide us reliable information in critically ill, unprepared and uncooperative patients. It also has the problem of exposing healthcare personnel to a consistent risk of infection and this limits its routine use in this setting. On the basis of the published data, US is mainly used for the hepatobiliary system, kidney and spleen evaluation, less for the pancreas and almost not utilized for gastrointestinal system assessment.

CT plays a pivotal role in identifying the signs of abdominal involvement, particularly for the gastrointestinal system, and is extremely useful for the evaluation of the vascular and parenchymal structures. Thanks to this method we can establish the severity and the evolution of the disease in COVID-19 patients. CT study should be performed at the baseline and after iodinated contrast medium injection in the various phases, paying attention to the arterial phase for ischemic lesions of the intestine and kidneys, in the portal-venous phase for wall thickening in colitis or for signs of acute pancreatitis or splenic infarcts and in the late phase for the evaluation of renal nephritis or ischemia.

Actually, magnetic resonance imaging has no defined role, and no significant experience is reported to our best knowledge. This is probably due to the fact that COVID-19 patients have difficulty undergoing magnetic resonance examination and the serious problems of management of these patients.

What is desirable in the future is that the abdominal and gastrointestinal manifestations identified by imaging may have a pathological correspondence in order to further clarify the etiopathogenetic mechanisms of the damage caused by COVID-19. Furthermore, larger series of COVID-19 patients studied with imaging methods could help us to identify the most characteristic signs of abdominal and gastrointestinal involvement. In this setting, magnetic resonance imaging could be particularly useful for the evaluation of the hepato- biliary system, pancreas and kidney.

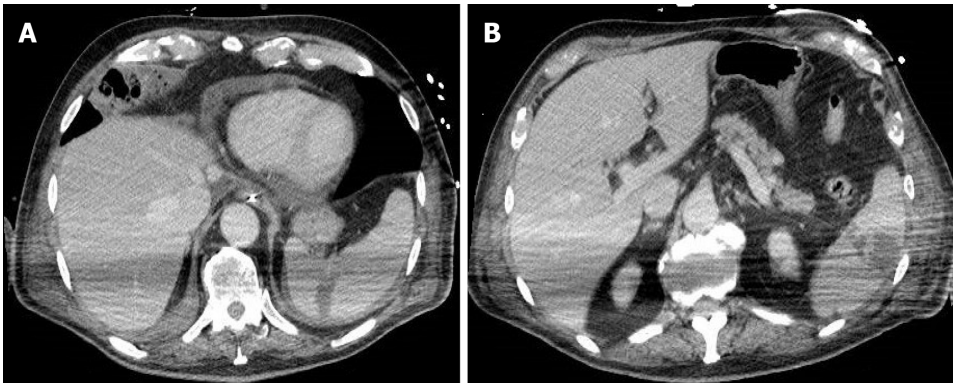

Figure 13 A 77-year-old man with abdominal tenderness. A: Contrast-enhanced portal-venous phase computed tomography image of the abdomen demonstrated a wedge-shaped low-attenuation area at the level of the spleen, typical of infarction. Pericardial effusion was also present; B: A further rounded low-attenuation area with peripheral localization was present in a lower portion of the spleen on contrast-enhanced portal-venous phase computed tomography scan.

CONCLUSION

Although radiological signs are not specific of abdominal and gastrointestinal involvement, the diagnostic imaging modalities and in particular CT are helpful for the clinician in the management, evaluation of the severity and evolution of COVID-19 patients.

REFERENCES

1 **Cucinotta D**, Vanelli M. WHO Declares COVID-19 a Pandemic. *Acta Biomed* 2020; **91**: 157-160 [PMID: 32191675 DOI: 10.23750/abm.v91i1.9397]
2 **Kallem VR**, Sharma D. COVID 19 in neonates. *J Matern Fetal Neonatal Med* 2020; 1-9 [PMID: 32419544 DOI: 10.1080/14767058.2020.1759542]
3 **Tan BS**, Dunnick NR, Gangi A, Goergen S, Jin ZY, Neri E, Nomura CH, Pitcher RD, Yee J, Mahmood U. RSNA International Trends: A Global Perspective on the COVID-19 Pandemic and Radiology in Late 2020. *Radiology* 2021; **299**: E193-E203 [PMID: 33289616 DOI: 10.1148/radiol.2020204267]
4 **Jutzeler CR**, Bourguignon L, Weis CV, Tong B, Wong C, Rieck B, Pargger H, Tschudin-Sutter S, Egli A, Borgwardt K, Walter M. Comorbidities, clinical signs and symptoms, laboratory findings, imaging features, treatment strategies, and outcomes in adult and pediatric patients with COVID-19: A systematic review and meta-analysis. *Travel Med Infect Dis* 2020; **37**: 101825 [PMID: 32763496 DOI: 10.1016/j.tmaid.2020.101825]
5 **Jayaweera M**, Perera H, Gunawardana B, Manatunge J. Transmission of COVID-19 virus by droplets and aerosols: A critical review on the unresolved dichotomy. *Environ Res* 2020; **188**: 109819 [PMID: 32569870 DOI: 10.1016/j.envres.2020.109819]
6 **Mahajan A**, Hirsch JA. Novel Coronavirus: What Neuroradiologists as Citizens of the World Need to Know. *AJNR Am J Neuroradiol* 2020; **41**: 552-554 [PMID: 32198164 DOI: 10.3174/ajnr.A6526]
7 **Stout AE**, André NM, Jaimes JA, Millet JK, Whittaker GR. Coronaviruses in cats and other companion animals: Where does SARS-CoV-2/COVID-19 fit? *Vet Microbiol* 2020; **247**: 108777 [PMID: 32768223 DOI: 10.1016/j.vetmic.2020.108777]
8 **Mizumoto K**, Kagaya K, Chowell G. Effect of a wet market on coronavirus disease (COVID-19) transmission dynamics in China, 2019-2020. *Int J Infect Dis* 2020; **97**: 96-101 [PMID: 32497812 DOI: 10.1016/j.ijid.2020.05.091]
9 **Kooraki S**, Hosseiny M, Myers L, Gholamrezanezhad A. Coronavirus (COVID-19) Outbreak: What the Department of Radiology Should Know. *J Am Coll Radiol* 2020; **17**: 447-451 [PMID: 32092296 DOI: 10.1016/j.jacr.2020.02.008]
10 **Chen J**, Jiang Q, Xia X, Liu K, Yu Z, Tao W, Gong W, Han JJ. Individual variation of the SARS-CoV-2 receptor ACE2 gene expression and regulation. *Aging Cell* 2020; **19** [PMID: 32558150 DOI: 10.1111/acel.13168]
11 **Varga Z**, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; **395**: 1417-1418 [PMID: 32325026 DOI: 10.1016/S0140-6736(20)30937-5]
12 **Boraschi P**. COVID-19 Pulmonary Involvement: Is Really an Interstitial Pneumonia? *Acad Radiol* 2020; **27**: 900 [PMID: 32312654 DOI: 10.1016/j.acra.2020.04.010]
13 **Ackermann M**, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, Jonigk D. Pulmonary Vascular Endothelialitis,

Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med* 2020; **383**: 120-128 [PMID: 32437596 DOI: 10.1056/NEJMoa2015432]

14 **Menter T**, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, Frank S, Turek D, Willi N, Pargger H, Bassetti S, Leuppi JD, Cathomas G, Tolnay M, Mertz KD, Tzankov A. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology* 2020; **77**: 198-209 [PMID: 32364264 DOI: 10.1111/his.14134]

15 **Porfidia A**, Pola R. Venous thromboembolism in COVID-19 patients. *J Thromb Haemost* 2020; **18**: 1516-1517 [PMID: 32294289 DOI: 10.1111/jth.14842]

16 **Han C**, Duan C, Zhang S, Spiegel B, Shi H, Wang W, Zhang L, Lin R, Liu J, Ding Z, Hou X. Digestive Symptoms in COVID-19 Patients With Mild Disease Severity: Clinical Presentation, Stool Viral RNA Testing, and Outcomes. *Am J Gastroenterol* 2020; **115**: 916-923 [PMID: 32301761 DOI: 10.14309/ajg.0000000000000664]

17 **Liu PP**, Blet A, Smyth D, Li H. The Science Underlying COVID-19: Implications for the Cardiovascular System. *Circulation* 2020; **142**: 68-78 [PMID: 32293910 DOI: 10.1161/CIRCULATIONAHA.120.047549]

18 **Luo S**, Zhang X, Xu H. Don't Overlook Digestive Symptoms in Patients With 2019 Novel Coronavirus Disease (COVID-19). *Clin Gastroenterol Hepatol* 2020; **18**: 1636-1637 [PMID: 32205220 DOI: 10.1016/j.cgh.2020.03.043]

19 **Holshue ML**, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, Diaz G, Cohn A, Fox L, Patel A, Gerber SI, Kim L, Tong S, Lu X, Lindstrom S, Pallansch MA, Weldon WC, Biggs HM, Uyeki TM, Pillai SK; Washington State 2019-nCoV Case Investigation Team. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med* 2020; **382**: 929-936 [PMID: 32004427 DOI: 10.1056/NEJMoa2001191]

20 **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]

21 **Siegel A**, Chang PJ, Jarou ZJ, Paushter DM, Harmath CB, Arevalo JB, Dachman A. Lung Base Findings of Coronavirus Disease (COVID-19) on Abdominal CT in Patients With Predominant Gastrointestinal Symptoms. *AJR Am J Roentgenol* 2020; **215**: 607-609 [PMID: 32301631 DOI: 10.2214/AJR.20.23232]

22 **Hamilton NE**, Adam GH, Ifan DL, Lam SS, Johnson K, Vedwan KAG, Shambrook JS, Peebles CR, Harden SP, Abbas A. Diagnostic utility of additional whole-chest CT as part of an acute abdominal pain CT imaging pathway during the COVID-19 pandemic. *Clin Radiol* 2020; **75**: 592-598 [PMID: 32546365 DOI: 10.1016/j.crad.2020.06.002]

23 **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]

24 **Lu R**, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; **395**: 565-574 [PMID: 32007145 DOI: 10.1016/S0140-6736(20)30251-8]

25 **Hung IF**, Lau SK, Woo PC, Yuen KY. Viral loads in clinical specimens and SARS manifestations. *Hong Kong Med J* 2009; **15** Suppl 9: 20-22 [PMID: 20393220]

26 **Effenberger M**, Grabherr F, Mayr L, Schwaerzler J, Nairz M, Seifert M, Hilbe R, Seiwald S, Scholl-Buergi S, Fritsche G, Bellmann-Weiler R, Weiss G, Müller T, Adolph TE, Tilg H. Faecal calprotectin indicates intestinal inflammation in COVID-19. *Gut* 2020; **69**: 1543-1544 [PMID: 32312790 DOI: 10.1136/gutjnl-2020-321388]

27 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]

28 **Carvalho A**, Alqusairi R, Adams A, Paul M, Kothari N, Peters S, DeBenedet AT. SARS-CoV-2 Gastrointestinal Infection Causing Hemorrhagic Colitis: Implications for Detection and Transmission of COVID-19 Disease. *Am J Gastroenterol* 2020; **115**: 942-946 [PMID: 32496741 DOI: 10.14309/ajg.0000000000000667]

29 **Goldberg-Stein S**, Fink A, Paroder V, Kobi M, Yee J, Chernyak V. Abdominopelvic CT findings in patients with novel coronavirus disease 2019 (COVID-19). *Abdom Radiol (NY)* 2020; **45**: 2613-2623 [PMID: 32761402 DOI: 10.1007/s00261-020-02669-2]

30 **Grief SN**, Loza JK. Guidelines for the Evaluation and Treatment of Pneumonia. *Prim Care* 2018; **45**: 485-503 [PMID: 30115336 DOI: 10.1016/j.pop.2018.04.001]

31 **Ravichandran D**, Burge DM. Pneumonia presenting with acute abdominal pain in children. *Br J Surg*

1996; **83**: 1707-1708 [PMID: 9038545 DOI: 10.1002/bjs.1800831214]
32 **Tirumani SH**, Rahnemai-Azar AA, Pierce JD, Parikh KD, Martin SS, Gilkeson R, Ramaiya NH. Are asymptomatic gastrointestinal findings on imaging more common in COVID-19 infection? *Abdom Radiol (NY)* 2021 [PMID: 33394096 DOI: 10.1007/s00261-020-02920-w]
33 **Noda S**, Ma J, Romberg EK, Hernandez RE, Ferguson MR. Severe COVID-19 initially presenting as mesenteric adenopathy. *Pediatr Radiol* 2021; **51**: 140-143 [PMID: 33037889 DOI: 10.1007/s00247-020-04789-9]
34 **Bhayana R**, Som A, Li MD, Carey DE, Anderson MA, Blake MA, Catalano O, Gee MS, Hahn PF, Harisinghani M, Kilcoyne A, Lee SI, Mojtahed A, Pandharipande PV, Pierce TT, Rosman DA, Saini S, Samir AE, Simeone JF, Gervais DA, Velmahos G, Misdraji J, Kambadakone A. Abdominal Imaging Findings in COVID-19: Preliminary Observations. *Radiology* 2020; **297**: E207-E215 [PMID: 32391742 DOI: 10.1148/radiol.2020201908]
35 **Kaafarani HMA**, El Moheb M, Hwabejire JO, Naar L, Christensen MA, Breen K, Gaitanidis A, Alser O, Mashbari H, Bankhead-Kendall B, Mokhtari A, Maurer L, Kapoen C, Langeveld K, El Hechi MW, Lee J, Mendoza AE, Saillant NN, Parks J, Fawley J, King DR, Fagenholz PJ, Velmahos GC. Gastrointestinal Complications in Critically Ill Patients With COVID-19. *Ann Surg* 2020; **272**: e61-e62 [PMID: 32675498 DOI: 10.1097/SLA.0000000000004004]
36 **Keshavarz P**, Rafiee F, Kavandi H, Goudarzi S, Heidari F, Gholamrezanezhad A. Ischemic gastrointestinal complications of COVID-19: a systematic review on imaging presentation. *Clin Imaging* 2021; **73**: 86-95 [PMID: 33341452 DOI: 10.1016/j.clinimag.2020.11.054]
37 **Iba T**, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of Coronavirus Disease 2019. *Crit Care Med* 2020; **48**: 1358-1364 [PMID: 32467443 DOI: 10.1097/CCM.0000000000004458]
38 **Revzin MV**, Raza S, Srivastava NC, Warshawsky R, D'Agostino C, Malhotra A, Bader AS, Patel RD, Chen K, Kyriakakos C, Pellerito JS. Multisystem Imaging Manifestations of COVID-19, Part 2: From Cardiac Complications to Pediatric Manifestations. *Radiographics* 2020; **40**: 1866-1892 [PMID: 33136488 DOI: 10.1148/rg.2020200195]
39 **Romano S**, Bartone G, Romano L. Ischemia and infarction of the intestine related to obstruction. *Radiol Clin North Am* 2008; **46**: 925-942, vi [PMID: 19103141 DOI: 10.1016/j.rcl.2008.07.004]
40 **Pear BL**. Pneumatosis intestinalis: a review. *Radiology* 1998; **207**: 13-19 [PMID: 9530294 DOI: 10.1148/radiology.207.1.9530294]
41 **Parry AH**, Wani AH, Yaseen M. Acute Mesenteric Ischemia in Severe Coronavirus-19 (COVID-19): Possible Mechanisms and Diagnostic Pathway. *Acad Radiol* 2020; **27**: 1190 [PMID: 32475635 DOI: 10.1016/j.acra.2020.05.016]
42 **Ward SE**, Curley GF, Lavin M, Fogarty H, Karampini E, McEvoy NL, Clarke J, Boylan M, Alalqam R, Worrall AP, Kelly C, de Barra E, Glavey S, Ni Cheallaigh C, Bergin C, Martin-Loeches I, Townsend L, Mallon PW, O'Sullivan JM, O'Donnell JS; Irish COVID-19 Vasculopathy Study (ICVS) Investigators. Von Willebrand factor propeptide in severe coronavirus disease 2019 (COVID-19): evidence of acute and sustained endothelial cell activation. *Br J Haematol* 2021; **192**: 714-719 [PMID: 33326604 DOI: 10.1111/bjh.17273]
43 **Escher R**, Breakey N, Lämmle B. Severe COVID-19 infection associated with endothelial activation. *Thromb Res* 2020; **190**: 62 [PMID: 32305740 DOI: 10.1016/j.thromres.2020.04.014]
44 **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]
45 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int* 2021; **41**: 20-32 [PMID: 33190346 DOI: 10.1111/liv.14730]
46 **Li H**, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspectives. *Int J Antimicrob Agents* 2020; **55**: 105951 [PMID: 32234466 DOI: 10.1016/j.ijantimicag.2020.105951]
47 **Zhao B**, Ni C, Gao R, Wang Y, Yang L, Wei J, Lv T, Liang J, Zhang Q, Xu W, Xie Y, Wang X, Yuan Z, Zhang R, Lin X. Recapitulation of SARS-CoV-2 infection and cholangiocyte damage with human liver ductal organoids. *Protein Cell* 2020; **11**: 771-775 [PMID: 32303993 DOI: 10.1007/s13238-020-00718-6]
48 **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]
49 **Li J**, Li RJ, Lv GY, Liu HQ. The mechanisms and strategies to protect from hepatic ischemia-reperfusion injury. *Eur Rev Med Pharmacol Sci* 2015; **19**: 2036-2047 [PMID: 26125267]
50 **Medeiros AK**, Barbisan CC, Cruz IR, de Araújo EM, Libânio BB, Albuquerque KS, Torres US. Higher frequency of hepatic steatosis at CT among COVID-19-positive patients. *Abdom Radiol (NY)* 2020; **45**: 2748-2754 [PMID: 32683613 DOI: 10.1007/s00261-020-02648-7]
51 **Phipps MM**, Barraza LH, LaSota ED, Sobieszczyk ME, Pereira MR, Zheng EX, Fox AN, Zucker J, Verna EC. Acute Liver Injury in COVID-19: Prevalence and Association with Clinical Outcomes in a Large U.S. Cohort. *Hepatology* 2020; **72**: 807-817 [PMID: 32473607 DOI: 10.1002/hep.31404]
52 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: 32145190 DOI: 10.1016/S2468-1253(20)30057-1]
53 **Wong SH**, Lui RN, Sung JJ. Covid-19 and the digestive system. *J Gastroenterol Hepatol* 2020; **35**:

744-748 [PMID: 32215956 DOI: 10.1111/jgh.15047]
54 **Sultan S**, Altayar O, Siddique SM, Davitkov P, Feuerstein JD, Lim JK, Falck-Ytter Y, El-Serag HB; AGA Institute. AGA Institute Rapid Review of the Gastrointestinal and Liver Manifestations of COVID-19, Meta-Analysis of International Data, and Recommendations for the Consultative Management of Patients with COVID-19. *Gastroenterology* 2020; **159**: 320-334. e27 [PMID: 32407808 DOI: 10.1053/j.gastro.2020.05.001]
55 **Kimura T**, Tanaka N, Tanaka E. What will happen in patients with advanced nonalcoholic fatty liver disease? *Hepatobiliary Surg Nutr* 2019; **8**: 283-285 [PMID: 31245415 DOI: 10.21037/hbsn.2019.01.04]
56 **Wei G**, An P, Vaid KA, Nasser I, Huang P, Tan L, Zhao S, Schuppan D, Popov YV. Comparison of murine steatohepatitis models identifies a dietary intervention with robust fibrosis, ductular reaction, and rapid progression to cirrhosis and cancer. *Am J Physiol Gastrointest Liver Physiol* 2020; **318**: G174-G188 [PMID: 31630534 DOI: 10.1152/ajpgi.00041.2019]
57 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: 32278005 DOI: 10.1016/j.jhep.2020.03.044]
58 **Singh S**, Khan A. Clinical Characteristics and Outcomes of Coronavirus Disease 2019 Among Patients With Preexisting Liver Disease in the United States: A Multicenter Research Network Study. *Gastroenterology* 2020; **159**: 768-771. e3 [PMID: 32376408 DOI: 10.1053/j.gastro.2020.04.064]
59 **Xiao AT**, Tong YX, Zhang S. Profile of RT-PCR for SARS-CoV-2: A Preliminary Study From 56 COVID-19 Patients. *Clin Infect Dis* 2020; **71**: 2249-2251 [PMID: 32306036 DOI: 10.1093/cid/ciaa460]
60 **Nagafuchi Y**, Sumitomo S, Soroida Y, Kanzaki T, Iwasaki Y, Michishita K, Iwai T, Ikeda H, Fujio K, Yamamoto K. The power Doppler twinkling artefact associated with periarticular calcification induced by intra-articular corticosteroid injection in patients with rheumatoid arthritis. *Ann Rheum Dis* 2013; **72**: 1267-1269 [PMID: 23505233 DOI: 10.1136/annrheumdis-2012-202897]
61 **Charles K**, McSherry, Frank Glenn, and Norman B. Javitt Composition of Basal and Stimulated Hepatic Bile in Baboons, and the Formation of Cholesterol Gallstones. *PNAS* 1971; **68**: 1564-1568 [DOI: 10.1073/pnas.68.7.1564]
62 **Liu F**, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection. *Clin Gastroenterol Hepatol* 2020; **18**: 2128-2130. e2 [PMID: 32334082 DOI: 10.1016/j.cgh.2020.04.040]
63 **Wang F**, Wang H, Fan J, Zhang Y, Zhao Q. Pancreatic Injury Patterns in Patients With Coronavirus Disease 19 Pneumonia. *Gastroenterology* 2020; **159**: 367-370 [PMID: 32247022 DOI: 10.1053/j.gastro.2020.03.055]
64 **de-Madaria E**, Capurso G. COVID-19 and acute pancreatitis: examining the causality. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 3-4 [PMID: 33203968 DOI: 10.1038/s41575-020-00389-y]
65 **Working Party of the British Society of Gastroenterology**; Association of Surgeons of Great Britain and Ireland; Pancreatic Society of Great Britain and Ireland; Association of Upper GI Surgeons of Great Britain and Ireland. UK guidelines for the management of acute pancreatitis. *Gut* 2005; **54** Suppl 3: iii1-iii9 [PMID: 15831893 DOI: 10.1136/gut.2004.057026]
66 **Shanbhogue AK**, Fasih N, Surabhi VR, Doherty GP, Shanbhogue DK, Sethi SK. A clinical and radiologic review of uncommon types and causes of pancreatitis. *Radiographics* 2009; **29**: 1003-1026 [PMID: 19605653 DOI: 10.1148/rg.294085748]
67 **Batlle D**, Soler MJ, Sparks MA, Hiremath S, South AM, Welling PA, Swaminathan S; COVID-19 and ACE2 in Cardiovascular, Lung; and Kidney Working Group. Acute Kidney Injury in COVID-19: Emerging Evidence of a Distinct Pathophysiology. *J Am Soc Nephrol* 2020; **31**: 1380-1383 [PMID: 32366514 DOI: 10.1681/ASN.2020040419]
68 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; 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; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]
69 **Ronco C**, Reis T, Husain-Syed F. Management of acute kidney injury in patients with COVID-19. *Lancet Respir Med* 2020; **8**: 738-742 [PMID: 32416769 DOI: 10.1016/S2213-2600(20)30229-0]
70 **Hirsch JS**, Ng JH, Ross DW, Sharma P, Shah HH, Barnett RL, Hazzan AD, Fishbane S, Jhaveri KD; Northwell COVID-19 Research Consortium; Northwell Nephrology COVID-19 Research Consortium. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int* 2020; **98**: 209-218 [PMID: 32416116 DOI: 10.1016/j.kint.2020.05.006]
71 **Su H**, Yang M, Wan C, Yi LX, Tang F, Zhu HY, Yi F, Yang HC, Fogo AB, Nie X, Zhang C. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int* 2020; **98**: 219-227 [PMID: 32327202 DOI: 10.1016/j.kint.2020.04.003]
72 **Pan XW**, Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Med* 2020; **46**: 1114-1116 [PMID: 32236644 DOI: 10.1007/s00134-020-06026-1]
73 **Hectors SJ**, Riyahi S, Dev H, Krishnan K, Margolis DJA, Prince MR. Multivariate analysis of CT

imaging, laboratory, and demographical features for prediction of acute kidney injury in COVID-19 patients: a Bi-centric analysis. *Abdom Radiol (NY)* 2020 [PMID: 33098478 DOI: 10.1007/s00261-020-02823-w]

74 **Huang Q**, Li J, Lyu S, Liang W, Yang R, Zhang R, Xiao W, Liu J, Yan S, Zheng L, Chen F. COVID-19 associated kidney impairment in adult: Qualitative and quantitative analyses with non-enhanced CT on admission. *Eur J Radiol* 2020; **131**: 109240 [PMID: 32920219 DOI: 10.1016/j.ejrad.2020.109240]

75 **Heller MT**, Haarer KA, Thomas E, Thaete F. Acute conditions affecting the perinephric space: imaging anatomy, pathways of disease spread, and differential diagnosis. *Emerg Radiol* 2012; **19**: 245-254 [PMID: 22286375 DOI: 10.1007/s10140-012-1022-7]

76 **Chan VW**, Chiu PK, Yee CH, Yuan Y, Ng CF, Teoh JY. A systematic review on COVID-19: urological manifestations, viral RNA detection and special considerations in urological conditions. *World J Urol* 2020 [PMID: 32462305 DOI: 10.1007/s00345-020-03246-4]

77 **Fang L**, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med* 2020; **8**: e21 [PMID: 32171062 DOI: 10.1016/S2213-2600(20)30116-8]

78 **Yousefifard M**, Zali A, Zarghi A, Madani Neishaboori A, Hosseini M, Safari S. Non-steroidal anti-inflammatory drugs in management of COVID-19; A systematic review on current evidence. *Int J Clin Pract* 2020; **74**: e13557 [PMID: 32460369 DOI: 10.1111/ijcp.13557]

79 **Xu X**, Chang XN, Pan HX, Su H, Huang B, Yang M, Luo DJ, Weng MX, Ma L, Nie X. [Pathological changes of the spleen in ten patients with coronavirus disease 2019(COVID-19) by postmortem needle autopsy]. *Zhonghua Bing Li Xue Za Zhi* 2020; **49**: 576-582 [PMID: 32340089 DOI: 10.3760/cma.j.cn112151-20200401-00278]

80 **Goerg C**, Schwerk WB. Splenic infarction: sonographic patterns, diagnosis, follow-up, and complications. *Radiology* 1990; **174**: 803-807 [PMID: 2406785 DOI: 10.1148/radiology.174.3.2406785]

81 **Balcar I**, Seltzer SE, Davis S, Geller S. CT patterns of splenic infarction: a clinical and experimental study. *Radiology* 1984; **151**: 723-729 [PMID: 6718733 DOI: 10.1148/radiology.151.3.6718733]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4160-4171

DOI: 10.3748/wjg.v27.i26.4160

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Inflammatory effect on the gastrointestinal system associated with COVID-19

Paulina Delgado-Gonzalez, Carlos A Gonzalez-Villarreal, Jorge A Roacho-Perez, Adriana G Quiroz-Reyes, Jose Francisco Islas, Juan Luis Delgado-Gallegos, Daniel Arellanos-Soto, Kame A Galan-Huerta, Elsa N Garza-Treviño

ORCID number: Paulina Delgado-Gonzalez 0000-0002-0492-7968; Carlos A Gonzalez-Villarreal 0000-0002-4890-1727; Jorge A Roacho-Perez 0000-0002-3801-135X; Adriana G Quiroz-Reyes 0000-0002-9405-7483; Jose Francisco Islas 0000-0002-0337-9912; Juan Luis Delgado-Gallegos 0000-0003-1580-0812; Daniel Arellanos-Soto 0000-0002-2707-9754; Kame A Galan-Huerta 0000-0002-3495-0528; Elsa N Garza-Treviño 0000-0002-1042-4603.

Author contributions: Delgado-Gonzalez P made the literature analysis and wrote; Gonzalez-Villarreal CA and Roacho-Perez J discussed the revised manuscript of this review; Quiroz-Reyes AG and Arellanos-Soto D design of the images, wrote, analyzed, and corrected the manuscript; Islas JF, Delgado-Gallegos JL and Galan-Huerta KA made the literature analysis and wrote part of the text and corrected the manuscript; Garza-Treviño EN supervised, directed and edited the manuscript; all authors read and approved the final manuscript.

Conflict-of-interest statement: All authors indicated no potential conflicts of interest in publishing this manuscript.

Paulina Delgado-Gonzalez, Jorge A Roacho-Perez, Adriana G Quiroz-Reyes, Jose Francisco Islas, Juan Luis Delgado-Gallegos, Daniel Arellanos-Soto, Kame A Galan-Huerta, Elsa N Garza-Treviño, Departamento de Bioquimica y Medicina Molecular, UANL, Monterrey 64610, Nuevo León, Mexico

Carlos A Gonzalez-Villarreal, Universidad de Monterrey, UDEM Vicerrectoria de Ciencias de la Salud, Monterrey 66238, Nuevo Leon, Mexico

Corresponding author: Elsa N Garza-Treviño, MSc, PhD, Research Associate, Bioquimica y Medicina Molecular, UANL, Ave. Universidad S/N Ciudad Universitaria San Nicolas de Los Garza, Monterrey 66455, Nuevo Leon, Mexico. egarza.nancy@gmail.com

Abstract

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) that causes coronavirus disease-2019 (COVID-19) has provoked a global pandemic, mainly affecting the respiratory tract; however, a percentage of infected individuals can develop gastrointestinal (GI) symptoms. Some studies describe the development of GI symptoms and how they affect the progression of COVID-19. In this review, we summarize the main mechanisms associated with gut damage during infection by SARS-CoV-2 as well as other organs such as the liver and pancreas. Not only are host factors associated with severe COVID-19 but intestinal microbiota dysbiosis is also observed in patients with severe disease.

Key Words: SARS-CoV-2; Gastrointestinal symptoms; COVID-19; Gastrointestinal system

Core Tip: Coronavirus disease-2019 (COVID-19) affects not only the respiratory systems but also gastrointestinal (GI) system and function of others organs. Until now, the mechanism of infection that severe acute respiratory syndrome, coronavirus 2 uses is not fully known. GI symptoms are rare but had great relevance in the severity of disease. We summarize the main known mechanisms that are associated with intestinal

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Mexico

Peer-review report's scientific quality classification

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

Received: January 28, 2021
Peer-review started: January 29, 2021
First decision: May 13, 2021
Revised: May 27, 2021
Accepted: June 17, 2021
Article in press: June 17, 2021
Published online: July 14, 2021

P-Reviewer: Amir M, Nazari A
S-Editor: Ma YJ
L-Editor: A
P-Editor: Zhang YL

damage, and the knowledge that is had about the impact of COVID-19 on the liver and pancreas.

Citation: Delgado-Gonzalez P, Gonzalez-Villarreal CA, Roacho-Perez JA, Quiroz-Reyes AG, Islas JF, Delgado-Gallegos JL, Arellanos-Soto D, Galan-Huerta KA, Garza-Treviño EN. Inflammatory effect on the gastrointestinal system associated with COVID-19. *World J Gastroenterol* 2021; 27(26): 4160-4171
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4160.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4160

INTRODUCTION

Coronaviruses are a family of viruses that cause illnesses such as the common cold, severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2), and Middle East respiratory syndrome (MERS)[1]. SARS-CoV-2 is the etiologic agent of coronavirus disease 2019 (COVID-19), designated as a pandemic by the World Health Organization on March 11, 2020. Up to January 1st, 2021, COVID-19 has caused globally over 85 million cases[2]. The impact that COVID-19 has had worldwide on health and the economy has been devastating since the number of deaths continues, partly because neither we fully understand the disease nor its transmission. Moreover, there are increasing long-term complications and sequelae after COVID-19 in some people[3,4].

The respiratory tract is the main entry route reported, and the transmission mechanism is *via* large droplets containing a high enough viral load. The virus is not motile by itself and depends on its rotational diffusivity to align its proteins (organized in hollow spikes called "peplomers") to its targets during the infection process[5]. Infected people in most cases do not develop symptoms (asymptomatic) or have mild symptoms such as fever, dry cough, fatigue, sore throat and/or headache, conjunctivitis, nausea, vomiting, skin rashes, and dysgeusia; which appear 2-14 d after being exposed to the virus[6]. It has been reported that the survival time of SARS-CoV-2 in aerosol form is 4 h, as the virus becomes inactive at 60°C. Propagation of the droplets in the air depends on the ventilation systems of the area where an infected person is spreading the virus while breathing without using personal protection equipment[7].

Additionally, gastrointestinal (GI) symptoms such as diarrhea, nausea, and vomiting have been reported[8], yet this seems to affect only about 1%–3.8% of the studied patients[9]. Nevertheless, the exact molecular mechanism with which SARS-CoV-2 produces GI damage is still unknown. Therefore, this review aims to describe the effect that SARS-CoV-2 produces in the GI tract.

MECHANISM ASSOCIATED WITH COVID-19 INFECTION IN THE GI SYSTEM

SARS-CoV-2 clinical manifestations include GI effects; however, there is insufficient research on the mechanisms that allow digestive colonization by a respiratory virus. With over 80% resemblance between SARS and SARS-CoV-2[8], several studies have shown tropism for the GI tract, as SARS-CoV-2 RNA was detected in stool specimens from COVID-19 patients with diarrhea, suggesting that it can be transmitted by the fecal-oral route[10].

The viral nucleocapsid protein of SARS-CoV-2 has been found in the GI lumen in the esophagus, stomach, duodenum, and the rectal glandular epithelial cells, suggesting this receptor as the entry point of the SARS-CoV-2 virus in the intestinal tract[10-12]. Also, the expression of angiotensin-converting enzyme 2 (ACE2) protein on glandular cells of gastric, duodenal, rectal epithelia (abundantly expression), and esophageal mucosa (less expression) was demonstrated, supporting the entry of SARS-CoV-2 into the host cells by immunofluorescent technique[13].

ACE2 is a receptor member of the angiotensin-converting enzyme (ACE) family of dipeptidyl-carboxypeptidase and is highly homologous to ACE1, which plays an important role in SARS-CoV-2 infection, through a high-affinity attachment to ACE2

receptors in human cells[11]. The primary function of ACE2 is the conversion of angiotensin (Ang) 1 to Ang 1-9 and Ang 2 into Ang 1-7. ACE receptors participate in cell proliferation and hypertrophy, inflammatory response, blood pressure, and fluid balance. Specifically, ACE2 has an important role in regulating cardiovascular, renal, and reproductive functions[10]. Besides its high expression in type II alveolar cells (AT2) in the lungs, the GI tract also expresses ACE2 receptor, particularly in the esophageal epithelium, glandular gastric mucosa, enterocytes, and colonocytes. ACE2 is present in the cytoplasm of the epithelial cells of the stomach and intestine and the cilia of glandular epithelial cells[10-12].

Recent studies have shown that SARS-CoV-2 may cause digestive symptoms by direct viral invasion of target cells and by inflammatory injury. The viral infection process involves a series of steps: (1) A direct cytopathic effect; (2) Downregulation of ACE2 expression with an increase of metalloproteinase action; and (3) Dysregulation of the immune system, with over secretion of proinflammatory cytokines[14]. Plasmatic and lymphocytic infiltration with interstitial edema[10]. Figure 1 includes more details about this process.

In general, all coronaviruses encode a surface glycoprotein and spike protein that binds to host cell receptors ACE2 and allows virus entry. The spike (S) protein of SARS-CoV-2 has a high affinity for human ACE2, which is the main entrance into the cell[12]. Furin is an enzyme that can be found on the small bowel, acting as a serine-protease that can divide the viral S-protein into two fragments: S1 and S2, allowing them to interact with ACE2. The separation of the S-spike into S1 and S2 is essential for the attachment of the virion to both the ACE receptor and the cell membrane[15]. S-protein proteases, such as cathepsins, expose the fusion domain to the endosome by acid-dependent proteolytic cleavage. Successful virus entry also requires a cellular serine protease, transmembrane protease serine 2 (TMPRSS2)[16]. TMPRSS2 cleaves the S protein of SARS-CoV-2 on the cell membrane, a process that is critical for the fusion of the viral and cell membranes. Importantly, both ACE2 and TMPRSS2 become highly expressed in the ileum and colon[16-18]. Hoffmann *et al*[19] demonstrated that inhibition of the TMPSSR (the serine protease responsible for splitting the S-spike) blocks the infection of cells by SARS-CoV-2.

After viral entry, RNA translates, and viral proteins become synthesized to form new virions released in the GI tract[13]. Thus, leading the CD4+ T cells to reach the small intestine, causing diarrhea and immune damage[12]. ACE2 participates in regulating intestinal inflammation and diarrhea by being a key enzyme in the renin-angiotensin system. It has been shown that loss of ACE2 leads to Ang 2 accumulation. Moreover, the plasma of COVID-19 patients with severe disease presents higher levels of interleukin (IL)-7, IL-10, granulocyte colony-stimulating factor, and recombinant human interferon-induced protein-10, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1A (MIP1A), and tumor necrosis factor alpha (TNF-α)[18]. The local inflammation could debilitate the epithelial barrier, and these inflammatory changes can be part of cell damage induced by viral replication and spreading [20]. This inflammation process disturbs the gut microbiota promoting the polarization of Th17 in the small intestine, promoting the recruitment of other immune cells such as neutrophils, and inducing intestinal immune damage, diarrhea, and other GI symptoms. Also, intestinal damage and gut microbiota alteration can affect the gut-liver axis by contamination of the liver with host and microbial metabolites through the portal vein[12].

A study by Xiao *et al*[13] showed that among 73 hospitalized patients, 53.42% tested positive for SARS-CoV-2 in the stool. The duration of positive stool results ranged from 1 to 12 d, and 23.29% of patients continued to have positive results in stool after being negative in respiratory samples and presenting positive staining for ACE2 receptor and viral nucleocapsid protein in stomach, duodenum, and rectum biopsies. Raising the question if COVID-19 can be transmitted by the fecal-oral route or transmitted by aerosols generated by toilet fumes has been shown with SARS-CoV-2 [21,22]. A study conducted by Zhang *et al*[23] showed that 39.6% of 140 confirmed COVID-19 patients presented GI symptoms among the most common clinical manifestations[23]. Another study reported that 10.1% of 138 confirmed COVID-19 patients, presented diarrhea and nausea[24], furthermore, a recent report showed that 11.4% of 651 patients showed GI symptoms associated with a more severe presentation of the disease[25]. Nonetheless, patients with SARS and MERS have reported more GI symptoms than COVID-19 patients[26]. There has been a high concern in how COVID-19 can affect the body with pre-existing diseases, inflammatory bowel diseases (IBD), such as Crohn disease and ulcerative colitis.

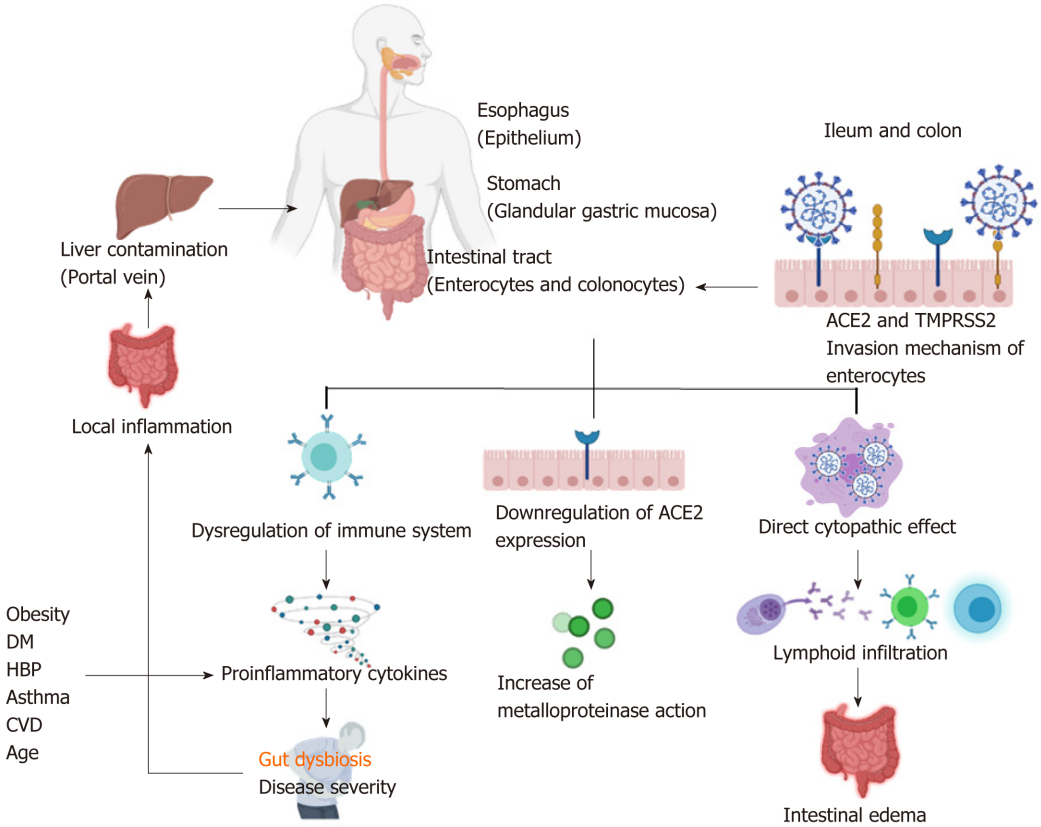

Figure 1 Mechanisms of severe acute respiratory syndrome-coronavirus-2 gastrointestinal infection. The same receptors mediate infections of the gastrointestinal system as in the respiratory system. This situation could begin at the intestinal tract by enterocyte invasion, which possesses angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 receptors recognized by severe acute respiratory syndrome-coronavirus-2. Once in cells, the virus can induce cell death-mediated dysregulation of the immune system by downregulation of ACE2 receptor expression and a direct cytopathic effect. All three mechanisms induce immune dysregulation and increase the inflammation mechanism. Some risk factors that accelerate immune inflammation are obesity, diabetes mellitus, high blood pressure, asthma, cardiovascular disease, and advanced age. Moreover, the virus could enter the liver by the portal vein and induce hepatic failure. ACE2: Angiotensin-converting enzyme 2; TMPRSS2: Transmembrane protease serine 2; DM: Diabetes mellitus; HPB: High blood pressure; CVD: Cardiovascular disease.

A study showed that immunosuppressors modulate the cytokine inflammatory response, thus preventing a more severe manifestation of COVID-19[27]. Also, GI symptoms derived from drug side effects of antibacterials (macrolides, fluoroquinolones, or cephalosporin) and antivirals (chloroquine phosphate, lopinavir, and remdesivir) administered during illness[12].

COVID-19 patients with preexisting comorbidities such as hypertension, asthma, diabetes, cardiovascular problems, and old age, have a higher susceptibility to inflammation. Recent studies have shown that the severity of the clinical course of COVID-19 is related to inflammation and higher levels of proinflammatory cytokines[14]. Studies show that SARS-CoV-2 rapidly activates T cells and induces the release of several inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 1 (IL-1), IL-6, monocyte chemoattractant protein-1, and interferon-gamma (IFN-γ). GM-CSF activates CD14+ cells, CD16+ cells, and monocytes, increasing inflammatory cytokine levels, stepping up the inflammatory cascade. This intense immune response causes tissue damage[28]. T cells from peripheral blood in COVID-19 infection present high cytotoxic activity with more cytotoxic granules, granulysin, and perforin, which shows that activated T cells could speed up systemic inflammation[29]. Also, ACE2 expressing cells release proinflammatory cytokines such as MCP-1, tumor growth factor (TGF-1), TNF-α, IL-1, and IL-6 [12].

Recently, COVID-19 intestinal pathogenesis mechanisms have been proposed since SARS-CoV-2 also might interfere with tryptophan absorption. Tryptophan stimulates the mTOR pathway for the production of antimicrobial peptides that maintain gut microbiota homeostasis. This process requires intestinal ACE2 to regulate the expression of neutral amino acid transporters. Tryptophan is absorbed by factors of

the B0AT1/ACE2 transport pathway on the lumen surface of intestinal epithelial cells. When there is not enough niacin or tryptophan intake, there is a high risk of developing pellagra, which eventually develops into colitis. As SARS-CoV-2 infection competes for available ACE2 receptors, it causes tryptophan deficiency and lower production of antimicrobial peptides[16]. COVID-19 murine models showed a deficiency of ACE2 receptors in the colon, which increase susceptibility to inflammation and colitis development due to decreased antimicrobial peptides and the alteration of gut microbiota, finalizing with diarrhea[12,29]. However, this mechanism needs to be proven in humans.

COVID-19 RELATED DAMAGE TO INTESTINAL MICROBIOTA

The human gut microbiota comprises 10^{14} resident microorganisms which include bacteria, *archae*, viruses, and fungi and has a key role in health through its protective function by regulating various host physiological functions, including dietary digestion, and imparting protective immunity against pathogens[30]. The defense mechanism of microbiota induces alpha-defensin, secretory IgA, and some other AMPs (antimicrobial peptides)[31], affecting innate lymphoid cells, but mainly they affect the innate and adaptive immune system by influencing epithelial or macrophage cell receptors, such as toll-like receptors (TLRs) or NOD-like receptors (NLRs). TLRs are involved in normal mucosal immune system development of the intestine, decreasing inflammatory responses and promoting immunological tolerance to the normal microbiota components. NLRs participate in the adjustment of the IL-18 level, the immune response, dysbiosis, and intestinal hyperplasia[32].

Healthy gut microbiota, primarily dominated by *Bifidobacterium* spp., *Faecalibacterium* spp., *Ruminococcus* spp., *and Prevotella* spp. Whom`s alterations in the balance between gut microbiota and the immune system, sometimes collectively called "gut dysbiosis" are associated with infections, inflammations, allergies, colorectal cancer, and autoimmune disease[30]. Studies have suggested that "gut-dysbiosis" might contribute to GI symptoms by SARS-CoV-2 infection, *i.e.*, *Bacteroides dorei, Bacteroides thetaiotaomicron, Bacteroides massiliensis*, and *Bacteroides ovatus*, can downregulate the expression of ACE2 during the hospitalization of COVID-19 patients[33].

Microbial dysbiosis with decreased levels of *Lactobacillus* and *Bifidobacterium* and the abundance of *Clostridium hathewayi, Clostridium ramosum*, and *Coprobacillus* positively correlated with the severity of the disease[33]. A study in a Chinese population reported that intestinal infection by SARS-CoV-2 can induce the production of proinflammatory factors such as IL-18. IL-18 is a proinflammatory cytokine produced by multiple enteric cells, including intestinal epithelial cells, immune cells, and the enteric nervous system, is shown increased in the serum of COVID-19 patients. IL-18 levels seem to correlate with an abundance of *Peptostreptococcus*, *Fusobacterium*, and *Citrobacter*, indicating changes in gut microbiota[34].

Obesity presents changes in microbiota, dysregulation of cytokine profiles, and higher levels of ACE2 in adipocytes[35]. As the opposite, an adequate fiber intake and whole grains diet improves intestinal microbiome composition, reduces intestinal inflammation markers like CRP, IL-6, and TNF-α[36]. Besides the colon and intestines, the liver is another organ the SARS-CoV-2 could affect[29].

LIVER INJURY IN COVID-19 PATIENTS

The few reports regarding liver damage by COVID-19 come from autopsies carried out in different hospital centers. The incidence of liver damage in patients with COVID 19 ranges from 14% to 53%[37]. Patients with elevated liver function tests were more likely to have a moderate-high degree fever, and these elevations were significantly more prevalent in male patients (68.67% *vs* 38.36%). It is important to mention that it is difficult to define how COVID-19 generates liver damage since patients at the time of hospitalization usually have chronic diseases such as non-alcoholic hepatic steatosis, which increases the progression of the disease, hepatitis C such as those reported by Schmit[20,38]. Among the biochemical indicators, there have been reports of elevated aminotransferases approximately on the tenth day of hospitalization[39].

Patient biopsies reveal the presence of hepatocyte mitosis with acidophilic bodies, moderate inflammation, and ballon degeneration. In the SARS virus epidemic in 2003, a study reported the elevation of aminotransferases in a range of 300-400, and prominent mitoses, which refers to what researchers have published in various studies

from these pandemics. The authors assumed that the prominent mitosis was likely due to a hyperproliferative state and cell cycle arrest[40].

Some researchers hypothesized that the direct action of the virus on liver cells causes centrilobular, periportal necrosis without significant inflammation compatible with acute liver damage. Also, the authors report that development of cholestasis and a great reactive biliary proliferation as consequences of the virus are to be expected. They further consider the possibility that the virus enters the liver through the portal vein[38]. The definitive mechanism by which liver injury occurs in COVID-19 patients remains unclear. There are multiple theories of the pathophysiology of the viral infection that could explain this phenomenon: (1) ACE2-mediated direct viral infection of hepatocytes; (2) Critically-ill status and immune-mediated injury; or (3) Drug hepatotoxicity[11] (Figure 2).

Liver damage by COVID-19 can be clarified thanks to the severe inflammatory response and cytotoxicity of the active replication with ACE2 receptors expressed in the liver, especially in cholangiocytes and epithelial cells of the bile duct, which is why the liver is also considered a target organ for SARS-CoV2 infection[41].

Fiel *et al*[42] found this elevation associated with pharmacological treatment with lopinavir/ritonavir. However, a review demonstrated that aminotransferases are only significantly elevated in severe COVID-19 cases. Drug toxicity has served as one mechanism for COVID-19-associated liver injury, damage that is secondary and does not make them susceptible to viral infection. However, little is known about the incidence of hepatotoxicity of various drugs used in COVID-19. Understandably, efforts are currently made regarding this concern. These efforts will prove important in developing a reasonable intervention and reducing the harmful effects of drug-induced hepatotoxicity for patients[29].

SARS-COV-2 AND THE PANCREAS

Expressed in the pancreas is the angiotensin converting enzyme 2 specifically in the exocrine glands, and islets[43] therefore, it is susceptible to SARS-CoV-2 infection. In a cohort of 121 patients with COVID-19 in China, 10% had increased lipase levels but only 4% showed pancreas enlargement or dilatation in computerized tomography (CT) scans[44].

In another cohort of 71 patients in the United States, 12% had increased lipase levels but only 3% exceeded three times the upper normal limit. None of the patients had abnormal pancreas images in CT scans[45]. In a cohort of 83 patients with COVID-19 in the United States, 16.8% had increased lipase levels (three times the upper limit). Researchers have associated high lipase levels with admission to the intensive care unit and intubation after a multivariable-adjusted model[46]. In a retrospective pooled analysis, the pooled prevalence of hyperlipasemia was 12% and the pooled odds ratio for severe COVID-19 was 3.143[47]. The ACE2 receptor is also highly expressed in pancreatic islet cells[43]; therefore, SARS-CoV-2 infection can theoretically cause islet damage resulting in acute diabetes, which associates to patients with pancreatic injury and high blood sugar. Mechanisms by which pancreatic injury could occur include the direct cytopathic effects of SARS-CoV-2 or indirect systemic inflammatory and immune-mediated cell responses, resulting in organ damage or secondary enzyme abnormalities. Antipyretics, which most of the patients in this study took before admission, could also cause drug-related pancreatic injury[48]. However, more information to understand the role of pancreatic injury in patients with COVID-19 is needed.

TREATMENTS OF GI SYMPTOMS

At the current stage of the COVID-19 pandemic, several vaccines are in their last stages of authorization for emergency use[49,50]. While full distribution will continue as a challenge, hopes of major population immunity are coming close. Yet, until we have a more resistant population, respiratory complications will continue as the major symptom reported during a COVID-19 infection. Interestingly, other lesser-known indicators that manifest, such as those of the GI which include vomiting, nausea, and diarrhea[12,51]. Many studies have shown that vomiting and nausea can be present in upwards of 30% and 15% of patients[12]. Interestingly enough, in a pediatric setting, one reported case showed patients with no-respiratory affliction who were all COVID-19 positive; all presented GI alterations. Several showed gastroenteritis, another

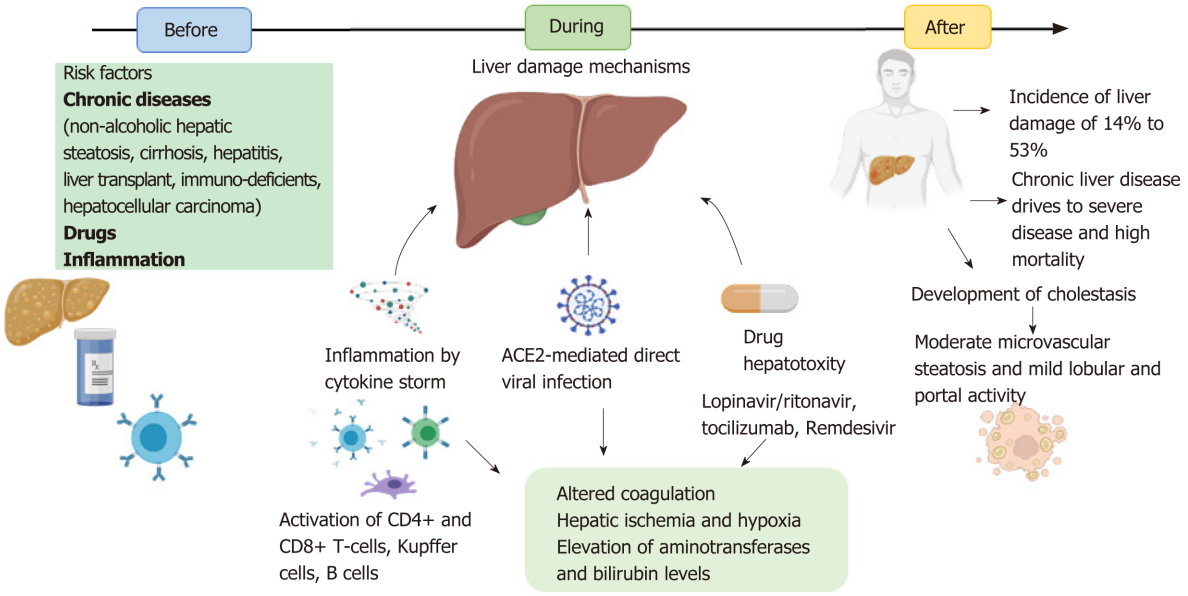

Figure 2 Proposed process of liver damage. Before severe acute respiratory syndrome-coronavirus-2 infection, there are risk factors considered that could be poor prognostic factors, such as chronic diseases, the use of drugs that affect the liver and the inflammation process. The virus can infect the liver through the portal vein. There are three proposed mechanisms of liver damage: inflammation induced by cytokine storm and activation of hepatic immunity, angiotensin-converting enzyme 2-mediated direct viral infection of hepatocytes, epithelial cells, and cholangiocytes, and drug hepatotoxicity mediated by some antivirals employed for coronavirus disease-2019 (COVID-19) treatment. The three mechanisms culminate in altered coagulation, hepatic ischemia, and elevation of aminotransferases and bilirubin levels. Following this, the incidence of liver damage derived from COVID-19 is up to 53%, which could develop cholestasis and reach high mortality risk. ACE2: Angiotensin-converting enzyme 2.

patient appendicitis, and yet another, hydronephrosis[52]. As more data becomes available, GI manifestations such as loss of appetite seem to be direct signs of COVID-19. Counter to the GI manifestations brought about by COVID-19, several drugs used to combat the effects of the virus have secondary side effects. Drugs like remdesivir, hydroxychloroquine, favipiravir, ivermectin, and azithromycin can induce side effects such as vomiting, nausea, elevated liver enzymes, weight loss, abdominal pain, and others[53]. The Table 1 display wide information.

In addition, COVID-19 patients can present a hyper-inflammatory state, with systemic response and cytokine storm mediated by IL-6, IL-8, and TNF-α, which can induce platelet activation and thrombosis, also presenting endothelial dysfunction due to direct virus damage and inflammation[54]. Heparin is used in COVID-19 patients as prophylactic therapy to prevent thrombosis. However, heparin-induced-thrombocytopenia (HIT) after administering low doses may not be enough to counteract the hypercoagulable state, leading to coagulation problems in these patients[55]. Heparin treatment, by a direct interaction between heparin and platelets, induces platelet clumping or sequestration. This event occurs within the first 48-72 h after starting treatment and generates mild and transient thrombocytopenia[56]. In some cases, thrombosis could be associated with HIT after heparin cessation[57].

In the GI system, the intestinal microbiota plays a crucial role in the correct balance and maintenance. If unbalanced component processing becomes inefficient, direct damage to the intestinal mucosa results in more accessible routes for viral infection[12,58]. Studies have confirmed that probiotics can assist in this treatment; both bifidobacterium and lactic acid can help induce antibody production[12]. It is important to mention that patients with severe GI symptoms require a nutritional risk assessment, as it becomes a predictor of outcome both in the long term and the short term[59].

COVID-19 individuals presenting irritable bowel disease are of particular interest since this condition warrants the use of immunosuppressants and steroids. Interestingly, vedolizumab and ustekinumab do not increase the risk of COVID-19, hence patients can continue its use safely. Yet, thiopurines, anti-tumor necrosis factor (anti-TNF) agents, and JAK inhibitors may continue to present a risk. In mild cases, 5-ASA and budesonide use are reasonable[53]. We should take special consideration to outweigh the benefit against the risk for each case. Also, unless emergent, patients should defer all surgical procedures until pandemic conditions rescind[60]. We should

Table 1 Side effects of most common drugs during coronavirus disease-2019 treatment

Pharmacological intervention	Mechanism of action	Adverse effects	Ref.
Hydroxychloroquine	Elevated endosomal pH; Disruption of lysosome-endosome fusion. Inhibition of cell-virus fusion when interacting with N-terminal domain of the SARS-CoV-2 peak	Q-T segment prolongation; Gastrointestinal Adverse Effects	[62-64]
Chloroquine	Inhibits RNA-dependent polymerases, decreases endosomal iron release required for DNA replication, and inhibits glycosylation of viral envelope glycoproteins	Gastrointestinal adverse effects; visual and extrapyramidal disturbances; Arrhythmogenic cardiotoxicity	[65-67]
Remdesivir	Transcription Inhibitor	Caution in patients with severe renal impairment [estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73] or severe liver disease	[68]
Lopinavir/Ritonavir	Lopinavir binds to the viral protease and prevents the cleavage of the Gag-Pol polyprotein, resulting in the production of non-infectious immature viral particles. Ritonavir increases the plasma concentration of lopinavir by inhibiting the metabolism of cytochrome P450 3A (CYP3A)	Gastrointestinal adverse effects	[64, 65,68-72]
Ribavirin	Interferes with RNA polymerase and viral protein synthesis	Hemolytic anemia; Leukopenia; Teratogenic	[68]
Interferon	Degradation of viral RNA; Alteration of RNA transcription; Inhibition of protein synthesis and apoptosis	Worsening psychiatric conditions, cytopenia, and uncontrolled seizures	[68]
Cortocosteroids, dexametasone	Inhibitor of the inflammatory process	Impair the immune response; Bacterial pneumonia risk; Hyperglycemia; Osteoporosis; Hypertension	[68-70]
Azithromycin	Bacteriostatic antibiotics; Anti-inflammatory effects Immunomodulatory effects	QTc with the risk of arrhythmias	[71, 73]
Heparin	Antiplatelet	Risk GI symptoms; Bleeding; Heparin-induced thrombocytopenia	[74]
Favipiravir	Competitive inhibitor of RNA-dependent RNA polymerase	GI adverse effects; liver injury	[75, 76]

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2; GI: Gastrointestinal.

consider patients with Crohn's disease for colectomy with end ileostomy. An important aspect to take into consideration is comorbidities, such as diabetes and hypertension, which are exacerbators of damage in COVID-19. As expected, comorbidities become paramount in symptom management, because of the high risk they represent[60,61].

CONCLUSION

By now, we know that GI symptoms in COVID-19 disease such as diarrhea are related to gut microbiota alterations that alter profile cytokines, either by SARS-Cov-2 ACE2 alterations or as a secondary effect of antibiotic and antiviral drugs employed in treatment. However, additional research is needed for the hepatic and pancreatic manifestations that aggravates the patient´s situation, and a deeper understanding of the sequelae after symptoms of the disease. Until now, the knowledge that we have mainly involves the host; however, we must not ignore the pathogenicity of the virus and the recent variants that are currently circulating since these could in the future serve to explain in greater detail the mechanisms involved in the intestinal damage or with the presentation of GI symptoms that can accompany COVID-19 respiratory disease.

ACKNOWLEDGEMENTS

Special thanks to Sergio Lozano for reviewing this manuscript.

REFERENCES

1 **National Institutes of Health**. Coronaviruses. [cited 13 November 2020] Available from: https://www.niaid.nih.gov/diseases-conditions/coronaviruses
2 **World Health Organization**. WHO Coronavirus disease. [cited 13 November 2020] Available from: https://covid19.who.int
3 **Yang C**, Wang J. Transmission rates and environmental reservoirs for COVID-19 - a modeling study. *J Biol Dyn* 2021; **15**: 86-108 [PMID: 33402047 DOI: 10.1080/17513758.2020.1869844]
4 **Center for Disease Control and Prevention**. Late sequelae of COVID-19. [cited 13 November 2020] Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/Late-sequelae.html
5 **Dbouk T**, Drikakis D. On coughing and airborne droplet transmission to humans. *Phys Fluids (1994)* 2020; **32**: 053310 [PMID: 32574229 DOI: 10.1063/5.0011960]
6 **Baj J**, Karakuła-Juchnowicz H, Teresiński G, Buszewicz G, Ciesielka M, Sitarz E, Forma A, Karakuła K, Flieger W, Portincasa P, Maciejewski R. COVID-19: Specific and Non-Specific Clinical Manifestations and Symptoms: The Current State of Knowledge. *J Clin Med* 2020; **9** [PMID: 32516940 DOI: 10.3390/jcm9061753]
7 **Nazari A**, Jafari M, Rezaei N, Taghizadeh-Hesary F. Jet fans in the underground car parking areas and virus transmission. *Phys Fluids (1994)* 2021; **33**: 013603 [PMID: 33746483 DOI: 10.1063/5.0033557]
8 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]
9 **Almeida JFM**, Chehter EZ. COVID-19 and the gastrointestinal tract: what do we already know? *Einstein (Sao Paulo)* 2020; **18**: eRW5909 [PMID: 33206816 DOI: 10.31744/einstein_journal/2020RW5909]
10 **Galanopoulos M**, Gkeros F, Doukatas A, Karianakis G, Pontas C, Tsoukalas N, Viazis N, Liatsos C, Mantzaris GJ. COVID-19 pandemic: Pathophysiology and manifestations from the gastrointestinal tract. *World J Gastroenterol* 2020; **26**: 4579-4588 [PMID: 32884218 DOI: 10.3748/wjg.v26.i31.4579]
11 **Wong SH**, Lui RN, Sung JJ. Covid-19 and the digestive system. *J Gastroenterol Hepatol* 2020; **35**: 744-748 [PMID: 32215956 DOI: 10.1111/jgh.15047]
12 **Ye Q**, Wang B, Zhang T, Xu J, Shang S. The mechanism and treatment of gastrointestinal symptoms in patients with COVID-19. *Am J Physiol Gastrointest Liver Physiol* 2020; **319**: G245-G252 [PMID: 32639848 DOI: 10.1152/ajpgi.00148.2020]
13 **Xiao F**, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 2020; **158**: 1831-1833. e3 [PMID: 32142773 DOI: 10.1053/j.gastro.2020.02.055]
14 **Eder P**, Łodyga M, Dobrowolska A, Rydzewska G, Kamhieh-Milz J. Addressing multiple gastroenterological aspects of coronavirus disease 2019. *Pol Arch Intern Med* 2020; **130**: 420-430 [PMID: 32356641 DOI: 10.20452/pamw.15332]
15 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]
16 **Wan Y**, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol* 2020; **94** [PMID: 31996437 DOI: 10.1128/jvi.00127-20]
17 **Trottein F**, Sokol H. Potential Causes and Consequences of Gastrointestinal Disorders during a SARS-CoV-2 Infection. *Cell Rep* 2020; **32**: 107915 [PMID: 32649864 DOI: 10.1016/j.celrep.2020.107915]
18 **Ding S**, Liang TJ. Is SARS-CoV-2 Also an Enteric Pathogen With Potential Fecal-Oral Transmission? *Gastroenterology* 2020; **159**: 53-61 [PMID: 32353371 DOI: 10.1053/j.gastro.2020.04.052]
19 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280. e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]
20 **Patel KP**, Patel PA, Vunnam RR, Hewlett AT, Jain R, Jing R, Vunnam SR. Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. *J Clin Virol* 2020; **128**: 104386 [PMID: 32388469 DOI: 10.1016/j.jcv.2020.104386]
21 **Kang M**, Wei J, Yuan J, Guo J, Zhang Y, Hang J, Qu Y, Qian H, Zhuang Y, Chen X, Peng X, Shi T, Wang J, Wu J, Song T, He J, Li Y, Zhong N. Probable Evidence of Fecal Aerosol Transmission of SARS-CoV-2 in a High-Rise Building. *Ann Intern Med* 2020; **173**: 974-980 [PMID: 32870707 DOI: 10.7326/M20-0928]
22 **McDermott CV**, Alicic RZ, Harden N, Cox EJ, Scanlan JM. Put a lid on it: are faecal bio-aerosols a route of transmission for SARS-CoV-2? *J Hosp Infect* 2020; **105**: 397-398 [PMID: 32315667 DOI: 10.1016/j.jhin.2020.04.024]

23 **Zhang JJ**, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; **75**: 1730-1741 [PMID: 32077115 DOI: 10.1111/all.14238]

24 **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; **323**: 1061-1069 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

25 **Jin X**, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, Hao SR, Jia HY, Cai H, Zhang XL, Yu GD, Xu KJ, Wang XY, Gu JQ, Zhang SY, Ye CY, Jin CL, Lu YF, Yu X, Yu XP, Huang JR, Xu KL, Ni Q, Yu CB, Zhu B, Li YT, Liu J, Zhao H, Zhang X, Yu L, Guo YZ, Su JW, Tao JJ, Lang GJ, Wu XX, Wu WR, Qv TT, Xiang DR, Yi P, Shi D, Chen Y, Ren Y, Qiu YQ, Li LJ, Sheng J, Yang Y. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020; **69**: 1002-1009 [PMID: 32213556 DOI: 10.1136/gutjnl-2020-320926]

26 **Luo X**, Zhou GZ, Zhang Y, Peng LH, Zou LP, Yang YS. Coronaviruses and gastrointestinal diseases. *Mil Med Res* 2020; **7**: 49 [PMID: 33054860 DOI: 10.1186/s40779-020-00279-z]

27 **Gou W**, Fu Y, Yue L, Chen G, Cai X, Shuai M, Xu F, Yi X, Chen H, Zhu Y, Xiao M, Jiang Z, Miao Z, Xiao C, Shen B, Wu X, Zhao H, Ling W, Wang J, Chen Y, Guo T, Zheng JS. Gut microbiota may underlie the predisposition of healthy individuals to COVID-19. medRxiv 2020; 1-44 [DOI: 10.1101/2020.04.22.20076091]

28 **Zhang Y**, Geng X, Tan Y, Li Q, Xu C, Xu J, Hao L, Zeng Z, Luo X, Liu F, Wang H. New understanding of the damage of SARS-CoV-2 infection outside the respiratory system. *Biomed Pharmacother* 2020; **127**: 110195 [PMID: 32361161 DOI: 10.1016/j.biopha.2020.110195]

29 **Ma C**, Cong Y, Zhang H. COVID-19 and the Digestive System. *Am J Gastroenterol* 2020; **115**: 1003-1006 [PMID: 32618648 DOI: 10.14309/ajg.0000000000000691]

30 **Dhar D**, Mohanty A. Gut microbiota and Covid-19- possible link and implications. *Virus Res* 2020; **285**: 198018 [PMID: 32430279 DOI: 10.1016/j.virusres.2020.198018]

31 **Muniz LR**, Knosp C, Yeretssian G. Intestinal antimicrobial peptides during homeostasis, infection, and disease. *Front Immunol* 2012; **3**: 310 [PMID: 23087688 DOI: 10.3389/fimmu.2012.00310]

32 **Baghbani T**, Nikzad H, Azadbakht J, Izadpanah F, Haddad Kashani H. Dual and mutual interaction between microbiota and viral infections: a possible treat for COVID-19. *Microb Cell Fact* 2020; **19**: 217 [PMID: 33243230 DOI: 10.1186/s12934-020-01483-1]

33 **Zuo T**, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology* 2020; **159**: 944-955. e8 [PMID: 32442562 DOI: 10.1053/j.gastro.2020.05.048]

34 **Tao W**, Zhang G, Wang X, Guo M, Zeng W, Xu Z, Liu L, Zhang K, Wang Y, Ma X, Chen Z, Jin T, Weng J, Zhu S. Analysis of the intestinal microbiota in COVID-19 patients and its correlation with the inflammatory factor IL-18 and SARS-CoV-2-specific IgA. *medRxiv* 2020; 1-9 [DOI: 10.1101/2020.08.12.20173781]

35 **Al Heialy S**, Hachim MY, Senok A, Gaudet M, Abou Tayoun A, Hamoudi R, Alsheikh-Ali A, Hamid Q. Regulation of Angiotensin- Converting Enzyme 2 in Obesity: Implications for COVID-19. *Front Physiol* 2020; **11**: 555039 [PMID: 33071815 DOI: 10.3389/fphys.2020.555039]

36 **Xu Y**, Wan Q, Feng J, Du L, Li K, Zhou Y. Whole grain diet reduces systemic inflammation: A meta-analysis of 9 randomized trials. *Medicine (Baltimore)* 2018; **97**: e12995 [PMID: 30412134 DOI: 10.1097/MD.0000000000012995]

37 **Jothimani D**, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol* 2020; **73**: 1231-1240 [PMID: 32553666 DOI: 10.1016/j.jhep.2020.06.006]

38 **Schmit G**, Lelotte J, Vanhaebost J, Horsmans Y, Van Bockstal M, Baldin P. The Liver in COVID-19-Related Death: Protagonist or Innocent Bystander? *Pathobiology* 2021; **88**: 88-94 [PMID: 33108789 DOI: 10.1159/000512008]

39 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: 32283325 DOI: 10.1016/j.cgh.2020.04.002]Available]

40 **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]

41 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int* 2021; **41**: 20-32 [PMID: 33190346 DOI: 10.1111/Liv.14730]

42 **Fiel MI**, El Jamal SM, Paniz-Mondolfi A, Gordon RE, Reidy J, Bandovic J, Advani R, Kilaru S, Pourmand K, Ward S, Thung SN, Schiano T. Findings of Severe Hepatic Severe Acute Respiratory Syndrome Coronavirus-2 Infection. *Cell Mol Gastroenterol Hepatol* 2021; 1-8 [DOI: 10.1016/j.jcmgh.2020.09.015]

43 **Wang F**, Wang H, Fan J, Zhang Y, Zhao Q. Pancreatic Injury Patterns in Patients With Coronavirus Disease 19 Pneumonia. *Gastroenterology* 2020; **159**: 367-370 [PMID: 32247022 DOI: 10.1053/j.gastro.2020.03.055]

44 **Liu F**, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection. *Clin Gastroenterol Hepatol* 2020; **18**: 2128-2130.

e2 [PMID: 32334082 DOI: 10.1016/j.cgh.2020.04.040]
45 **McNabb-Baltar J**, Jin DX, Grover AS, Redd WD, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Shen L, Chan WW. Lipase Elevation in Patients With COVID-19. *Am J Gastroenterol* 2020; **115**: 1286-1288 [PMID: 32496339 DOI: 10.14309/ajg.0000000000000732]
46 **Barlass U**, Wiliams B, Dhana K, Adnan D, Khan SR, Mahdavinia M, Bishehsari F. Marked Elevation of Lipase in COVID-19 Disease: A Cohort Study. *Clin Transl Gastroenterol* 2020; **11**: e00215 [PMID: 32764201 DOI: 10.14309/ctg.0000000000000215]
47 **Mobasheri A**, Batt M. An update on the pathophysiology of osteoarthritis. *Ann Phys Rehabil Med* 2016; **59**: 333-339 [PMID: 27546496]
48 **Alves AM**, Yvamoto EY, Marzinotto MAN, Teixeira ACS, Carrilho FJ. SARS-CoV-2 Leading to acute pancreatitis: an unusual presentation. *Braz J Infect Dis* 2020; **24**: 561-564 [PMID: 32961108 DOI: 10.1016/j.bjid.2020.08.011]
49 **Mahase E**. Covid-19: Pfizer and BioNTech submit vaccine for US authorisation. *BMJ* 2020; **371**: m4552 [PMID: 33219049 DOI: 10.1136/bmj.m4552]
50 **Callaway E**. COVID vaccine excitement builds as Moderna reports third positive result. *Nature* 2020; **587**: 337-338 [PMID: 33199901 DOI: 10.1038/d41586-020-03248-7]
51 **Su S**, Shen J, Zhu L, Qiu Y, He JS, Tan JY, Iacucci M, Ng SC, Ghosh S, Mao R, Liang J. Involvement of digestive system in COVID-19: manifestations, pathology, management and challenges. *Therap Adv Gastroenterol* 2020; **13**: 1756284820934626 [PMID: 32595762 DOI: 10.1177/1756284820934626]
52 **Cai X**, Ma Y, Li S, Chen Y, Rong Z, Li W. Clinical Characteristics of 5 COVID-19 Cases With Non-respiratory Symptoms as the First Manifestation in Children. *Front Pediatr* 2020; **8**: 258 [PMID: 32574284 DOI: 10.3389/fped.2020.00258]
53 **Dhar J**, Samanta J, Kochhar R. Corona Virus Disease-19 pandemic: The gastroenterologists' perspective. *Indian J Gastroenterol* 2020; **39**: 220-231 [PMID: 32783168 DOI: 10.1007/s12664-020-01075-2]
54 **Carfora V**, Spiniello G, Ricciolino R, Di Mauro M, Migliaccio MG, Mottola FF, Verde N, Coppola N; Vanvitelli COVID-19 group. Anticoagulant treatment in COVID-19: a narrative review. *J Thromb Thrombolysis* 2021; **51**: 642-648 [PMID: 32809158 DOI: 10.1007/s11239-020-02242-0]
55 **Porfidia A**, Pola R. Venous Thromboembolism and Heparin Use in COVID-19 Patients: Juggling between Pragmatic Choices, Suggestions of Medical Societies and the Lack of Guidelines. *J Thromb Thrombolysis* 2020; **50**: 68-71 [PMID: 32367471 DOI: 10.1007/s11239-020-02125-4]
56 **Ahmed I**, Majeed A, Powell R. Heparin induced thrombocytopenia: diagnosis and management update. *Postgrad Med J* 2007; **83**: 575-582 [PMID: 17823223 DOI: 10.1136/pgmj.2007.059188]
57 **Jang IK**, Hursting MJ. When heparins promote thrombosis: review of heparin-induced thrombocytopenia. *Circulation* 2005; **111**: 2671-2683 [PMID: 15911718 DOI: 10.1161/CIRCULATIONAHA.104.518563]
58 **Marciani L**, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Fillery-Travis AJ. Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1227-G1233 [PMID: 11352816 DOI: 10.1152/ajpgi.2001.280.6.g1227]
59 **Hersberger L**, Bargetzi L, Bargetzi A, Tribolet P, Fehr R, Baechli V, Geiser M, Deiss M, Gomes F, Kutz A, Kägi-Braun N, Hoess C, Pavlicek V, Schmid S, Bilz S, Sigrist S, Brändle M, Benz C, Henzen C, Nigg M, Thomann R, Brand C, Rutishauser J, Aujesky D, Rodondi N, Donzé J, Stanga Z, Mueller B, Schuetz P. Nutritional risk screening (NRS 2002) is a strong and modifiable predictor risk score for short-term and long-term clinical outcomes: secondary analysis of a prospective randomised trial. *Clin Nutr* 2020; **39**: 2720-2729 [PMID: 31882232 DOI: 10.1016/j.clnu.2019.11.041]
60 **Baryah ANS**, Midha V, Mahajan R, Sood A. Impact of Corona Virus Disease-19 (COVID-19) pandemic on gastrointestinal disorders. *Indian J Gastroenterol* 2020; **39**: 214-219 [PMID: 32749642 DOI: 10.1007/s12664-020-01071-6]
61 **Remzi FH**, Panis Y, Spinelli A, Kotze PG, Mantzaris G, Söderholm JD, d'Hoore A, Bemelman WA, Yamamoto T, Pemberton JH, Tiret E, Øresland T, Fleshner P. International Organization for the Study of IBD Recommendations for Surgery in Patients With IBD During the Coronavirus Disease 2019 Pandemic. *Dis Colon Rectum* 2020; **63**: 870-873 [PMID: 32355056 DOI: 10.1097/DCR.0000000000001718]
62 **Esposito S**, Noviello S, Pagliano P. Update on treatment of COVID-19: ongoing studies between promising and disappointing results. *Infez Med* 2020; **28**: 198-211 [PMID: 32335561]
63 **Meo SA**, Klonoff DC, Akram J. Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19. *Eur Rev Med Pharmacol Sci* 2020; **24**: 4539-4547 [PMID: 32373993 DOI: 10.26355/eurrev_202004_21038]
64 **Lofgren SM**, Nicol MR, Bangdiwala AS, Pastick KA, Okafor EC, Skipper CP, Pullen MF, Engen NW, Abassi M, Williams DA, Nascene AA, Axelrod ML, Lother SA, MacKenzie LJ, Drobot G, Marten N, Cheng MP, Zarychanski R, Schwartz IS, Silverman M, Chagla Z, Kelly LE, McDonald EG, Lee TC, Hullsiek KH, Boulware DR, Rajasingham R. Safety of Hydroxychloroquine Among Outpatient Clinical Trial Participants for COVID-19. *Open Forum Infect Dis* 2020; **7**: ofaa500 [PMID: 33204764 DOI: 10.1093/ofid/ofaa500]
65 **Becker RC**. Covid-19 treatment update: follow the scientific evidence. *J Thromb Thrombolysis* 2020; **50**: 43-53 [PMID: 32338320 DOI: 10.1007/s11239-020-02120-9]
66 **Gao J**, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in

treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* 2020; **14**: 72-73 [PMID: 32074550 DOI: 10.5582/BST.2020.01047]

67 **Pascarella G**, Strumia A, Piliego C, Bruno F, Del Buono R, Costa F, Scarlata S, Agrò FE. COVID-19 diagnosis and management: a comprehensive review. *J Intern Med* 2020; **288**: 192-206 [PMID: 32348588 DOI: 10.1111/joim.13091]

68 **Song Y**, Zhang M, Yin L, Wang K, Zhou Y, Zhou M, Lu Y. COVID-19 treatment: close to a cure? *Int J Antimicrob Agents* 2020; **56**: 106080 [PMID: 32634603 DOI: 10.1016/j.ijantimicag.2020.106080]

69 **Fatima SA**, Asif M, Khan KA, Siddique N, Khan AZ. Comparison of efficacy of dexamethasone and methylprednisolone in moderate to severe covid 19 disease. *Ann Med Surg (Lond)* 2020; **60**: 413-416 [PMID: 33200031 DOI: 10.1016/j.amsu.2020.11.027]

70 **Mattos-Silva P**, Felix NS, Silva PL, Robba C, Battaglini D, Pelosi P, Rocco PRM, Cruz FF. Pros and cons of corticosteroid therapy for COVID-19 patients. *Respir Physiol Neurobiol* 2020; **280**: 103492 [PMID: 32659271 DOI: 10.1016/j.resp.2020.103492]

71 **Pani A**, Lauriola M, Romandini A, Scaglione F. Macrolides and viral infections: focus on azithromycin in COVID-19 pathology. *Int J Antimicrob Agents* 2020; **56**: 106053 [PMID: 32534189 DOI: 10.1016/j.ijantimicag.2020.106053]

72 **Cao B**, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, Li X, Xia J, Chen N, Xiang J, Yu T, Bai T, Xie X, Zhang L, Li C, Yuan Y, Chen H, Li H, Huang H, Tu S, Gong F, Liu Y, Wei Y, Dong C, Zhou F, Gu X, Xu J, Liu Z, Zhang Y, Shang L, Wang K, Li K, Zhou X, Dong X, Qu Z, Lu S, Hu X, Ruan S, Luo S, Wu J, Peng L, Cheng F, Pan L, Zou J, Jia C, Liu X, Wang S, Wu X, Ge Q, He J, Zhan H, Qiu F, Guo L, Huang C, Jaki T, Hayden FG, Horby PW, Zhang D, Wang C. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; **382**: 1787-1799 [PMID: 32187464 DOI: 10.1056/nejmoa2001282]

73 **Sultana J**, Cutroneo PM, Crisafulli S, Puglisi G, Caramori G, Trifirò G. Azithromycin in COVID-19 Patients: Pharmacological Mechanism, Clinical Evidence and Prescribing Guidelines. *Drug Saf* 2020; **43**: 691-698 [PMID: 32696429 DOI: 10.1007/s40264-020-00976-7]

74 **Hippensteel JA**, LaRiviere WB, Colbert JF, Langouët-Astrié CJ, Schmidt EP. Heparin as a therapy for COVID-19: current evidence and future possibilities. *Am J Physiol Lung Cell Mol Physiol* 2020; **319**: L211-L217 [PMID: 32519894 DOI: 10.1152/AJPLUNG.00199.2020]

75 **Cai Q**, Yang M, Liu D, Chen J, Shu D, Xia J, Liao X, Gu Y, Cai Q, Yang Y, Shen C, Li X, Peng L, Huang D, Zhang J, Zhang S, Wang F, Liu J, Chen L, Chen S, Wang Z, Zhang Z, Cao R, Zhong W, Liu Y, Liu L. Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. *Engineering (Beijing)* 2020; **6**: 1192-1198 [PMID: 32346491 DOI: 10.1016/j.eng.2020.03.007]

76 **Coomes EA**, Haghbayan H. Favipiravir, an antiviral for COVID-19? *J Antimicrob Chemother* 2020; **75**: 2013-2014 [PMID: 32417899 DOI: 10.1093/jac/dkaa171]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4172-4181

DOI: 10.3748/wjg.v27.i26.4172

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Adult pancreatoblastoma: Current concepts in pathology

Ayo O Omiyale

ORCID number: Ayo O Omiyale 0000-0001-9955-9207.

Author contributions: Omiyale AO reviewed the literature and wrote the manuscript.

Conflict-of-interest statement: The author declares no conflict of interest for this article.

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: United Kingdom

Peer-review report's scientific quality classification

Grade A (Excellent): A

Grade B (Very good): B

Ayo O Omiyale, Department of Cellular Pathology, Imperial College Healthcare NHS Trust, London W6 8RF, United Kingdom

Corresponding author: Ayo O Omiyale, MBChB, MPH, Doctor, Department of Cellular Pathology, Imperial College Healthcare NHS Trust, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, United Kingdom. ayodeji.omiyale@nhs.net

Abstract

Adult pancreatoblastoma is an exceptionally rare malignant tumour of the pancreas that mimics other solid cellular neoplasms of the pancreas, which may pose diagnostic difficulties. Because of its rarity, little is known about its clinical and pathologic features. This article reviews the clinical and pathologic features of pancreatoblastoma in adults including differential diagnosis, treatment, and follow-up. Although pancreatoblastoma commonly occurs in childhood, there have now been more than 70 adult pancreatoblastomas described in the literature. There is a slight male predominance. There are no symptoms unique to pancreatoblastomas and adult patients are frequently symptomatic. The most common presenting symptom is abdominal pain. Grossly, the tumours are often large and well-circumscribed. Microscopically, pancreatoblastomas are composed of neoplastic cells with predominantly acinar differentiation and characteristic squamoid nests. These tumours are positive for trypsin, chymotrypsin, lipase, and BCL10. Loss of heterozygosity on chromosome 11p is the most common molecular alteration in pancreatoblastomas. Adult pancreatoblastomas are aggressive tumours with frequent local invasion, recurrence, and distant metastasis. Treatment consists of surgical resection. Chemotherapy and radiotherapy may have a role in the treatment of recurrent, residual, unresectable, and metastatic disease. It is important to distinguish pancreatoblastomas from morphological mimics such as acinar cell carcinomas, solid pseudopapillary neoplasms, and pancreatic neuroendocrine neoplasms.

Key Words: Pancreas; Adult pancreatoblastoma; Pancreatic cancer; Solid pancreatic mass; Non-ductal pancreatic tumours

Core Tip: Adult pancreatoblastomas are extremely rare tumours of the pancreas. They are composed of neoplastic cells with multiple lines of differentiation and characteristic

Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: January 30, 2021
Peer-review started: January 30, 2021.
First decision: March 6, 2021
Revised: March 23, 2021
Accepted: June 22, 2021
Article in press: June 22, 2021
Published online: July 14, 2021

P-Reviewer: Kankaria J, Sergi C
S-Editor: Fan JR
L-Editor: A
P-Editor: Liu JH

squamoid nests. They mimic other neoplasms of the pancreas, which may give rise to diagnostic difficulties. This article provides an up-to-date review of the clinical and pathologic features of pancreatoblastoma in adults, including differential diagnosis, treatment, and follow-up.

Citation: Omiyale AO. Adult pancreatoblastoma: Current concepts in pathology. *World J Gastroenterol* 2021; 27(26): 4172-4181
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4172.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4172

INTRODUCTION

Pancreatoblastoma is a malignant epithelial neoplasm of the pancreas composed of cells with predominantly acinar differentiation and characteristic squamoid nests. Neuroendocrine, ductal and less commonly, mesenchymal differentiation can be seen but are often less extensive[1-3]. Less than 1% of pancreatic neoplasms are pancreatoblastomas[4,5]. Pancreatoblastoma commonly occurs in childhood, accounting for 25% of pancreatic neoplasms occurring in the first decade of life, with a mean age of approximately 4 years[1].

Adult pancreatoblastoma is extremely rare. Hence, little is known about its clinical and pathologic features. Furthermore, pre-operative diagnosis can be quite challenging because of the considerable overlap with other cellular neoplasms of the pancreas.

This article provides an up-to-date review of the clinical and pathologic features of pancreatoblastoma in adults, including cytology, molecular pathology, differential diagnosis, treatment, and follow-up.

EPIDEMIOLOGY

Adult pancreatoblastomas are exceptionally rare. To date, only 74 adult pancreatoblastomas have been reported in the literature, mostly in the form of isolated case reports and small series[6-10]. The mean age at diagnosis is 41 years (range, 18-78 years). There is a slight male predilection, with a male-to-female ratio of 1.2:1.

AETIOLOGY

The aetiology is unknown. Although most tumours are sporadic[11-15], few adult pancreatoblastomas have been described in the setting of familial adenomatous polyposis (FAP)[8,9]. Rare cases in children have been associated with Beckwith-Wiedemann syndrome[16,17].

CLINICAL PRESENTATION

Most patients are symptomatic, with very few cases discovered incidentally during routine examination and imaging[4,10,11]. There are no symptoms unique to pancreatoblastomas. The most common presenting symptom is abdominal pain[2]. Other clinical features include abdominal mass, weight loss, nausea, jaundice, and diarrhoea [6,14,15,18,19]. Rarely, patients may present with upper gastrointestinal bleeding[12].

Most adult pancreatoblastomas arise in the head of the pancreas. Of the 74 adult pancreatoblastomas described in the literature, localization data were available in 69 cases. The head of the pancreas was involved in 52.1% of cases (36 patients); the tail in 30.4% of cases (21 patients); the body in 14.5% of cases (10 patients); the body and tail in 1.5% of cases (1 patient); and the ampulla of vater in 1.5% of cases (1 patient).

Elevated serum levels of CA19-9[14,15] as well as corticotropin releasing hormone secretion[20] rarely occurs in adult pancreatoblastomas. Serum alpha fetoprotein

(AFP) is elevated in some pancreatoblastomas[4,7,13,21,22]. In addition, AFP may be detected immunohistochemically in tumours associated with elevated serum levels of AFP[3,4,22]. Serum AFP is frequently elevated in children[4,10,13] with levels often in excess of 1000 μg/L[3]. In contrast, AFP is not consistently elevated in adults[2,7,10,13]. When present, elevated serum AFP has been used as a marker of tumour recurrence or disease progression because AFP levels should decrease or normalize with successful treatment[7,13,21].

It is important to note that elevated AFP is not specific for pancreatoblastoma in a patient with a pancreatic mass. Pancreatic ductal adenocarcinomas[23] and pancreatic acinar cell carcinomas[24,25] have been associated with elevated serum AFP. Furthermore, AFP is widely used as a tumour marker for hepatocellular carcinoma (HCC). However, the limitations of AFP in detecting HCC includes the poor sensitivity in detecting small tumours and elevated levels of AFP in patients with chronic liver disease without HCC. To overcome this limitation, the *Lens culinaris* agglutinin-reactive AFP (AFP-L3) has been found to be highly specific and useful not only for early detection of HCC but also for predicting the risk of development of HCC in patients with chronic liver disease[26,27]. However, AFP-L3 or other isoforms of AFP are yet to be extensively studied in pancreatoblastomas.

Malignant behaviour is prominent in adult pancreatoblastomas. Approximately 59% of adult patients with pancreatoblastoma develop metastases at the time of diagnosis or afterwards in the course of the disease. The liver is the most common site of metastasis[2,12,15,28] followed by lymph nodes[4,5,29], and lung[4,5,7,15]. Chest wall[5], breast[15], bone[30], and brain metastases[31] are extremely rare. Tumours can invade adjacent structures such as the duodenum, spleen, common bile duct, portal vein, and superior mesenteric vessels[2,4,7,19,29].

IMAGING

There are no significant differences in the imaging findings of adult and paediatric patients[2,32]. Pancreatoblastomas are large well-defined heterogenous masses with low to intermediate signal intensity on T1-weighted images and high signal intensity on T2-weighted images. Enhancement is a common feature on contrast-enhanced computed tomography images and may be present on magnetic resonance imaging. Calcifications when present may be rim-like or clustered[19,33]. On ultrasound, pancreatoblastomas are well-demarcated solid masses inseparable from the pancreas with mixed echogenicity[33].

CYTOLOGY

Fine needle aspiration specimens are composed of cellular singly dispersed and/or clustered polygonal cells. The cells have round to oval nuclei with fine chromatin pattern, small indistinct nucleoli, and moderate amounts of amphophilic or eosinophilic cytoplasm[3,9]. Squamoid nests or corpuscles are best appreciated in cell block preparations. They are composed of plump epithelioid cells with abundant cytoplasm [7,9].

PATHOLOGY

Grossly, the tumours are solitary, solid, well-circumscribed, and often encapsulated. Pancreatoblastomas are usually large, averaging 8 cm in diameter (range, 1.8–30 cm)[2,3,6]. On cut section, the tumours have yellow to tan fleshy lobules separated by dense fibrous bands. Foci of haemorrhage and necrosis may be present. Rarely, pancreatoblastomas may undergo cystic change or show gross extension into the adjacent peripancreatic soft tissue[4].

Microscopically, pancreatoblastomas are composed of cellular well-delineated lobules separated by dense fibrous bands, often imparting a geographic low power appearance (Figure 1A). The dense fibrous bands between the lobules are composed of spindled cells with varying amounts of collagen (Figure 1B). Tumours predominantly show acinar differentiation; however, ductal, neuroendocrine and less commonly, mesenchymal differentiation may be present[1-3]. Solid areas with sheets of cells often alternate with areas with acinar differentiation. The acinar units comprise small cells

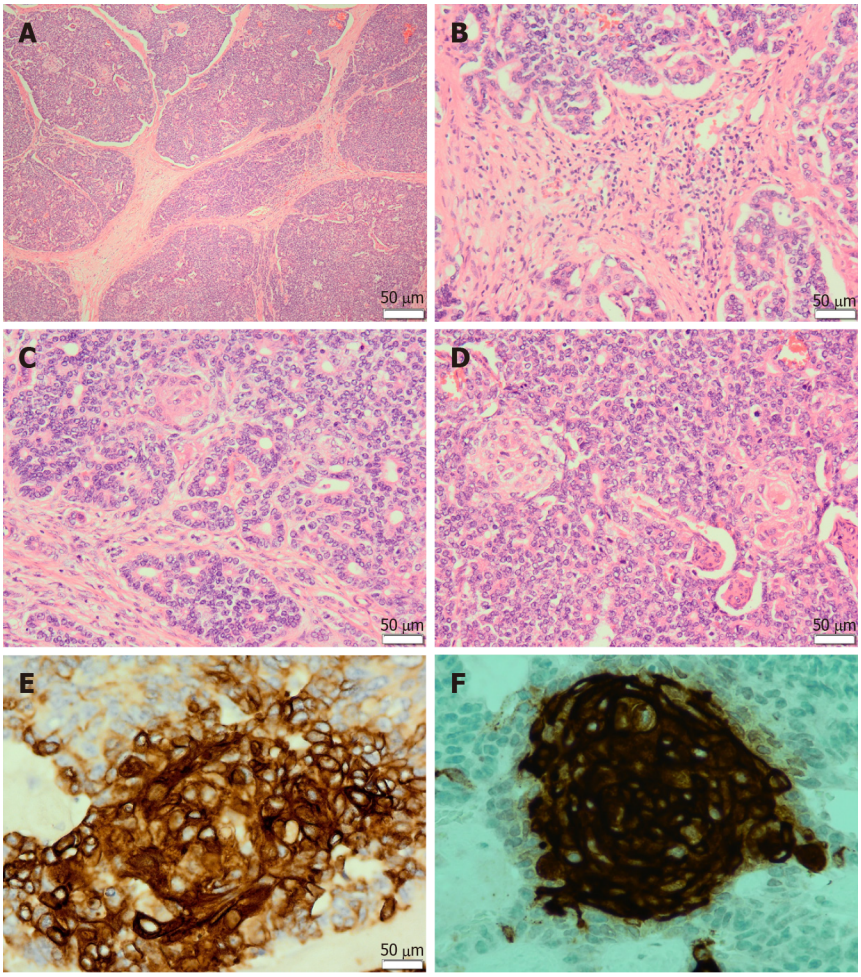

Figure 1 Pancreatoblastoma. A: The tumour is composed of lobules separated by dense fibrous bands, imparting a geographic low power appearance [Haematoxylin and Eosin (H&E) staining, 40 ×]; B: The dense fibrous bands between the lobules are composed of spindled cells with varying amounts of collagen (H&E staining, 200 ×); C: The tumour predominantly shows acinar differentiation. The acinar units are composed of neoplastic cells arranged around central lumina (H&E staining, 200 ×); D: The tumour shows characteristic squamoid nests. Squamoid nests are large islands of plump epithelioid cells with abundant eosinophilic cytoplasm (H&E staining, 200 ×); E: The squamoid nests are immunoreactive for AE1/AE3 (400 ×); F: The tumour shows immunolabeling for CD10 limited to the squamoid nests (400 ×).

with granular cytoplasm arranged around central lumina (Figure 1C). The cells have round to oval nuclei with single prominent nucleoli[1,3,4].

The defining histological feature of pancreatoblastoma is the squamoid nests. Squamoid nests vary from large islands of plump epithelioid cells to whorled nests of spindled cells showing mild to frank keratinization. The cells of the squamoid nests are often distinct from surrounding acinar cells. They are larger than surrounding cells with abundant eosinophilic to clear cytoplasm and without cytological atypia (Figure 1D). The amount of squamoid nests can vary both within and between tumours.

Pancreatoblastomas typically express trypsin, chymotrypsin, lipase, and BCL10. The granules are periodic acid-Schiff (PAS)-positive and resistant to diastase (PASD)[3,4]. Focal expression of chromogranin A and synaptophysin may be present. Squamoid nests may be positive for EMA, AE1/AE3 (Figure 1E) or CD10 (Figure 1F). In addition, patchy nuclear and cytoplasmic expression of β-catenin may be seen.

The staging of pancreatoblastoma follows the TNM classification of carcinoma of the exocrine pancreas[3].

MOLECULAR PATHOLOGY

Loss of heterozygosity on chromosome 11p is the most common molecular alteration in pancreatoblastomas, occurring in 86% of cases. Molecular alterations in the adenomatous polyposis coli (APC)/β-catenin signalling pathway have also been

identified in 67% of pancreatoblastomas, including biallelic inactivation of the *APC* gene and activating mutations of CTNNB1 (β-catenin) gene. Biallelic inactivation of the *APC* gene has been identified in a patient with pancreatoblastoma arising in the setting of FAP[8]. Interestingly, aberrations in the APC/β-catenin pathway have been implicated in the development of hepatoblastoma, a tumour associated with Beckwith-Wiedemann syndrome[8,34].

Recent RNA sequencing studies have identified molecular aberrations in the fibroblast growth factor receptor (FGFR) signalling pathway. These include somatic FGFR1 mutation, *FGFR2* gene rearrangement, and a high mRNA expression of fibroblast growth factor (FGF) receptors 1, 3 and 4 as well as of their ligands, FGF3 and FGF4[18].

The most frequent recurrent molecular alterations identified in pancreatic ductal adenocarcinomas, including mutations in *KRAS*, *TP53*, and *CDKN2A/p16* genes, are typically lacking in pancreatoblastomas, suggesting that pancreatoblastomas are genetically distinct from pancreatic ductal adenocarcinomas[8]. Loss of SMAD4/DPC4 expression is rare in pancreatoblastomas[8,35].

DIFFERENTIAL DIAGNOSIS

Pancreatoblastomas are distinct from the more common pancreatic ductal adenocarcinoma, and it is generally easy to differentiate them on the basis of morphology. The differential diagnosis of pancreatoblastoma includes solid cellular neoplasms of the pancreas such as acinar cell carcinomas, solid pseudopapillary neoplasms, and pancreatic neuroendocrine neoplasms (PanNENs).

There are a number of clinical and morphological similarities between acinar cell carcinomas and pancreatoblastomas. Acinar cell carcinomas are rare, accounting for 1%-2% of pancreatic neoplasms in adults and about 15% in children[1]. Acinar cell carcinomas have a poor prognosis, with a mean survival of 18-24 mo and a 3-year survival rate of 26%[36,37]. Both acinar cell carcinomas and pancreatoblastomas present with non-specific clinical symptoms such as abdominal pain, abdominal mass, and weight loss. In addition, both tumours are cellular neoplasms with acinar differentiation. Neoplastic cells are often polarized around central lumina. The cells contain PASD-positive cytoplasmic granules. Furthermore, acinar cell carcinomas and pancreatoblastomas are typically immunoreactive for trypsin, chymotrypsin, lipase, and BCL10. However, the distinguishing feature is the characteristic squamoid nests seen in pancreatoblastomas.

Solid pseudopapillary neoplasm of the pancreas is a low-grade malignant neoplasm characterized by cells with solid and pseudopapillary growth patterns. Approximately 1%-2% of pancreatic neoplasms are solid pseudopapillary neoplasms, and they frequently occur in girls and young women[3]. Microscopically, solid pseudopapillary neoplasms are composed of poorly cohesive monomorphic epithelial cells arranged around hyalinized fibrovascular stalks, forming solid and pseudopapillary structures. The nuclei frequently show indentations, clefts, and grooves. Typically, these tumours contain scattered PASD-positive hyaline globules, foamy histiocytes, cholesterol clefts, and foreign body giant cells[1]. Solid pseudopapillary neoplasms are positive for nuclear and/or cytoplasmic β-catenin, CD56, CD10, vimentin, and cyclin D1. Unlike pancreatoblastomas, the prognosis of solid pseudopapillary neoplasm of the pancreas is excellent.

PanNENs constitute about 2%-5% of pancreatic neoplasms[3]. They are architecturally diverse and can be confused with pancreatoblastomas. In addition, pancreatoblastomas can focally express neuroendocrine markers. In contrast, pancreatic neuroendocrine tumours are composed of cells with amphophilic to eosinophilic cytoplasm and the nuclei have characteristic salt and pepper chromatin. Typically, pancreatic neuroendocrine tumours strongly express synaptophysin, chromogranin A, and CD56. Features that favour a diagnosis of pancreatoblastoma include predominant acinar differentiation, squamoid nests, PASD-positive cytoplasmic granules, and expression of trypsin, chymotrypsin, lipase, and BCL10.

OUTCOME

There are no established treatment guidelines for pancreatoblastoma. Treatment consists of surgical resection with a variable combination of chemotherapy, radiotherapy, or targeted therapy (Table 1).

Table 1 Treatment and outcome of adult pancreatoblastoma

Ref.	Treatment	Follow-up (mo)	Outcome
Charlton-Ouw *et al*[39], 2008	Surgical resection, chemotherapy, RT	60	NED
Levey and Banner[40], 1996	Surgical resection	4	DOD
Palosaari *et al*[29], 1986	Surgical resection, chemotherapy, RT	15	AWD
Rajpal *et al*[13], 2006	Surgical resection, chemotherapy	17	DOD
Dunn and Longnecker[41], 1995	Surgical resection, chemotherapy	11	DFUD
Zhu *et al*[42], 2005	Chemotherapy	9	AWD
Du *et al*[14], 2003	Surgical resection	6	NED
Hoorens *et al*[43], 1994	Surgical resection	30	NED
Robin *et al*[44], 1997	Surgical resection, chemotherapy	7	DOD
Gruppioni *et al*[45], 2002	Surgical resection	10	NED
Benoist *et al*[12], 2001	Surgical resection, chemotherapy	36	NED
Mumme *et al*[46], 2001	Surgical resection, chemotherapy, RT	9	DOD
Salman *et al*[5], 2013	Surgical resection	30	NED
Salman *et al*[5], 2013	Surgical resection, chemotherapy, RT	41	NED
Salman *et al*[5], 2013	Surgical resection, chemotherapy, ablation of liver mets	51	DOD
Hayasaki *et al*[47], 1999	Surgical resection	15	NED
Sheng *et al*[48], 2005	Surgical resection, chemotherapy, RT, TACE	26	DOD
Balasundaram *et al*[15], 2012	Chemotherapy	1	DFUD
Klimstra *et al*[4], 1995	Surgical resection	5	NED
Klimstra *et al*[4], 1995	None	5	DOD
Klimstra *et al*[4], 1995	Surgical resection	10	DOD
Klimstra *et al*[4], 1995	Surgical resection	15	NED
Klimstra *et al*[4], 1995	Chemotherapy, RT	38	DOD
Rosebrook *et al*[32], 2005	Surgical resection	NA	NA
Montemarano *et al*[19], 2000	Surgical resection	NA	NA
Abraham *et al*[8], 2001	NA	NA	NA
Abraham *et al*[8], 2001	NA	NA	NA
Boix *et al*[20], 2010	Surgical resection	3	DOD
Pitman and Faquin[7], 2004	Surgical resection, chemotherapy, RT	108	AWD
Savastano *et al*[49], 2009	Surgical resection, chemotherapy, RT	NA	NED
Cavallini *et al*[10], 2009	Surgical resection	51	NED
Cavallini *et al*[10], 2009	Surgical resection	15	NED
Hammer and Owens[28], 2013	Surgical resection	NA	NA
Zhang *et al*[50], 2015	Surgical resection, chemotherapy	NA	NED
Ohike *et al*[51], 2008	Surgical resection	108	NED
Chen *et al*[52], 2018	Hepatic transarterial chemoembolization (TACE)	48	DOD
Yamaguchi *et al*[53], 2018	Surgical resection, chemotherapy	13	DOD
Nunes *et al*[54], 2018	Palliative care	3	DOD
Vilaverde *et al*[55], 2016	Surgical resection, chemotherapy	12	DOD
Zouros *et al*[30], 2015	Surgical resection, chemotherapy, RT	13	DOD
Kuxhaus *et al*[56], 2005	NA	NA	NA

Comper *et al*[57], 2009	Surgical resection	NA	NA
Comper *et al*[57], 2009	Surgical resection	NA	NA
Gringeri *et al*[58], 2012	Surgical resection, chemotherapy, stereotactic RT	44	NED
Redelman *et al*[59], 2014	Surgical resection	NA	NA
Tabusso *et al*[6], 2017	Surgical resection, chemotherapy, RT	10	AWD
Tabusso *et al*[6], 2017	Surgical resection	15	NED
Liu *et al*[60], 2020	Surgical resection	24	NED
Reid *et al*[9], 2019	NA	72.2	DOD
Reid *et al*[9], 2019	NA	17.9	AWD
Reid *et al*[9], 2019	NA	3.6	DOD
Reid *et al*[9], 2019	NA	85	DOD
Reid *et al*[9], 2019	NA	143.7	DOD
Reid *et al*[9], 2019	NA	13.6	NED
Reid *et al*[9], 2019	NA	0.8	DOD
Reid *et al*[9], 2019	NA	6.5	NED
Reid *et al*[9], 2019	NA	348	AWD
Reid *et al*[9], 2019	NA	88	AWD
Reid *et al*[9], 2019	NA	91	AWD
Terino *et al*[61], 2018	Chemotherapy	NA	NA
Morrissey et *al*[62], 2020	Surgical resection, chemotherapy	2	NED
Berger *et al*[8], 2020	Surgical resection, chemotherapy	18	DOD
Berger *et al*[8], 2020	Surgical resection, chemotherapy, RT, splenectomy	24	DOD
Berger *et al*[8], 2020	Surgical resection, chemotherapy, immunotherapy, RT	17	DOD
Berger *et al*[8], 2020	Chemotherapy, tyrosine kinase inhibitor therapy	15	DOD
Zhang *et al*[11], 2020	NA	NA	NA
Zhang *et al*[11], 2020	NA	NA	NA
Zhang *et al*[11], 2020	NA	NA	NA
Zhang *et al*[11], 2020	NA	NA	NA
Zhang *et al*[11], 2020	NA	NA	NA
Zhang *et al*[11], 2020	NA	NA	NA
Zhang *et al*[11], 2020	NA	NA	NA
Elghawy *et al*[31], 2021	Chemotherapy, autologous hematopoetic cell transplantation	57	AWD
Snyder *et al*[63], 2020	Surgical resection, chemotherapy, GKRS	63	NED

RT: Radiotherapy; GKRS: Gamma knife radiosurgery; DOD: Died of disease; AWD: Alive with disease; DFUD: Died from unrelated disease; NED: No evidence of disease; NA: Not available.

Of the 74 cases of adult pancreatoblastomas described in the literature, outcome data were available in 57 cases. The mean follow-up time was 36 mo (range, 0.8-348 mo). Forty-two percent (24 cases) of patients died of the disease at a mean interval of 27 mo (range, 0.8-143.7 mo); 4% (2 cases) of patients died from unrelated causes (cerebral haemorrhage and pulmonary artery embolus); 16% (9 cases) of patients were alive with disease; and 38% (22 cases) of patients had no evidence of disease (Table 1).

Although long-term survival has been observed in some adults, the prognosis of pancreatoblastoma in children may be more favourable than in adults[1,4,13,14]. Poor prognostic factors include the presence of metastases and unresectable disease[3]. Chemotherapy and radiotherapy may have a role in the treatment of recurrent, residual, unresectable and metastatic disease[3,38]. Because of the tendency for

recurrence and metastasis, long-term follow-up is advised for these patients[38].

CONCLUSION

In summary, adult pancreatoblastomas are extremely rare. Although these tumours typically occur in children, pancreatoblastomas should be considered in the differential diagnosis of solid pancreatic tumours in adults. An appreciation of distinctive squamoid nests, predominant acinar differentiation, and expression of trypsin, chymotrypsin, lipase, and BCL10 are important for the accurate diagnosis of pancreatoblastomas. These tumours are aggressive with frequent local invasion, recurrence, and distant metastasis. They must be distinguished from morphological mimics. There is a need for further research to better understand the molecular drivers of pancreatoblastomas, identify druggable molecular targets, and, most importantly, improve patient care.

REFERENCES

1 **Klimstra DS**. Nonductal neoplasms of the pancreas. *Mod Pathol* 2007; **20** Suppl 1: S94-S112 [PMID: 17486055 DOI: 10.1038/modpathol.3800686]

2 **Omiyale AO**. Clinicopathological review of pancreatoblastoma in adults. *Gland Surg* 2015; **4**: 322-328 [PMID: 26312218 DOI: 10.3978/j.issn.2227-684X.2015.04.05]

3 **Carneiro F**, Chan JKC, Cheung NYA. (Eds): WHO Classification of Tumors of the Digestive System. 5th edition. Lyon: International Agency for Research on Cancer, 2019

4 **Klimstra DS**, Wenig BM, Adair CF, Heffess CS. Pancreatoblastoma. A clinicopathologic study and review of the literature. *Am J Surg Pathol* 1995; **19**: 1371-1389 [PMID: 7503360 DOI: 10.1097/00000478-199512000-00005]

5 **Salman B**, Brat G, Yoon YS, Hruban RH, Singhi AD, Fishman EK, Herman JM, Wolfgang CL. The diagnosis and surgical treatment of pancreatoblastoma in adults: a case series and review of the literature. *J Gastrointest Surg* 2013; **17**: 2153-2161 [PMID: 24081396 DOI: 10.1007/s11605-013-2294-2]

6 **Tabusso FY**, Placencia RMF, Figueroa ER. Two cases of adult pancreatoblastoma: an infrequent differential diagnosis. JOP 2017. [cited 10 January 2021]. Available from: https://pancreas.imedpub.com/two-cases-of-adult-pancreatoblastoma-an-infrequent-differential-diagnosis.php?aid=19308

7 **Pitman MB**, Faquin WC. The fine-needle aspiration biopsy cytology of pancreatoblastoma. *Diagn Cytopathol* 2004; **31**: 402-406 [PMID: 15540188 DOI: 10.1002/dc.20128]

8 **Abraham SC**, Wu TT, Klimstra DS, Finn LS, Lee JH, Yeo CJ, Cameron JL, Hruban RH. Distinctive molecular genetic alterations in sporadic and familial adenomatous polyposis-associated pancreatoblastomas : frequent alterations in the APC/beta-catenin pathway and chromosome 11p. *Am J Pathol* 2001; **159**: 1619-1627 [PMID: 11696422 DOI: 10.1016/s0002-9440(10)63008-8]

9 **Reid MD**, Bhattarai S, Graham RP, Pehlivanoglu B, Sigel CS, Shi J, Saqi A, Shirazi M, Xue Y, Basturk O, Adsay V. Pancreatoblastoma: Cytologic and histologic analysis of 12 adult cases reveals helpful criteria in their diagnosis and distinction from common mimics. *Cancer Cytopathol* 2019; **127**: 708-719 [PMID: 31581358 DOI: 10.1002/cncy.22187]

10 **Cavallini A**, Falconi M, Bortesi L, Crippa S, Barugola G, Butturini G. Pancreatoblastoma in adults: a review of the literature. *Pancreatology* 2009; **9**: 73-80 [PMID: 19077457 DOI: 10.1159/000178877]

11 **Zhang X**, Ni SJ, Wang XH, Huang D, Tang W. Adult pancreatoblastoma: clinical features and Imaging findings. *Sci Rep* 2020; **10**: 11285 [PMID: 32647222 DOI: 10.1038/s41598-020-68083-2]

12 **Benoist S**, Penna C, Julié C, Malafosse R, Rougier P, Nordlinger B. Prolonged survival after resection of pancreatoblastoma and synchronous liver metastases in an adult. *Hepatogastroenterology* 2001; **48**: 1340-1342 [PMID: 11677959]

13 **Rajpal S**, Warren RS, Alexander M, Yeh BM, Grenert JP, Hintzen S, Ljung BM, Bergsland EK. Pancreatoblastoma in an adult: case report and review of the literature. *J Gastrointest Surg* 2006; **10**: 829-836 [PMID: 16769539 DOI: 10.1016/j.gassur.2005.11.011]

14 **Du E**, Katz M, Weidner N, Yoder S, Moossa AR, Shabaik A. Ampullary pancreatoblastoma in an elderly patient: a case report and review of the literature. *Arch Pathol Lab Med* 2003; **127**: 1501-1505 [PMID: 14567752 DOI: 10.5858/2003-127-1501-APIAEP]

15 **Balasundaram C**, Luthra M, Chavalitdhamrong D, Chow J, Khan H, Endres PJ. Pancreatoblastoma: a rare tumor still evolving in clinical presentation and histology. *JOP* 2012; **13**: 301-303 [PMID: 22572137]

16 **Koh TH**, Cooper JE, Newman CL, Walker TM, Kiely EM, Hoffmann EB. Pancreatoblastoma in a neonate with Wiedemann-Beckwith syndrome. *Eur J Pediatr* 1986; **145**: 435-438 [PMID: 3792392 DOI: 10.1007/BF00439255]

17 **Drut R**, Jones MC. Congenital pancreatoblastoma in Beckwith-Wiedemann syndrome: an emerging association. *Pediatr Pathol* 1988; **8**: 331-339 [PMID: 2845376 DOI: 10.3109/15513818809042976]

18 **Berger AK**, Mughal SS, Allgäuer M, Springfeld C, Hackert T, Weber TF, Naumann P, Hutter B, Horak P, Jahn A, Schröck E, Haag GM, Apostolidis L, Jäger D, Stenzinger A, Fröhling S, Glimm H, Heining C. Metastatic adult pancreatoblastoma: Multimodal treatment and molecular characterization of a very rare disease. *Pancreatology* 2020; **20**: 425-432 [PMID: 32156527 DOI: 10.1016/j.pan.2020.02.017]

19 **Montemarano H**, Lonergan GJ, Bulas DI, Selby DM. Pancreatoblastoma: imaging findings in 10 patients and review of the literature. *Radiology* 2000; **214**: 476-482 [PMID: 10671596 DOI: 10.1148/radiology.214.2.r00fe36476]

20 **Boix E**, Yuste A, Meana A, Alcaraz E, Payá A, Arnold C, Picó A, Lluis F. Corticotropin-releasing hormone-secreting pancreatoblastoma in an adult patient. *Pancreas* 2010; **39**: 938-939 [PMID: 20664482 DOI: 10.1097/MPA.0b013e3181d36444]

21 **Huang Y**, Yang W, Hu J, Zhu Z, Qin H, Han W, Wang H. Diagnosis and treatment of pancreatoblastoma in children: a retrospective study in a single pediatric center. *Pediatr Surg Int* 2019; **35**: 1231-1238 [PMID: 31338582 DOI: 10.1007/s00383-019-04524-y]

22 **Défachelles AS**, Martin De Lassalle E, Boutard P, Nelken B, Schneider P, Patte C. Pancreatoblastoma in childhood: clinical course and therapeutic management of seven patients. *Med Pediatr Oncol* 2001; **37**: 47-52 [PMID: 11466723 DOI: 10.1002/mpo.1162]

23 **Scheithauer W**, Chott A, Knoflach P. Alpha-fetoprotein-positive adenocarcinoma of the pancreas. *Int J Pancreatol* 1989; **4**: 99-103 [PMID: 2466920 DOI: 10.1007/BF02924151]

24 **Shinagawa T**, Tadokoro M, Maeyama S, Maeda C, Yamaguchi S, Morohoshi T, Ishikawa E. Alpha fetoprotein-producing acinar cell carcinoma of the pancreas showing multiple lines of differentiation. *Virchows Arch* 1995; **426**: 419-423 [PMID: 7541276 DOI: 10.1007/BF00191352]

25 **Kolb-van Harten P**, Rosien U, Klöppel G, Layer P. Pancreatic acinar cell carcinoma with excessive alpha-fetoprotein expression. *Pancreatology* 2007; **7**: 370-372 [PMID: 17703084 DOI: 10.1159/000107397]

26 **Hanaoka T**, Sato S, Tobita H, Miyake T, Ishihara S, Akagi S, Amano Y, Kinoshita Y. Clinical significance of the highly sensitive fucosylated fraction of α-fetoprotein in patients with chronic liver disease. *J Gastroenterol Hepatol* 2011; **26**: 739-744 [PMID: 21083609 DOI: 10.1111/j.1440-1746.2010.06585.x]

27 **Egashira Y**, Suganuma M, Kataoka Y, Higa Y, Ide N, Morishita K, Kamada Y, Gu J, Fukagawa K, Miyoshi E. Establishment and characterization of a fucosylated α-fetoprotein-specific monoclonal antibody: a potential application for clinical research. *Sci Rep* 2019; **9**: 12359 [PMID: 31451706 DOI: 10.1038/s41598-019-48821-x]

28 **Hammer ST**, Owens SR. Pancreatoblastoma: a rare, adult pancreatic tumor with many faces. *Arch Pathol Lab Med* 2013; **137**: 1224-1226 [PMID: 23991735 DOI: 10.5858/arpa.2013-0272-CR]

29 **Palosaari D**, Clayton F, Seaman J. Pancreatoblastoma in an adult. *Arch Pathol Lab Med* 1986; **110**: 650-652 [PMID: 3013120]

30 **Zouros E**, Manatakis DK, Delis SG, Agalianos C, Triantopoulou C, Dervenis C. Adult pancreatoblastoma: A case report and review of the literature. *Oncol Lett* 2015; **9**: 2293-2298 [PMID: 26137059 DOI: 10.3892/ol.2015.3001]

31 **Elghawy O**, Wang JS, Whitehair RM, Grosh W, Kindwall-Keller TL. Successful treatment of metastatic pancreatoblastoma in an adult with autologous hematopoietic cell transplant. *Pancreatology* 2021; **21**: 188-191 [PMID: 33199137 DOI: 10.1016/j.pan.2020.10.049]

32 **Rosebrook JL**, Glickman JN, Mortele KJ. Pancreatoblastoma in an adult woman: sonography, CT, and dynamic gadolinium-enhanced MRI features. *AJR Am J Roentgenol* 2005; **184**: S78-S81 [PMID: 15728031 DOI: 10.2214/ajr.184.3_supplement.01840s78]

33 **Lee JY**, Kim IO, Kim WS, Kim CW, Yeon KM. CT and US findings of pancreatoblastoma. *J Comput Assist Tomogr* 1996; **20**: 370-374 [PMID: 8626892 DOI: 10.1097/00004728-199605000-00007]

34 **Bläker H**, Hofmann WJ, Rieker RJ, Penzel R, Graf M, Otto HF. Beta-catenin accumulation and mutation of the CTNNB1 gene in hepatoblastoma. *Genes Chromosomes Cancer* 1999; **25**: 399-402 [PMID: 10398436]

35 **Jiao Y**, Yonescu R, Offerhaus GJ, Klimstra DS, Maitra A, Eshleman JR, Herman JG, Poh W, Pelosof L, Wolfgang CL, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N, Wood LD. Whole-exome sequencing of pancreatic neoplasms with acinar differentiation. *J Pathol* 2014; **232**: 428-435 [PMID: 24293293 DOI: 10.1002/path.4310]

36 **Klimstra DS**, Heffess CS, Oertel JE, Rosai J. Acinar cell carcinoma of the pancreas. A clinicopathologic study of 28 cases. *Am J Surg Pathol* 1992; **16**: 815-837 [PMID: 1384374 DOI: 10.1097/00000478-199209000-00001]

37 **La Rosa S**, Adsay V, Albarello L, Asioli S, Casnedi S, Franzi F, Marando A, Notohara K, Sessa F, Vanoli A, Zhang L, Capella C. Clinicopathologic study of 62 acinar cell carcinomas of the pancreas: insights into the morphology and immunophenotype and search for prognostic markers. *Am J Surg Pathol* 2012; **36**: 1782-1795 [PMID: 23026929 DOI: 10.1097/PAS.0b013e318263209d]

38 **Glick RD**, Pashankar FD, Pappo A, Laquaglia MP. Management of pancreatoblastoma in children and young adults. *J Pediatr Hematol Oncol* 2012; **34** Suppl 2: S47-S50 [PMID: 22525406 DOI: 10.1097/MPH.0b013e31824e3839]

39 **Charlton-Ouw KM**, Kaiser CL, Tong GX, Allendorf JD, Chabot JA. Revisiting metastatic adult pancreatoblastoma. A case and review of the literature. *JOP* 2008; **9**: 733-738 [PMID: 18981556]

40 **Levey JM**, Banner BF. Adult pancreatoblastoma: a case report and review of the literature. *Am J Gastroenterol* 1996; **91**: 1841-1844 [PMID: 8792711]

41 **Dunn JL**, Longnecker DS. Pancreatoblastoma in an older adult. *Arch Pathol Lab Med* 1995; **119**: 547-551 [PMID: 7605173]

42 **Zhu LC**, Sidhu GS, Cassai ND, Yang GC. Fine-needle aspiration cytology of pancreatoblastoma in a young woman: report of a case and review of the literature. *Diagn Cytopathol* 2005; **33**: 258-262 [PMID: 16138370 DOI: 10.1002/dc.20355]

43 **Hoorens A**, Gebhard F, Kraft K, Lemoine NR, Klöppel G. Pancreatoblastoma in an adult: its separation from acinar cell carcinoma. *Virchows Arch* 1994; **424**: 485-490 [PMID: 8032529 DOI: 10.1007/BF00191433]

44 **Robin E**, Terris B, Valverde A, Molas G, Belghiti J, Bernades P, Ruszniewski P. [Pancreatoblastoma in adults]. *Gastroenterol Clin Biol* 1997; **21**: 880-883 [PMID: 9587540]

45 **Gruppioni F**, Casadei R, Fusco F, Calculli L, Marrano D, Gavelli G. Adult pancreatoblastoma. A case report. *Radiol Med* 2002; **103**: 119-122 [PMID: 11859308]

46 **Mumme T**, Büttner R, Peiper C, Schumpelick V. [Pancreatoblastoma: a rare malignant neoplasm in early adulthood]. *Chirurg* 2001; **72**: 806-811 [PMID: 11490758 DOI: 10.1007/s001040170108]

47 **Hayasaki N**, Miyake N, Takahashi H, Nakamura E, Yamagishi S, Kuno Y, Mori N, Shinoda M, Kimura M, Suzuki T, Tashiro K. [A case of pancreatoblastoma in an adult]. *Nihon Shokakibyo Gakkai Zasshi* 1999; **96**: 558-563 [PMID: 10369002]

48 **Sheng L**, Weixia Z, Longhai Y, Jinming Y. Clinical and biologic analysis of pancreatoblastoma. *Pancreas* 2005; **30**: 87-90 [PMID: 15632705]

49 **Savastano S**, d'Amore ES, Zuccarotto D, Banzato O, Beghetto M, Famengo B. Pancreatoblastoma in an adult patient. A case report. *JOP* 2009; **10**: 192-195 [PMID: 19287117]

50 **Zhang D**, Tang N, Liu Y, Wang EH. Pancreatoblastoma in an adult. *Indian J Pathol Microbiol* 2015; **58**: 93-95 [PMID: 25673604 DOI: 10.4103/0377-4929.151199]

51 **Ohike N**, Yamochi T, Shiokawa A, Yoshida T, Yamazaki T, Date Y, Morohoshi T. A peculiar variant of pancreatoblastoma in an adult. *Pancreas* 2008; **36**: 320-322 [PMID: 18362850 DOI: 10.1097/MPA.0b013e31815842c0]

52 **Chen M**, Zhang H, Hu Y, Liu K, Deng Y, Yu Y, Wu Y, Qi A, Li Y, Wen G. Adult pancreatoblastoma: A case report and clinicopathological review of the literature. *Clin Imaging* 2018; **50**: 324-329 [PMID: 29753278 DOI: 10.1016/j.clinimag.2018.05.001]

53 **Yamaguchi S**, Fujii T, Izumi Y, Fukumura Y, Han M, Yamaguchi H, Akita T, Yamashita C, Kato S, Sekiya T. Identification and characterization of a novel *adenomatous polyposis coli* mutation in adult pancreatoblastoma. *Oncotarget* 2018; **9**: 10818-10827 [PMID: 29535845 DOI: 10.18632/oncotarget.24017]

54 **Nunes G**, Coelho H, Patita M, Barosa R, Pinto Marques P, Roque Ramos L, Brito MJ, Tomaz A, Fonseca J. Pancreatoblastoma: an unusual diagnosis in an adult patient. *Clin J Gastroenterol* 2018; **11**: 161-166 [PMID: 29285688 DOI: 10.1007/s12328-017-0812-6]

55 **Vilaverde F**, Reis A, Rodrigues P, Carvalho A, Scigliano H. Adult pancreatoblastoma - Case report and review of literature. *J Radiol Case Rep* 2016; **10**: 28-38 [PMID: 27761191 DOI: 10.3941/jrcr.v10i8.2737]

56 **Kuxhaus L**, Swayne LC, Chevinsky A, Samli B. Adult metastatic pancreaticoblastoma detected with Tc-99m MDP bone scan. *Clin Nucl Med* 2005; **30**: 577-578 [PMID: 16024962 DOI: 10.1097/01.rlu.0000170228.87136.a6]

57 **Comper F**, Antonello D, Beghelli S, Gobbo S, Montagna L, Pederzoli P, Chilosi M, Scarpa A. Expression pattern of claudins 5 and 7 distinguishes solid-pseudopapillary from pancreatoblastoma, acinar cell and endocrine tumors of the pancreas. *Am J Surg Pathol* 2009; **33**: 768-774 [PMID: 19194274 DOI: 10.1097/PAS.0b013e3181957bc4]

58 **Gringeri E**, Polacco M, D'Amico FE, Bassi D, Boetto R, Tuci F, Bonsignore P, Noaro G, D'Amico F, Vitale A, Feltracco P, Barbieri S, Neri D, Zanus G, Cillo U. Liver autotransplantation for the treatment of unresectable hepatic metastasis: an uncommon indication-a case report. *Transplant Proc* 2012; **44**: 1930-1933 [PMID: 22974875 DOI: 10.1016/j.transproceed.2012.06.032]

59 **Redelman M**, Cramer HM, Wu HH. Pancreatic fine-needle aspiration cytology in patients < 35-years of age: a retrospective review of 174 cases spanning a 17-year period. *Diagn Cytopathol* 2014; **42**: 297-301 [PMID: 24273058 DOI: 10.1002/dc.23070]

60 **Liu ET**, Liu C, Wang HL, Sun TT, Wang SX. Adult Pancreatoblastoma With Liver Metastasis Diagnosed by 18F-FDG PET/CT and 2 Years' Postoperative Follow-up. *Clin Nucl Med* 2020; **45**: e24-e28 [PMID: 31274558 DOI: 10.1097/RLU.0000000000002684]

61 **Terino M**, Plotkin E, Karagozian R. Pancreatoblastoma: an Atypical Presentation and a Literature Review. *J Gastrointest Cancer* 2018; **49**: 361-364 [PMID: 28194556 DOI: 10.1007/s12029-017-9925-x]

62 **Morrissey G**, Cohen P, Julve M. Rare case of adult pancreatoblastoma. *BMJ Case Rep* 2020; **13** [PMID: 32265211 DOI: 10.1136/bcr-2019-233884]

63 **Snyder MH**, Ampie L, Mandell JW, Helm GA, Syed HR. A Rare Case of Pancreatoblastoma with Intracranial Seeding. *World Neurosurg* 2020; **142**: 334-338 [PMID: 32622062 DOI: 10.1016/j.wneu.2020.06.210]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4182-4193

DOI: 10.3748/wjg.v27.i26.4182

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Prevention of vertical transmission of hepatitis B virus infection

Piero Veronese, Icilio Dodi, Susanna Esposito, Giuseppe Indolfi

ORCID number: Piero Veronese 0000-0002-4416-2269; Icilio Dodi 0000-0002-6772-4180; Susanna Esposito 0000-0003-4103-2837; Giuseppe Indolfi 0000-0003-3830-9823.

Author contributions: Veronese P and Indolfi G wrote the paper; Dodi I and Esposito S revised it critically for significant intellectual content.

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

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Italy

Piero Veronese, Susanna Esposito, Department of Medicine and Surgery, University of Parma, Parma 43121, Italy

Icilio Dodi, Department of Pediatrics, Pietro Barilla Children's Hospital, Parma 43121, Italy

Giuseppe Indolfi, Department Neurofarba, University of Florence, Florence 50129, Italy

Giuseppe Indolfi, Department Neurofarba, Meyer Children's University Hospital, Florence 50129, Italy

Corresponding author: Giuseppe Indolfi, MD, Professor, Department Neurofarba, University of Florence, Viale Gaetano Pieraccini, 24, Florence 50129, Italy. giuseppe.indolfi@meyer.it

Abstract

Hepatitis B virus (HBV) is the leading cause of chronic viral hepatitis. Annually, almost two million children younger than 5 years acquire the infection, mostly through vertical or horizontal transmission in early life. Vertical transmission of HBV is a high efficacy phenomenon ranging, in the absence of any preventive interventions, from 70% to 90% for hepatitis e antigen positive mothers and from 10% to 40% for hepatitis e antigen-negative mothers. Maternal viraemia is a preeminent risk factor for vertical transmission of HBV. Maternal screening is the first step to prevent vertical transmission of HBV. Hepatitis B passive and active immunoprophylaxis at birth together with antiviral treatment of highly viraemic mothers are the key strategies for global elimination of HBV infection. Strategies are needed to promote implementation of birth-dose vaccination and hepatitis B immunoglobulins in low- and middle-income countries where the prevalence of the infection is at the highest.

Key Words: Hepatitis B; Vertical transmission; Hepatitis B vaccine; Hepatitis B immune globulin; Neonatal immunoprophylaxis; Tenofovir alafenamide fumarate

Core Tip: Hepatitis B is one of the main causes of morbidity and mortality worldwide. Vertical transmission is the main transmission route, especially in areas with high prevalence of the infection. Maternal viraemia is a preeminent risk factor for vertical transmission of hepatitis B virus (HBV). Breastfeeding is recommended, although all the conditions leading to maternal-foetal microtransfusions with HBV-infected

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

Received: February 5, 2021
Peer-review started: February 5, 2021
First decision: March 14, 2021
Revised: March 29, 2021
Accepted: June 2, 2021
Article in press: June 2, 2021
Published online: July 14, 2021

P-Reviewer: Gao P, Okajima H, Rigopoulou EI, Xu XY
S-Editor: Gao CC
L-Editor: Filipodia
P-Editor: Liu JH

maternal blood increase the risk of vertical transmission. Neonatal immunoprophylaxis at birth represent the most important approach to prevent HBV infection. The aim of the present narrative review is to summarise the knowledge on prevention of vertical transmission of HBV infection.

Citation: Veronese P, Dodi I, Esposito S, Indolfi G. Prevention of vertical transmission of hepatitis B virus infection. *World J Gastroenterol* 2021; 27(26): 4182-4193
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4182.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4182

INTRODUCTION

Hepatitis B virus (HBV) is the leading cause of chronic viral hepatitis and a major cause of acute and chronic liver disease and associated morbidity and mortality worldwide[1]. According to the latest estimation, in 2016 there were 291 million people chronically infected with HBV in the world corresponding to a global prevalence of 3.9%. Annually, almost two million children younger than 5 years acquire the infection. The highest prevalence has been reported in Africa and in the Western Pacific area. In these regions the coverage with the birth vaccination dose is at the lowest, mostly through vertical transmission in early life[1]. Vertical transmission or infections acquired during early infancy are still responsible for most chronic HBV infections in adults, especially in the areas with high prevalence of the infection[2,3]. Hepatitis B passive and active immunoprophylaxis at birth together with antiviral treatment of highly viraemic mothers are the key strategies for global of HBV infection [4]. According to latest World Health Organization (WHO) estimates, the relative amount of children under 5 years of age chronically infected with HBV dropped to under 1% in 2019, down from around 5% in the pre-vaccine era[5]. In 2019, coverage of three doses of the vaccine reached 85% worldwide compared to around 30% in 2000. However, coverage of the hepatitis B vaccine birth dose remains uneven. Global coverage of the HBV birth dose is 43%, while coverage in the WHO African Region is only 6%[5].

Breast-feeding does not entail any additional risk of transmission in infants who receive a correct immunoprophylaxis[6]. The aim of the present narrative review is to summarise the knowledge on prevention of vertical transmission of HBV infection.

VERTICAL TRANSMISSION OF HBV: DEFINITION, TIMING AND TRANSMISSION RATE

Vertical transmission of HBV is defined as transmission occurring during pregnancy and in the perinatal period from the HBV-infected mother to the foetus or to the child, resulting in positivity at 6-12 mo of life of the hepatitis B surface antigen (HBsAg) or HBV DNA in infants[7]. Overall, vertical transmission of HBV is a high efficacy phenomenon ranging, in the absence of any preventive interventions, from 70% to 90% for hepatitis e antigen (HBeAg) positive mothers and from 10% to 40% for HBeAg-negative mothers. The high success rate of immunoprophylaxis provided to newborns in reducing the incidence of HBV transmission suggests that most vertical transmissions occur at or near the time of birth. Intrauterine infections take place in < 15% of pregnancies.

RISK FACTORS FOR VERTICAL TRANSMISSION OF HBV

Maternal viraemia, identified through the detection of HBV DNA or through the positivity of its surrogate markers HBsAg and HBeAg, is a preeminent risk factor for vertical transmission of HBV. HBeAg-positive mothers and mothers with high circulating concentrations of HBV DNA (> 10^6 IU/mL) have the highest risk of transmission[8,9]. All the conditions leading to maternal-foetal microtransfusions with HBV-infected maternal blood increase the risk of vertical transmission. Microtrans-

fusions could occur intrauterine, during labour, or at delivery. Placental leakages due to threatened preterm delivery or abortion, amniocentesis or chorionic villus sampling, and prolonged uterine contractions could be associated with maternal microtransfusions. The exposure of the neonate to the maternal HBV-infected cervical secretions and blood is possible during labour and delivery.

Mode of delivery

The mode of delivery has been examined as a potential risk factor for vertical transmission of HBV, but the resulting evidence is conflicting. In a large study from China, the effect of Caesarean section delivery on vertical transmission of HBV was evaluated in 1409 infants born to 1401 HBsAg-positive mothers of whom 61.5% (863 of 1401) had detectable levels of HBV DNA. All the children enrolled completed appropriate immunization against HBV. A lower vertical transmission rate was observed among infants in the group delivered by elective Caesarean section (1.4%) compared with that of those in the vaginal delivery group (3.4%). In the multivariate analysis, elective Caesarean section was beneficial for vertical transmission prevention only in mothers with maternal HBV DNA levels > 200000 IU/mL. In line with this study, two recent systematic reviews with meta-analysis showed that Caesarean section reduced the risk of vertical transmission in infants of HBeAg-positive mothers who did not receive antiviral therapy during pregnancy[10]. Other previous studies had contradictory results regarding the benefit of elective Caesarean section. Overall, there is no robust evidence to support Caesarean section as the mode of choice for the prevention of HBV transmission. The possible beneficial effect of Caesarean section should be weighed against the efficacy of the other well recognised practices for prevention of transmission, (*i.e.* antiviral therapy during pregnancy and passive and active immunoprophylaxis at birth). Thus far, regardless of viraemia, the mode of delivery of mothers with chronic HBV infection should follow the usual obstetric indications and is not influenced by the presence of the infection.

Amniocentesis and other obstetric procedures

Invasive diagnostic procedures during pregnancy, such as amniocentesis, occur before the timing for immunoprophylaxis and may favour the mixing of maternal and foetal blood. Different studies[11-15] conducted before the routine use of HBV viral load testing did not demonstrate an augmented risk for *in utero* infection after amniocentesis in women with chronic infection. In a recent study enrolling 642 consecutive Chinese infants born to HBsAg positive mothers without antiviral exposure and who completed appropriate immunization, 63 infants with amniocentesis were compared with 198 matched infants selected from the remaining 579 infants without amniocentesis. There was a significantly higher vertical transmission rate in infants with amniocentesis than in those without amniocentesis if the maternal HBV DNA levels were ≥ 2×10^6 IU/mL (50% *vs* 4.5%, respectively, $P = 0.006$). On the basis of this result, adequate counselling is advised for HBV-infected women who may necessitate invasive testing (*e.g.*, amniocentesis or chorionic villus sampling) including the possible increased risk for maternal-foetal transmission with HBV viral load ≥ 2×10^6 IU/mL[16].

All the procedures that break the skin and mucosal barrier including foetal scalp electrodes and blood sampling and vigorous suctioning of the newborn's airway at birth should be avoided. The risk of traumatizing the foetal skin is lower with vacuum extraction and forceps, and its use should follow obstetric indications.

Breastfeeding

We identified three major questions concerning breastfeeding and vertical transmission of HBV: (1) Does breastfeeding increase the risk of vertical transmission of HBV? (2) Does breastfeeding interfere with the immune response to vaccine? and (3) Is breastfeeding from HBV-infected mothers on antiviral treatment contraindicated? The role of breastfeeding in the transmission of hepatitis B has been discussed for many years. Examination of relevant studies indicates that there is no evidence that breastfeeding poses any additional risk to infants of HBV carrier mothers[17-19]. The risk of vertical transmission of HBV through breastfeeding is negligible if infants born to HBV-positive mothers who receive the hepatitis B immunoglobulins (HBIG)/hepatitis B vaccine at birth, and the benefits of breastfeeding outweigh any potential risk of infection. HBV infection should not be considered a contraindication to breastfeeding of infants who receive the HBIG and HBV vaccine[20]. Data are insufficient to say whether it is safe or not for the HBV-positive mother to breastfeed if her nipples are cracked and bleeding. Breastfeeding should be temporarily stopped to avoid any

potential exposure to blood, and once nipples are no longer cracked or bleeding, the HBV-positive mother may fully resume breastfeeding.

Wang *et al*[21] have showed that breastfeeding does not interfere with the immune response to the HBV vaccine. A total of 230 babies with HBV immunoprophylaxis at birth were followed up for 1 year in order to measure rates of anti-HBs antibodies at different ages. There were no significant differences in the incidence of immunoprophylaxis failure between breast-fed and formula-fed babies[21]. For mothers who received antivirals during pregnancy, the safety of continuing these drugs after delivery during breastfeeding has been and is a matter of concern and discussion. Although the risk of *in utero* exposure to drugs is likely higher than for infants through breast milk, antivirals are recommended for use during pregnancy but many experts remain concerned about long-term consequences of prolonged antiviral agent exposure in the neonate and of its possible impact on growth and development. However, breastfeeding is advantageous on many issues, especially in low-income countries where formula feeding is not widely available. Furthermore, in human-immunodeficiency setting, antiretroviral treatment could continue during the breast-feeding period in infected women. Only a small quantity of oral nucleoside analogues is secreted in breast milk[22], and the effect on bone growth of exposed children is not significantly different after a follow-up period[23]. In women treated with tenofovir, presence of the drug in breast milk has been reported, but its oral bioavailability is limited, and thus infants are exposed to only small concentrations. Current recommendations by the European Association for the Study of Liver Disease stated that breastfeeding is not contraindicated in HBV-positive mothers on tenofovir-based treatment or prophylaxis.

PREVENTION OF VERTICAL TRANSMISSION OF HBV: MANAGEMENT STRATEGIES DURING PREGNANCY

Maternal screening

The first step to prevent vertical transmission of HBV is to test all pregnant women in the first trimester in order to identify the best management strategy for mothers and the correct immunoprophylaxis schedule for future newborns[24]. In case of positive HBsAg, it is necessary to perform further investigations (hepatitis B core antibody, HBeAg, hepatitis B e antibody, serum aminotransferase levels, quantification of serum HBV DNA, liver imaging) to determine the woman's hepatitis B phase and therefore the possible requirement for treatment during or after pregnancy[25]. In HBsAg negative women with an increased risk of infection (infected partners, infected family members, at risk habits) the evaluation of maternal serological status should also be repeated when entering the hospital at the time of delivery.

In recent years there is a growing interest in new biomarkers of HBV infection, such as covalently-closed circular DNA (cccDNA), hepatitis B core-related antigen, and circulating HBV RNA. cccDNA is a key factor for the persistence of infection and represents a specific marker of replication[26] and was shown to persist in the liver, serum, and peripheral mononuclear cells[27].

Hepatitis B vaccination during pregnancy

Vaccination against HBV during pregnancy is safe and effective[28,29]. There is agreement that pregnant women who are not immune or infected with HBV, whether or not at high risk for HBV infection (as defined by having > one sex partner during the previous 6 mo, a current diagnosis of a sexually transmitted disease, having had an HBsAg-positive sex partner or a recent or current injection drug use), should be vaccinated[16,25]. Following the vaccination, maternal antibodies are passively transferred across the placenta to newborns, although without the active vaccination at birth, its titres rapidly wane over time[28]. Pregnant women can be considered HBV-immune when anti-HBs levels are higher than 10 mIU/mL. Sheffield *et al*[30] have shown that an accelerated vaccination schedule at 0, 1, and 4 mo in high-risk pregnant women is effective and well tolerated.

Hepatitis B immunoglobulin during pregnancy

The rationale behind the possible use of HBIG and/or of antiviral treatment during pregnancy is that up to 10% of infants born to HBV-infected mothers still have HBV infection despite receiving HBIG and HBV vaccine at birth. This suggests that additional interventions during the pre-birth phase could be favourable to decrease

the transmission rate.

HBIG is a purified solution of human immunoglobulin that could be administered to the mother, newborn, or both. When HBIG is administered to pregnant women, the antibodies passively diffuse across the placenta to the foetus. The maternal-foetal diffusion is maximal during the third trimester of pregnancy. Several studies have explored the efficacy of the administration of HBIG to HBV-infected pregnant women [31-34]. Unfortunately, the studies are quite heterogeneous in term of HBIG doses and routes of administration and of definitions of maternal and neonatal infection. A recent Cochrane review found varying effects of maternal antenatal HBIG in preventing vertical transmission of HBV. This review selected 36 trials originated from China including 6044 pregnant women who were HBsAg, HBeAg, or HBV DNA positive. Most of the trials (30/36; 83%) assessed HBIG 200 IU at 28, 32, and 36 wk of pregnancy. Serological signs of hepatitis B infection of the newborns were reported as HBsAg, HBeAg, and HBV DNA positive results at end of follow-up. Although, overall HBIG seemed to impact the HBsAg and HBV DNA status of the newborn, due to low quality evidence found in the review, the authors concluded for the uncertainty of the effect of benefit of antenatal HBIG administration to the HBV-infected mothers on newborn outcomes as compared with no intervention[35].

Antiviral treatment during pregnancy

The use of nucleoside or nucleotide analogues (lamivudine, telbivudine, or tenofovir [36-38]) during the last trimester of pregnancy in highly viraemic, HBeAg positive mothers, in combination with standard infant immune-prophylaxis, has been shown to be effective in further reducing the vertical transmission of HBV[36,37].

Antiviral treatment should be considered based on HBV DNA quantification, and it has been generally suggested in pregnant women with HBV DNA levels of more than 2×10^5 IU/mL. The appropriate time to start and stop antiretroviral drug in pregnant women is still debated. The aim of therapy is to reduce HBV DNA levels below the threshold of transmission or immunoprophylaxis failure at the time of delivery, and for this reason treatment is mainly started around 28 wk to 32 wk of gestation. Earlier may be beneficial and has been suggested for prevention of early placental infection and intrauterine transmission[39]. When the treatment is started only to prevent vertical transmission, it could be discontinued as early as at delivery or, as suggested by the major international societies, prolonged until 12 wk after delivery. While small amounts of drugs are usually present in breast milk, there is a potential risk of maternal hepatitis flare following the end of treatment, most of which are asymptomatic. However, there is no additional benefit in the aspect of hepatitis flare prevention in women who carry on treatment to 4 wk postpartum[40]. Close check of transaminase levels is needed after the end of treatment. Lamivudine[41], telbivudine [42], and tenofovir disoproxil fumarate[43] are the antiretroviral drugs that are considered safe to use during pregnancy. Telbivudine and lamivudine could significantly reduce transmission in infants compared with cases with no treatment, but both drugs have a low genetic barrier to resistance barrier. Therefore, tenofovir disoproxil fumarate is the treatment of choice for HBV-positive mothers because of its potent antiviral activity and high genetic barrier to resistance. Tenofovir alafenamide fumarate is a prodrug of tenofovir that can be administered at a lower dose compared with tenofovir disoproxil fumarate, as its active metabolite could be delivered to the target organs with lower circulating drug levels. The efficacy and safety of tenofovir alafenamide fumarate in HBV-infected pregnant women need to be evaluated before recommending it for use.

Treatment guidelines differ mainly with regard to the type of treatment, the threshold viraemia level, and timing for starting antiviral treatment. Consistency across the different guidelines seems a desirable and achievable target in order to standardise the global approach to mothers with HBV infection and antenatal prevention of vertical transmission.

Indications for treatment including which drug, the threshold of HBV DNA level, when to start, and when to stop treatment, as recommended by the main international scientific societies are summarised in Table 1[44]. Despite the different indications provided by the current guidelines, all societies agree to start antiviral treatment when HBV DNA levels are higher than 2×10^5 IU/mL, regardless of maternal serological status (HBeAg positive or negative).

In 2018, a large, double-blinded randomised placebo-controlled trial of tenofovir disoproxil fumarate given from 28 wk of gestational age to 8 wk postpartum to HBeAg-positive pregnant women with a mean HBV DNA of 10^8 IU/mL in Thailand, plus birth-dose vaccination and HBIG, did not find a significantly lower vertical transmission rate beyond the low rate already achieved in the comparison group that

Table 1 Recommendations for antiviral treatment in pregnant women with chronic hepatitis B virus infection

Societies	Antivirals	HBV-DNA level	When to start treatment	When to stop treatment
American Association for the Study of Liver Diseases[25]	Tenofovir disoproxil fumarate	> 2×10^5 IU/mL	28-32 wk	At birth to 3 mo
European Association for the Study of the Liver[24]	Tenofovir disoproxil fumarate	> 2×10^5 IU/mL	24-28 wk	Up to 12 wk after delivery
Asian Pacific Association for the Study of the Liver[70]	Tenofovir disoproxil fumarate, telbivudine	> 10^{6-7} IU/mL	28-32 wk	At delivery
Chinese Medical Association[71]	Tenofovir disoproxil fumarate, telbivudine, lamivudine	> 2×10^6 IU/mL	24-28 wk	At delivery
National Institute for Health and Care Excellence[72]	Tenofovir disoproxil fumarate	> 10^7 IU/mL	3rd trimester	4-12 wk after birth

HBV: Hepatitis B virus.

was given infant HBIG and HBV vaccination initiated at birth[45]. The study confirmed a significant drop at delivery of HBV DNA for the pregnant women treated with tenofovir. However, all infants received HBV vaccine and immunoglobulin at a mean time of 1.2 and 1.3 h after delivery, and the vertical transmission rate with the administration of HBIG and vaccine in the placebo group was low (2% instead of the expected 12%). Furthermore, mothers with signs of HBV-related liver disease (alanine aminotransferase > 30 IU/L) were excluded and both the tenofovir and the placebo groups consisted of mothers with low viral loads at baseline, possibly impacting the results of the study.

PREVENTION OF VERTICAL TRANSMISSION OF HBV: MANAGEMENT STRATEGIES AT BIRTH

Neonatal immunoprophylaxis: The birth vaccine dose

Post-exposure combined immunoprophylaxis through early administration of the first dose of vaccine and of HBIG is the most effective weapon to prevent vertical transmission of HBV. Without any preventative measures, the risk of vertical transmission for HBeAg-positive and HBeAg negative mothers ranges from 70% to 90% and from 10% to 40%, respectively[46]. The administration of HBV vaccine within 12 h of birth, followed by at least two more doses of vaccine within 6-12 mo[47], is 90%-95% effective in preventing vertical transmission[48,49]. If the administration of HBV vaccine is delayed until 48 h after birth, it would cause significant reduction in neonatal immunoprophylaxis efficacy. The recommendation by the WHO is to provide the first dose of hepatitis B vaccine as soon as possible after birth, preferably within 24 h[50], even in areas where HBV is of low endemicity. The combined approach with hepatitis B vaccine and HBIG at birth is not affordable in most of the endemic low and middle income countries. In these countries, considering the limited resources and the lack of access of HBIG, the WHO identifies HBV vaccination within 24 h of birth as the minimum intervention level and the main strategy to prevent infection[51]. In 2016 the coverage for the three-dose series of hepatitis B vaccine in infancy was estimated to be 84% (compared with 1% in 1990), and birth-dose coverage was estimated to be 39%.

Neonatal immunoprophylaxis: The combined vaccine and hepatitis B immunoglobulin approach

In addition to the HBV vaccination, providing a dose of HBIG at birth to the vaccinated infants can further reduce the risk of transmission, especially in highly viraemic mothers, to less than 5%[52-54]. This was first demonstrated by Wong and collaborators[55] in 1984 in a prospective study enrolling 189 infants who were randomly assigned to receive (1) vaccine at birth and at 1, 2, and 6 mo with seven monthly HBIG injections (100 IU); (2) the same vaccine schedule but only one HBIG injection at birth; (3) only the vaccine, at months 0, 1, 2, and 6; and (4) placebos for both vaccine and HBIG. In all three treatment groups, development of the persistent carrier state was significantly less frequent than in the placebo group (2.9%, 6.8%, 21%, and

73.2%, respectively). Vaccination alone was associated with a remarkable protection toward vertical transmission but was significantly less protective than vaccination plus multiple HBIG injections.

HBIG are obtained from plasma donors with high levels of anti-HBs antibodies. Standard immunoglobulins are not indicated for prevention of vertical transmission of HBV because they contain too low antibody titres against HBV. Timely administration of HBIG and hepatitis B vaccine is critical for interrupting vertical transmission[47]. The Centers for Disease Control and Prevention recommends that the birth dose of HBIG and hepatitis B vaccine be given within 12 h after birth through intra-muscular injection but in an anatomic site different from that of the vaccine[47,56,57]. The earlier the administration of HBIG, the higher is the efficacy of the intervention that is unlikely to exceed the 7th day of birth. After administration of HBV vaccination combined with HBIG, infection can still occur in 2%-10% of HBeAg-positive or highly viraemic mothers[8,45,58]. Failure of the vaccine and immune-prophylaxis regimen or transplacental or intrauterine infection could account for this[8,9,59]. HBeAg-positive mothers and mothers with high circulating levels of HBV DNA (> 10^6 IU/mL) have the highest risk of transmission[8,9]. The dose of HBIG generally used in infants is between 100 and 200 IU, corresponding to 30-40 IU/kg. It is important to note that the availability of HBIG in many countries, especially in those with low and middle income, that also have the higher endemicity is still low. The need for refrigerated storage, short shelf life, and low cost of the product should be addressed in order to make the use of HBIG feasible in all the different settings[60].

Specific indications for immunoprophylaxis according to the HBsAg status of the mother and the weight of the child

According to the Advisory Committee on Immunization Practice of the Center for Disease Control (ACIP-CDC) and the Committee on Infection Diseases of the American Academy of Pediatrics, the choice of the post-exposure immunoprophylaxis schedule is based on the mother's antigenic status (HBsAg) and the birth weight of the child (higher or lower than 2000 g)[47,61,62].

Infants born to HBsAg positive mothers

All newborns born to a mother with HBsAg must receive the birth dose of vaccine and HBIG within 12 h of birth regardless of the birth weight. The completion of HBV vaccine is different according to the birth weight. According to the ACIP-CDC, newborns of mothers with HBsAg test not available during pregnancy but with highly suggestive evidence of HBV infection (presence of HBV DNA, HBeAg-positive, or mother known to be chronically infected with HBV) must be considered as born to HBsAg positive mothers[47].

Infants born to women with unknow HBsAg status

Women with unknown HBsAg status at the time of delivery must be tested as soon as possible. In the meantime, newborns must receive the birth dose of the hepatitis B vaccine within 12 h of birth, regardless of birth weight. If the mother is positive, HBIG should be administered as soon as possible within 7 d of birth. If the mother is negative, the vaccination scheme should be completed as scheduled. In children weighing less than 2000 g, considering the potential reduced immunogenicity of the HBV vaccine in these children, it is recommended to administer HBIG within 12 h of birth even if the maternal status is still unknown. The vaccination schedule should be completed as indicated for HBsAg positive mothers[47].

Infants born to HBsAg negative mothers

The WHO Strategic Advisory Groups of Experts recommends that infants receive the HBV vaccine at birth, preferably within 24 h, but administration up to 7 d after birth followed by two or three additional doses can still be effective[63]. In the case of newborns weighing less than 2000 g, the first dose should be administered after 1 mo of life or at the discharge if this occurs earlier.

Completion of HBV vaccine series after the birth dose

The birth HBV vaccine dose should be followed by completion of a vaccine series. A study from the United States enrolling 17951 mother-infant pairs showed that the number of HBV vaccine doses was associated with risk of infant infection[64]. Overall, vertical HBV infection occurred among 1% of infants who received HBV vaccine and HBIG. Infection was detected in 6.7% (3 of 45 infants) of infants who received < three vaccine doses, compared with 1.1% (97 of 9207 infants) of infants who received ≥ three

doses. The ACIP recommends immunoprophylaxis consisting of hepatitis B vaccine and HBIG within 12 h of birth, followed by completion of an HBV vaccine series.

According to the indications from WHO, if the birth weight is more than 2000 g, the vaccination schedule must be completed with two or three more doses[60], starting within the 2nd month of life and administering the final dose after the 24th week of life (164 d). In case of birth weight less than 2000 g, the birth dose should not be considered as part of the vaccination schedule but three additional doses of vaccine will be required for a total of four, starting when the child has reached 1 mo of age[65, 66]. This recommendation is provided because some studies showed that seroconversion rates may decrease among infants with a birth weight < 2000 g after administration of hepatitis B vaccine at birth. However, within the 1st month of age, all medically stable preterm newborns, regardless of their initial birth weight or gestational age, are as likely to respond to HBV immunization as term and larger infants.

Testing infants for anti-HBs and HBsAg

Newborns to HBsAg positive mother should be tested after 1-2 mo from the final vaccine dose and normally at the age of 9-12 mo, through the evaluation of HBsAg and anti-HBs[67,68]. Test should not be executed before 9 mo of age to avoid detection of passive anti-HBs from HBIG administered at birth and to maximise the probability of detecting late HBV infection. Detection of anti-core antibodies is not recommended in infants born to HBsAg positive mothers because can be passively acquired and detected up to the age of 24 mo[47]. HBsAg negative and vaccinated children with anti-HBs titre greater than or equal to 10 mUI/mL have an adequate protection. If anti-HBs titres < 10 mIU/mL, a fourth additional dose should be administered and the test must be repeated after 1-2 mo. In case of persistence of anti-HBs < 10 mIU/mL after four vaccine doses, two additional doses for a total of six may be administered. The test should be repeated 1-2 mo after the sixth dose. In case of non-response, no further doses are expected[69].

CONCLUSION

Vertical transmission of HBV is the leading mode of acquisition of the infection worldwide. Prevention of vertical transmission is possible in the majority of cases through the correct administration of the birth dose of HBV vaccine and HBIG to the neonate. Strategies are needed to promote implementation of birth-dose vaccination and HBIG in low- and middle-income countries where the prevalence of the infection is at the highest. Breastfeeding should be encouraged as long as the infant receives immunoprophylaxis at birth. Further studies on the use of antivirals (tenofovir alafenamide and tenofovir disoproxil fumarate) during pregnancy are required to increase prevention of HBV infection and their effectiveness in preventing vertical HBV infection when used together with to early active and passive immunoprophylaxis.

REFERENCES

1 **Zhou YH**. Global prevalence of hepatitis B virus infection and prevention of mother-to-child transmission. *Lancet Gastroenterol Hepatol* 2018; **3**: 598 [PMID: 30102180 DOI: 10.1016/S2468-1253(18)30176-6]

2 **Schweitzer A**, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015; **386**: 1546-1555 [PMID: 26231459 DOI: 10.1016/S0140-6736(15)61412-X]

3 **Ott JJ**, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212-2219 [PMID: 22273662 DOI: 10.1016/j.vaccine.2011.12.116]

4 **World Health Organization**. Global hepatitis report, 2017. Global Hepatitis Programme, 2017

5 **World Health Organization**. Hepatitis B. [cited 5 February 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

6 **Tran TT**. Breastfeeding by mothers infected with hepatitis B carries no increased risk of transmission to infants who receive proper immunoprophylaxis: a meta-analysis. *Evid Based Med* 2012; **17**: 125-126 [PMID: 22187494 DOI: 10.1136/ebmed.2011.100378]

7 **Borgia G**, Carleo MA, Gaeta GB, Gentile I. Hepatitis B in pregnancy. *World J Gastroenterol* 2012; **18**: 4677-4683 [PMID: 23002336 DOI: 10.3748/wjg.v18.i34.4677]

8 **Lin X**, Guo Y, Zhou A, Zhang Y, Cao J, Yang M, Xiao F, Zhang B, Du Y. Immunoprophylaxis Failure Against Vertical Transmission of Hepatitis B Virus in the Chinese Population: A Hospital-based Study and a Meta-analysis. *Pediatr Infect Dis J* 2014; **33**: 897-903 [DOI: 10.1097/INF.0000000000000315]

9 **Wen WH**, Chang MH, Zhao LL, Ni YH, Hsu HY, Wu JF, Chen PJ, Chen DS, Chen HL. Mother-to-infant transmission of hepatitis B virus infection: significance of maternal viral load and strategies for intervention. *J Hepatol* 2013; **59**: 24-30 [PMID: 23485519 DOI: 10.1016/j.jhep.2013.02.015]

10 **Pan YC**, Jia ZF, Wang YQ, Yang N, Liu JX, Zhai XJ, Song Y, Wang C, Li J, Jiang J. The role of caesarean section and nonbreastfeeding in preventing mother-to-child transmission of hepatitis B virus in HBsAg-and HBeAg-positive mothers: results from a prospective cohort study and a meta-analysis. *J Viral Hepat* 2020; **27**: 1032-1043 [PMID: 32362050 DOI: 10.1111/jvh.13314]

11 **Ko TM**, Tseng LH, Chang MH, Chen DS, Hsieh FJ, Chuang SM, Lee TY. Amniocentesis in mothers who are hepatitis B virus carriers does not expose the infant to an increased risk of hepatitis B virus infection. *Arch Gynecol Obstet* 1994; **255**: 25-30 [PMID: 8042875 DOI: 10.1007/BF02390671]

12 **Chen LZ**, Zhou WQ, Zhao SS, Liu ZY, Wen SW. A nested case-control study of maternal-neonatal transmission of hepatitis B virus in a Chinese population. *World J Gastroenterol* 2011; **17**: 3640-3644 [PMID: 21987612 DOI: 10.3748/wjg.v17.i31.3640]

13 **Song YM**, Sung J, Yang S, Choe YH, Chang YS, Park WS. Factors associated with immunoprophylaxis failure against vertical transmission of hepatitis B virus. *Eur J Pediatr* 2007; **166**: 813-818 [PMID: 17120036 DOI: 10.1007/s00431-006-0327-5]

14 **Towers CV**, Asrat T, Rumney P. The presence of hepatitis B surface antigen and deoxyribonucleic acid in amniotic fluid and cord blood. *Am J Obstet Gynecol* 2001; **184**: 1514-1518; discussion 1518 [PMID: 11408875 DOI: 10.1067/mob.2001.114866]

15 **Gagnon A**, Davies G, Wilson RD; GENETICS COMMITTEE. Prenatal invasive procedures in women with hepatitis B, hepatitis C, and/or human immunodeficiency virus infections. *J Obstet Gynaecol Can* 2014; **36**: 648-653 [PMID: 25184985 DOI: 10.1016/S1701-2163(15)30546-6]

16 **Society for Maternal-Fetal Medicine (SMFM)**. Dionne-Odom J, Tita AT, Silverman NS. #38: Hepatitis B in pregnancy screening, treatment, and prevention of vertical transmission. *Am J Obstet Gynecol* 2016; **214**: 6-14 [PMID: 26454123 DOI: 10.1016/j.ajog.2015.09.100]

17 **Hill JB**, Sheffield JS, Kim MJ, Alexander JM, Sercely B, Wendel GD. Risk of hepatitis B transmission in breast-fed infants of chronic hepatitis B carriers. *Obstet Gynecol* 2002; **99**: 1049-1052 [PMID: 12052598 DOI: 10.1016/s0029-7844(02)02000-8]

18 **Beasley RP**, Stevens CE, Shiao IS, Meng HC. Evidence against breast-feeding as a mechanism for vertical transmission of hepatitis B. *Lancet* 1975; **2**: 740-741 [PMID: 52772 DOI: 10.1016/s0140-6736(75)90724-2]

19 **de Martino M**, Appendino C, Resti M, Rossi ME, Muccioli AT, Vierucci A. Should hepatitis B surface antigen positive mothers breast feed? *Arch Dis Child* 1985; **60**: 972-974 [PMID: 4062350 DOI: 10.1136/adc.60.10.972]

20 **World Health Organization**. Hepatitis B and Breastfeeding. [cited 5 February 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/maternal_child_adolescent/documents/pdfs/hepatitis_b_and_breastfeeding.pdf

21 **Wang JS**, Zhu QR, Wang XH. Breastfeeding does not pose any additional risk of immunoprophylaxis failure on infants of HBV carrier mothers. *Int J Clin Pract* 2003; **57**: 100-102 [PMID: 12661791]

22 **Benaboud S**, Pruvost A, Coffie PA, Ekouévi DK, Urien S, Arrivé E, Blanche S, Théodoro F, Avit D, Dabis F, Tréluyer JM, Hirt D. Concentrations of tenofovir and emtricitabine in breast milk of HIV-1-infected women in Abidjan, Cote d'Ivoire, in the ANRS 12109 TEmAA Study, Step 2. *Antimicrob Agents Chemother* 2011; **55**: 1315-1317 [PMID: 21173182 DOI: 10.1128/AAC.00514-10]

23 **Jacobson DL**, Patel K, Williams PL, Geffner ME, Siberry GK, DiMeglio LA, Crain MJ, Mirza A, Chen JS, McFarland E, Kacanek D, Silio M, Rich K, Borkowsky W, Van Dyke RB, Miller TL; Pediatric HIVAIDS Cohort Study. Growth at 2 Years of Age in HIV-exposed Uninfected Children in the United States by Trimester of Maternal Antiretroviral Initiation. *Pediatr Infect Dis J* 2017; **36**: 189-197 [PMID: 27798548 DOI: 10.1097/INF.0000000000001387]

24 **European Association for the Study of the Liver**. ; European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]

25 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

26 **Singh M**, Dicaire A, Wakil AE, Luscombe C, Sacks SL. Quantitation of hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) in the liver of HBV-infected patients by LightCycler real-time PCR. *J Virol Methods* 2004; **118**: 159-167 [PMID: 15081611 DOI: 10.1016/j.jviromet.2004.02.006]

27 **Shi X**, Wang X, Xu X, Feng Y, Li S, Feng S, Wang B, Wang S. Impact of HBV replication in peripheral blood mononuclear cell on HBV intrauterine transmission. *Front Med* 2017; **11**: 548-553 [PMID: 29170913 DOI: 10.1007/s11684-017-0597-5]

28 **Gupta I**, Ratho RK. Immunogenicity and safety of two schedules of Hepatitis B vaccination during pregnancy. *J Obstet Gynaecol Res* 2003; **29**: 84-86 [PMID: 12755527 DOI: 10.1046/j.1341-8076.2002.00076.x]

29 **Liu CJ**, Jeng YM, Chen CL, Cheng HR, Chen PJ, Chen TC, Liu CH, Lai MY, Chen DS, Kao JH.

Hepatitis B virus basal core promoter mutation and DNA load correlate with expression of hepatitis B core antigen in patients with chronic hepatitis B. *J Infect Dis* 2009; **199**: 742-749 [PMID: 19199543 DOI: 10.1086/596655]

30 **Sheffield JS**, Hickman A, Tang J, Moss K, Kourosh A, Crawford NM, Wendel GD Jr. Efficacy of an accelerated hepatitis B vaccination program during pregnancy. *Obstet Gynecol* 2011; **117**: 1130-1135 [PMID: 21508752 DOI: 10.1097/AOG.0b013e3182148efe]

31 **Li XM**, Shi MF, Yang YB, Shi ZJ, Hou HY, Shen HM, Teng BQ. Effect of hepatitis B immunoglobulin on interruption of HBV intrauterine infection. *World J Gastroenterol* 2004; **10**: 3215-3217 [PMID: 15457579 DOI: 10.3748/wjg.v10.i21.3215]

32 **Xiao XM**, Li AZ, Chen X, Zhu YK, Miao J. Prevention of vertical hepatitis B transmission by hepatitis B immunoglobulin in the third trimester of pregnancy. *Int J Gynaecol Obstet* 2007; **96**: 167-170 [PMID: 17296201 DOI: 10.1016/j.ijgo.2006.11.011]

33 **Yuan J**, Lin J, Xu A, Li H, Hu B, Chen J, Yao J, Dong H, Jiang M. Antepartum immunoprophylaxis of three doses of hepatitis B immunoglobulin is not effective: a single-centre randomized study. *J Viral Hepat* 2006; **13**: 597-604 [PMID: 16907846 DOI: 10.1111/j.1365-2893.2006.00738.x]

34 **Xu Q**, Xiao L, Lu XB, Zhang YX, Cai X. A randomized controlled clinical trial: interruption of intrauterine transmission of hepatitis B virus infection with HBIG. *World J Gastroenterol* 2006; **12**: 3434-3437 [PMID: 16733865 DOI: 10.3748/wjg.v12.i21.3434]

35 **Eke AC**, Eleje GU, Eke UA, Xia Y, Liu J. Hepatitis B immunoglobulin during pregnancy for prevention of mother-to-child transmission of hepatitis B virus. *Cochrane Database Syst Rev* 2017; **2**: CD008545 [PMID: 28188612 DOI: 10.1002/14651858.CD008545.pub2]

36 **Pan CQ**, Duan Z, Dai E, Zhang S, Han G, Wang Y, Zhang H, Zou H, Zhu B, Zhao W, Jiang H; China Study Group for the Mother-to-Child Transmission of Hepatitis B. Tenofovir to Prevent Hepatitis B Transmission in Mothers with High Viral Load. *N Engl J Med* 2016; **374**: 2324-2334 [PMID: 27305192 DOI: 10.1056/NEJMoa1508660]

37 **Zhang H**, Pan CQ, Pang Q, Tian R, Yan M, Liu X. Telbivudine or lamivudine use in late pregnancy safely reduces perinatal transmission of hepatitis B virus in real-life practice. *Hepatology* 2014; **60**: 468-476 [PMID: 25187919 DOI: 10.1002/hep.27034]

38 **Brown RS Jr**, McMahon BJ, Lok AS, Wong JB, Ahmed AT, Mouchli MA, Wang Z, Prokop LJ, Murad MH, Mohammed K. Antiviral therapy in chronic hepatitis B viral infection during pregnancy: A systematic review and meta-analysis. *Hepatology* 2016; **63**: 319-333 [PMID: 26565396 DOI: 10.1002/hep.28302]

39 **Sirilert S**, Tongsong T. Hepatitis B Virus Infection in Pregnancy: An Update on Evidence-Based Management. *Obstet Gynecol Surv* 2020; **75**: 557-565 [PMID: 32997148 DOI: 10.1097/OGX.0000000000000831]

40 **Nguyen V**, Tan PK, Greenup AJ, Glass A, Davison S, Samarasinghe D, Holdaway S, Strasser SI, Chatterjee U, Jackson K, Locarnini SA, Levy MT. Anti-viral therapy for prevention of perinatal HBV transmission: extending therapy beyond birth does not protect against post-partum flare. *Aliment Pharmacol Ther* 2014; **39**: 1225-1234 [PMID: 24666381 DOI: 10.1111/apt.12726]

41 **Xu WM**, Cui YT, Wang L, Yang H, Liang ZQ, Li XM, Zhang SL, Qiao FY, Campbell F, Chang CN, Gardner S, Atkins M. Lamivudine in late pregnancy to prevent perinatal transmission of hepatitis B virus infection: a multicentre, randomized, double-blind, placebo-controlled study. *J Viral Hepat* 2009; **16**: 94-103 [PMID: 19175878 DOI: 10.1111/j.1365-2893.2008.01056.x]

42 **Han LF**, Zheng JM, Zheng LQ, Gao HB, Chen LX, Xu QL, Chai YH, Zhang X, Pan C, Yao LF. Telbivudine can safely reduce mother-to-child transmission in chronic hepatitis B women after 12 wk of gestation. *BMC Infect Dis* 2019; **19**: 614 [PMID: 31299917 DOI: 10.1186/s12879-019-4250-6]

43 **Hyun MH**, Lee YS, Kim JH, Je JH, Yoo YJ, Yeon JE, Byun KS. Systematic review with meta-analysis: the efficacy and safety of tenofovir to prevent mother-to-child transmission of hepatitis B virus. *Aliment Pharmacol Ther* 2017; **45**: 1493-1505 [PMID: 28436552 DOI: 10.1111/apt.14068]

44 **Hou J**, Cui F, Ding Y, Dou X, Duan Z, Han G, Jia J, Mao Q, Li J, Li Z, Liu Z, Wei L, Xie Q, Yang X, Zhang H, Zhuang H. Management Algorithm for Interrupting Mother-to-Child Transmission of Hepatitis B Virus. *Clin Gastroenterol Hepatol* 2019; **17**: 1929-1936. e1 [PMID: 30312789 DOI: 10.1016/j.cgh.2018.10.007]

45 **Jourdain G**, Ngo-Giang-Huong N, Harrison L, Decker L, Khamduang W, Tierney C, Salvadori N, Cressey TR, Sirirungsi W, Achalapong J, Yuthavisuthi P, Kanjanavikai P, Na Ayudhaya OP, Siriwachirachai T, Prommas S, Sabsanong P, Limtrakul A, Varadisai S, Putiyanun C, Suriyachai P, Liampongsabuddhi P, Sangsawang S, Matanasarawut W, Buranabanjasatean S, Puernngooluerm P, Bowonwatanuwong C, Puthanakit T, Klinbuayaem V, Thongsawat S, Thanprasertsuk S, Siberry GK, Watts DH, Chakhtoura N, Murphy TV, Nelson NP, Chung RT, Pol S, Chotivanich N. Tenofovir *vs* Placebo to Prevent Perinatal Transmission of Hepatitis B. *N Engl J Med* 2018; **378**: 911-923 [PMID: 29514030 DOI: 10.1056/NEJMoa1708131]

46 **Thio CL**, Guo N, Xie C, Nelson KE, Ehrhardt S. Global elimination of mother-to-child transmission of hepatitis B: revisiting the current strategy. *Lancet Infect Dis* 2015; **15**: 981-985 [PMID: 26145195 DOI: 10.1016/S1473-3099(15)00158-9]

47 **Schillie S**, Vellozzi C, Reingold A, Harris A, Haber P, Ward JW, Nelson NP. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2018; **67**: 1-31 [PMID: 29939980 DOI: 10.15585/mmwr.rr6701a1]

48 **Zhang L**, Xu A, Yan B, Song L, Li M, Xiao Z, Xu Q, Li L. A significant reduction in hepatitis B

virus infection among the children of Shandong Province, China: the effect of 15 years of universal infant hepatitis B vaccination. *Int J Infect Dis* 2010; **14**: e483-e488 [PMID: 19939719 DOI: 10.1016/j.ijid.2009.08.005]

49 **Ni YH**, Huang LM, Chang MH, Yen CJ, Lu CY, You SL, Kao JH, Lin YC, Chen HL, Hsu HY, Chen DS. Two decades of universal hepatitis B vaccination in taiwan: impact and implication for future strategies. *Gastroenterology* 2007; **132**: 1287-1293 [PMID: 17433322 DOI: 10.1053/j.gastro.2007.02.055]

50 **Polaris Observatory Collaborators**. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* 2018; **3**: 383-403 [PMID: 29599078 DOI: 10.1016/S2468-1253(18)30056-6]

51 **World Health Organization**. Hepatitis B Control Through Immunization: A Reference Guide. [cited 5 February 2021]. In: World Health Organization [Internet]. Available from: http://iris.wpro.who.int/bitstream/handle/10665.1/10820/9789290616696_eng.pdf;jsessionid=FEDDD4672274909D7E6998B86BED45C8?sequence=3%0Ahttp://www.who.int/immunization/sage/meetings/2015/october/8_WPRO_Hepatitis_B_Prevention_Through_Immunization_Regional_R

52 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]

53 **Lo KJ**, Tsai YT, Lee SD, Yeh CL, Wang JY, Chiang BN, Wu TC, Yeh PS, Goudeau A, Coursaget P. Combined passive and active immunization for interruption of perinatal transmission of hepatitis B virus in Taiwan. *Hepatogastroenterology* 1985; **32**: 65-68 [PMID: 3159639]

54 **Lee C**, Gong Y, Brok J, Boxall EH, Gluud C. Effect of hepatitis B immunisation in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. *BMJ* 2006; **332**: 328-336 [PMID: 16443611 DOI: 10.1136/bmj.38719.435833.7C]

55 **Wong VC**, Ip HM, Reesink HW, Lelie PN, Reerink-Brongers EE, Yeung CY, Ma HK. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis-B vaccine and hepatitis-B immunoglobulin. Double-blind randomised placebo-controlled study. *Lancet* 1984; **1**: 921-926 [PMID: 6143868 DOI: 10.1016/S0140-6736(84)92388-2]

56 **Weinbaum CM**, Mast EE, Ward JW. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *Hepatology* 2009; **49**: S35-S44 [PMID: 19399812 DOI: 10.1002/hep.22882]

57 **Mast EE**, Weinbaum CM, Fiore AE, Alter MJ, Bell BP, Finelli L, Rodewald LE, Douglas JM Jr, Janssen RS, Ward JW; Advisory Committee on Immunization Practices (ACIP) Centers for Disease Control and Prevention (CDC). A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults. *MMWR Recomm Rep* 2006; **55**: 1-33; quiz CE1 [PMID: 17159833]

58 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Epidemiological serosurvey of hepatitis B in China--declining HBV prevalence due to hepatitis B vaccination. *Vaccine* 2009; **27**: 6550-6557 [PMID: 19729084 DOI: 10.1016/j.vaccine.2009.08.048]

59 **Chen HL**, Lin LH, Hu FC, Lee JT, Lin WT, Yang YJ, Huang FC, Wu SF, Chen SC, Wen WH, Chu CH, Ni YH, Hsu HY, Tsai PL, Chiang CL, Shyu MK, Lee PI, Chang FY, Chang MH. Effects of maternal screening and universal immunization to prevent mother-to-infant transmission of HBV. *Gastroenterology* 2012; **142**: 773-781. e2 [PMID: 22198276 DOI: 10.1053/j.gastro.2011.12.035]

60 **World Health Organization**. Guideline for the prevention, care and treatment of persons with chronic hepatitis B infection. World Health Organization, 2015: 124

61 **World Health Organization**. Preventing Perinatal Hepatitis B Virus Transmission: A Guide for Introducing and Strengthening Hepatitis B Birth Dose Vaccination. [cited 5 February 2021]. In: World Health Organization [Internet]. Available from: http://www.who.int/iris/handle/10665/208278

62 **Losonsky GA**, Wasserman SS, Stephens I, Mahoney F, Armstrong P, Gumpper K, Dulkerian S, West DJ, Gewolb IH. Hepatitis B vaccination of premature infants: a reassessment of current recommendations for delayed immunization. *Pediatrics* 1999; **103**: E14 [PMID: 9925860 DOI: 10.1542/peds.103.2.e14]

63 **World Health Organization**. Hepatitis B vaccines: WHO position paper – July 2017. [cited 5 February 2021]. In: World Health Organization [Internet]. Available from: https://www.who.int/immunization/policy/position_papers/hepatitis_b/en/

64 **Schillie S**, Walker T, Veselsky S, Crowley S, Dusek C, Lazaroff J, Morris SA, Onye K, Ko S, Fenlon N, Nelson NP, Murphy TV. Outcomes of infants born to women infected with hepatitis B. *Pediatrics* 2015; **135**: e1141-e1147 [PMID: 25896839 DOI: 10.1542/peds.2014-3213]

65 **Saari TN**; American Academy of Pediatrics Committee on Infectious Diseases. Immunization of preterm and low birth weight infants. American Academy of Pediatrics Committee on Infectious Diseases. *Pediatrics* 2003; **112**: 193-198 [PMID: 12837889 DOI: 10.1542/peds.112.1.193]

66 **World Health Organization**. HBV vaccination among low birth weight children (LBW). [cited 5 February 2021]. In: World Health Organization [Internet]. Available from: http://www.who.int/immunization/sage/meetings/2016/october/4_Systematic_review_of_safety_efficacy_hep_b.pdf?ua=1

67 **Schillie S**, Murphy TV, Fenlon N, Ko S, Ward JW. Update: Shortened Interval for Postvaccination

Serologic Testing of Infants Born to Hepatitis B-Infected Mothers. *MMWR Morb Mortal Wkly Rep* 2015; **64**: 1118-1120 [PMID: 26447601 DOI: 10.15585/mmwr.mm6439a6]

68 **Committee on Infectious Diseases**; Committee on Fetus and Newborn. Elimination of Perinatal Hepatitis B: Providing the First Vaccine Dose Within 24 Hours of Birth. *Pediatrics* 2017; **140** [PMID: 28847980 DOI: 10.1542/peds.2017-1870]

69 **Committee on Infectious Disease**. Hepatitis B. In: Kimberlin DW. Red Book. Committee on Infectious Disease, 2018: 401-428

70 **Sarin SK**, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016; **10**: 1-98 [PMID: 26563120 DOI: 10.1007/s12072-015-9675-4]

71 **Hou J**, Wang G, Wang F, Cheng J, Ren H, Zhuang H, Sun J, Li L, Li J, Meng Q, Zhao J, Duan Z, Jia J, Tang H, Sheng J, Peng J, Lu F, Xie Q, Wei L; Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases; Chinese Medical Association. Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update). *J Clin Transl Hepatol* 2017; **5**: 297-318 [PMID: 29226097 DOI: 10.14218/JCTH.2016.00019]

72 **National Institute for Health and Care Excellence**. Hepatitis B (chronic): diagnosis and management. Clinical guidelines [CG165]. [cited 5 February 2021]. In: National Institute for Health and Care Excellence [Internet]. Available from: https://www.nice.org.uk/guidance/cg165

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4194-4207

DOI: 10.3748/wjg.v27.i26.4194

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for pancreatic masses, subepithelial lesions, and lymph nodes

Irving Levine, Arvind J Trindade

ORCID number: Irving Levine 0000-0003-4922-1835; Arvind J Trindade 0000-0002-4132-0014.

Author contributions: Levine I and Trindade AJ contributed equally to this work; Levine I and Trindade AJ designed the research study; Levine I and Trindade AJ performed the research; Levine I and Trindade AJ analyzed the data and wrote the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: Trindade AJ has received research funding from Ninepoint Medical. Trindade AJ is a consultant for Pentax Medical and Olympus America.

Irving Levine, Arvind J Trindade, Division of Gastroenterology, Long Island Jewish Medical Center, Zucker School of Medicine at Hofstra/Northwell, Northwell Health System, New Hyde Park, NY 11040, United States

Corresponding author: Arvind J Trindade, MD, Associate Professor, Division of Gastroenterology, Long Island Jewish Medical Center, Zucker School of Medicine at Hofstra/Northwell, Northwell Health System, 270-05 76th Avenue, New Hyde Park, NY 11040, United States. arvind.trindade@gmail.com

Abstract

Endoscopic ultrasound tissue acquisition, in the form of both fine needle aspiration (EUS-FNA) and fine needle biopsy (EUS-FNB), is utilized for pancreatic mass lesions, subepithelial lesions, and lymph node biopsy. Both procedures are safe and yield high diagnostic value. Despite its high diagnostic yield, EUS-FNA has potential limitations associated with cytological aspirations, including inability to determine histologic architecture, and a small quantitative sample for further immunohistochemical staining. EUS-FNB, with its larger core biopsy needle, was designed to overcome these potential limitations. However, it remains unclear which technique should be used and for which lesions. Comparative trials are plagued by heterogeneity at every stage of comparison; including variable needles used, and different definitions of endpoints, which therefore limit generalizability. Thus, we present a review of prospective trials, systematic reviews, and meta-analyses on studies examining EUS-FNA *vs* EUS-FNB. Prospective comparative trials of EUS-FNA *vs* EUS-FNB primarily focus on pancreatic mass lesions, and yield conflicting results in terms of demonstrating the superiority of one method. However, consistent among trials is the potential for diagnosis with fewer passes, and a larger quantity of sample achieved for next generation sequencing. With regard to subepithelial lesions and lymph node biopsy, fewer prospective trials exist, and larger prospective studies are necessary. Based on the available literature, we would recommend EUS-FNB for peri-hepatic lymph nodes.

Key Words: Endoscopic ultrasound fine needle aspiration; Endoscopic ultrasound fine needle biopsy; Pancreatic lesions; Subepithelial lesions; Lymph node biopsy

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: United States

Peer-review report's scientific quality classification

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

Received: February 26, 2021
Peer-review started: February 26, 2021
First decision: April 18, 2021
Revised: April 28, 2021
Accepted: June 18, 2021
Article in press: June 18, 2021
Published online: July 14, 2021

P-Reviewer: Havre RF, Tomizawa M, Zhang Y
S-Editor: Zhang H
L-Editor: Webster JR
P-Editor: Zhang YL

Core Tip: Endoscopic ultrasound fine needle aspiration (EUS-FNA) and fine needle biopsy (EUS-FNB) provide two methods for endoscopic ultrasound tissue acquisition for pancreatic mass lesions, subepithelial lesions, and lymph node biopsy. Both methods are safe and provide high diagnostic yield. Prospective comparative trials of EUS-FNA *vs* EUS-FNB primarily focus on pancreatic lesions. EUS-FNB provides diagnostic accuracy with fewer needle passes, and may provide higher diagnostic yield for peri-hepatic lymph nodes.

Citation: Levine I, Trindade AJ. Endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for pancreatic masses, subepithelial lesions, and lymph nodes. *World J Gastroenterol* 2021; 27(26): 4194-4207
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4194.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4194

INTRODUCTION

As medical and surgical therapeutics continue to evolve, there is a renewed emphasis on timely diagnosis of various illnesses. This mantra certainly holds true in gastrointestinal (GI) diseases, including pancreatic malignancies and GI tumors, where early and specific diagnosis guides management and impacts morbidity and mortality [1]. While cross-sectional imaging can characterize lesions, a tissue diagnosis is often required for a definitive diagnosis prior to therapy[2-5]. Endoscopic ultrasound tissue acquisition (EUS-TA) has improved the ability for tissue diagnosis using a minimally invasive technique. The two modalities for EUS-TA, endoscopic ultrasound fine needle aspiration (EUS-FNA) and endoscopic ultrasound fine needle biopsy (EUS-FNB) vary in technique and utility. Common indications for EUS-TA include the diagnosis and staging of pancreaticobiliary and luminal GI malignancy, and assessing lymphadenopathy associated with luminal GI and lung cancers[6]. Additionally, EUS-TA aids in the evaluation of potentially neoplastic GI subepithelial lesions[6]. Comparative studies on the diagnostic ability of EUS-FNA and EUS-FNB have yielded conflicting results. Here we review prospective comparative data on EUS-FNA *vs* EUS-FNB for pancreatic masses, subepithelial lesions, and lymph node biopsy (Table 1-3).

EUS-FNA

EUS-FNA was first introduced in 1992. It is often combined with rapid onsite evaluation (ROSE) to improve diagnostic ability[7,8]. EUS-FNA is now standard of care for sampling pancreatic solid masses, subepithelial lesions, and lymph nodes, among others. The European Society of Gastroenterology and American Society of Gastroenterology recommend EUS-FNA as first line for diagnosing pancreatic lesions [9-11].

Marked variability exists in EUS-FNA equipment and technique. Several different needle sizes are available including 19 G, 20 G, 22 G, and 25 G. Additionally, variability exists in aspiration technique, including the use of negative pressure suction (used with either a 5 mL or 10 mL syringe) or slow stylet pull. The aspirate from EUS-FNA is often sufficient for cytology and adequate for diagnosis, with diagnostic accuracy ranging from 77% to 95% for pancreatic masses[9,10]. Given its minimally invasive technique and small needle size, EUS-FNA has low rates of morbidity[12].

However, several limitations exist for EUS-FNA which obtains a cytological specimen. EUS-FNA is limited by an inability to obtain histological architecture, and the inability to perform immunohistochemical analysis and molecular profiling. This is of particular importance as certain neoplasms, such as stromal cell tumors and lymphomas, may be difficult to diagnose without histologic samples, as their tissue architecture and morphology are essential for accurate pathologic assessment and histochemical studies[9,13-17]. Furthermore, with the increased attention on personalized or precision medicine in oncology, a sufficient tissue sample to perform next generation sequencing is required. Current National Comprehensive Cancer Network guidelines recommend germline testing for any patient with confirmed pancreatic

Table 1 Prospective comparative trials of endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for solid pancreatic mass lesions

Ref.	Study design	Number of subjects	Needle size (FNA, FNB)	Diagnostic yield/specimen adequacy (EUS-FNA *vs* EUS-FNB)	Diagnostic accuracy (EUS-FNA *vs* EUS-FNB)	Number of passes needed (EUS-FNA *vs* EUS-FNB)	Comments
Bang *et al*[9], 2012	RCT	56	22 G, 22 G Procore	66.7% *vs* 80% (NS)	N/A	1.61 *vs* 1.28 (NS)	
Aadam *et al*[30], 2015	RCT	73	Variable, variable	78.4% *vs* 91.7% (NS)	67.5% *vs* 83.3% (NS)	N/A	
Tian *et al*[31], 2018	RCT	36	22 G, 22 G ProCore	83.3% *vs* 83.3%	N/A	1.83 *vs* 1.11 (P = 0.049)	
Hedenstrom *et al* [33], 2018	RCT, crossover	68	25G, 22G reverse bevel Wilson Cook	N/A	78% *vs* 69% (NS)	N/A	In a subset of non-pancreatic adenocarcinoma, combined modality (EUS-FNA + FNB) was significantly higher compared to EUS-FNA alone
Oppong *et al*[34], 2020	RCT, crossover	108	Variable, variable Sharkcore	71% *vs* 82% (OR 3.23, sig)	64% *vs* 79% (OR 4.79, sig)	N/A	Shorter sampling time and pathology viewing time with EUS-FNB. Equivalent cost analysis.
Kandel *et al*[35], 2020	RCT, crossover	50	25 G, variable Sharkcore	100% *vs* 86% (NS)	100% *vs* 100%	N/A	Primary outcome of DNA concentration, significantly higher in EUS-FNB than in EUS-FNA
Wang *et al*[26], 2017	Meta-analysis	921	Variable, variable	81.4% *vs* 88.3% (OR 0.57, sig)	84.0% *vs* 87.8% (NS)	Fewer in EUS-FNB	
Li *et al*[27], 2018	Meta-analysis	1382	Variable, variable	82.3% *vs* 89.4% (OR 1.83, sig)	84.3% *vs* 89.6% (OR 1.62, sig)	Fewer in EUS-FNB	

EUS-FNA: Endoscopic ultrasound fine needle aspiration; EUS-FNB: Endoscopic ultrasound fine needle biopsy; RCT: Randomized controlled trial; N/A: Not applicable; NS: Not significant.

cancer using comprehensive gene panels for hereditary cancer syndromes, as well as tumor/somatic gene profiling for patients with locally advanced or metastatic disease to identify mutations that may benefit from anti-cancer therapy. Testing on tumor tissue is preferred; however, cell-free DNA testing can also be considered[18]. There is uncertainty whether EUS-FNA will be able to routinely provide adequate material for these studies[6].

EUS-FNB

In an attempt to overcome the limitations of EUS-FNA, EUS-FNB was first introduced in the early 2000s to obtain tissue specimens as opposed to aspiration-based cytology. With the goal of evaluating tissue core, EUS-FNB provided novel needles for improved diagnostic accuracy.

Table 2 Prospective comparative trials of endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for subepithelial lesions

Ref.	Study design	Number of subjects	Needle size (FNA, FNB)	Lesions sampled	Diagnostic yield/specimen adequacy (EUS-FNA *vs* EUS-FNB)	Diagnostic accuracy (EUS-FNA *vs* EUS-FNB)	Number of needle passes needed (EUS-FNA *vs* EUS-FNB)	Comments
Kim *et al*[47], 2014	RCT	22	22 G, 22 G Procore	All SELs	20% *vs* 75% (P = 0.01)	N/A	4 *vs* 2 (P = 0.025)	
Iwai *et al*[43], 2017	RCT, crossover	23	Variable, variable Procore	Gastric SELs	73.9% *vs* 91.3% (P = 0.12)	N/A	N/A	Histology positive significantly higher in EUS-FNB for 21 mm-30 mm lesions
Hedenstrom *et al*[48], 2018	RCT, crossover	70	Variable, variable reverse-bevel Wilson-Cook	All SELs	N/A	49% *vs* 83% (P < 0.001)	N/A	Extramural lesions lower sensitivity for EUS-FNA but not EUS-FNB)
Nagula *et al*[49], 2018	RCT	18	Variable, variable Procore	All SELs	83.3% *vs* 75% (NS)	N/A	2 *vs* 2 (NS)	

EUS-FNA: Endoscopic ultrasound fine needle aspiration; EUS-FNB: Endoscopic ultrasound fine needle biopsy; RCT: Randomized controlled trial; N/A: Not applicable; NS: Not significant; SELs: Subepithelial lesions.

Table 3 Prospective comparative trials of endoscopic ultrasound fine needle aspiration *vs* fine needle biopsy for lymph node biopsy

Ref.	Study design	Number of subjects	Needle size (FNA, FNB)	Lymph nodes sampled	Diagnostic yield/specimen adequacy (EUS-FNA *vs* EUS-FNB)	Diagnostic accuracy (EUS-FNA *vs* EUS-FNB)	Number of needle passes needed (EUS-FNA *vs* EUS-FNB)	Comments
Nagula *et al*[49], 2018)	RCT	46	Variable, variable Procore	All lymph nodes	92.9% *vs* 94.4% (NS)	N/A	2 *vs* 2 (NS)	
de Moura *et al*[52], 2020)	Retrospective study of prospectively collected data	209	Variable, variable	All lymph nodes	N/A	78.8% *vs* 83.2% (NS)	N/A	For peri-hepatic lesions, EUS-FNB was significantly more accurate

EUS-FNA: Endoscopic ultrasound fine needle aspiration; EUS-FNB: Endoscopic ultrasound fine needle biopsy; RCT: Randomized controlled trial; N/A: Not applicable; NS: Not significant.

Early models of EUS-FNB utilized Trucut needle biopsy, with a tissue penetrating stylet within an outer cannula. The 19 G Trucut FNB proved more accurate than EUS-FNA for diagnosing lymphomas and stromal tumors, but was limited by mechanical failure when attempting to biopsy pancreatic head masses and duodenal lesions due to the torqued echoendoscope and mechanical friction[16,19]. Newer models, including EchoTip HD ProCore™ (Wilson-Cook Medical Inc., Winston-Salem, NC, United States) is available in 19-25 G, and provides two cutting surfaces, a tip and reverse bevel, to further preserve histological architecture[1]. The reverse or opposing cutting bevel design of the EUS-FNB needle allows for the biopsy of core histopathologic tissue. This

aspect has the potential advantage of improving diagnostic performance, but also allowing a wide range of follow-up testing[20]. Immunohistochemistry, which is required for the diagnosis of autoimmune pancreatitis, lymphoma, and metastasis, can be performed on the tissue core. Furthermore, molecular analysis, which is now standard of care for pancreatic malignancies, can also be performed. Other needles, including SharkCore™ (Medtronic Inc., Sunnyvale, CA, United States) and Acquire™ (Boston Scientific, Malborough, MA, United States), (Figure 1) may achieve even higher diagnostic accuracy[21,22].

Studies have demonstrated high diagnostic yields of core specimens with EUS-FNB with fewer needle passes[23-25]. The potential concern for increased bleeding when using EUS-FNB is offset by the fewer passes required for diagnosis.

EUS-FNA *VS* EUS-FNB: OVERVIEW

Several comparative trials have evaluated EUS-FNA *vs* EUS-FNB. Interpreting the conflicting data is challenging, as trials are plagued by heterogeneity in every stage of comparison. Reported outcomes as well as definition of those outcomes vary between studies. For example, inconsistent use of the term "diagnostic accuracy" and "diagnostic adequacy" creates confusion. Furthermore, heterogeneity exists within equipment use (needle size), and technique (suction *vs* slow pull; specified number of passes). Additionally, designing strong randomized trials is limited by the inability to blind endosonographers, and sometimes cytopathologists, to the type of needle used [26-57].

METHODS

In compiling this review article, we performed a literature search utilizing PUBMED, EMBASE, and Google Scholar for comparative trials of EUS-FNA *vs* EUS-FNB for pancreatic mass lesions, subepithelial lesions, and lymph nodes. A total of 77 articles were identified. Trials were excluded if they were retrospective (n = 26), if they did not directly compare EUS-FNA and EUS-FNB (n = 18), or if they were incomplete manuscripts (n = 6). Any study performed on a variety of mass lesions without subcategories for the aforementioned groups was also excluded (n = 4).

PANCREATIC LESIONS

Pancreatic adenocarcinoma is characterized by a poor prognosis, with a 5-year survival rate of 5%-6%[27]. Pancreatic adenocarcinoma may be difficult to differentiate from other pancreatic mass lesions based on cross-sectional imaging and abdominal ultrasound[2-5]. The reported sensitivity of EUS in the detection of pancreatic cancer is between 94% and 100%[28]. Compared to computed tomography (CT), EUS can detect up to 14% of pancreatic tumors that were not visualized on CT, especially tumors smaller than 20 mm[11]. As such, EUS is currently the standard method for tissue diagnosis of pancreatic masses[11].

Tissue sampling of pancreatic mass lesions by EUS is vital in diagnosis. Several sampling approaches are possible depending on the location of the pancreatic mass lesion. A trans-duodenal approach may be optimal for lesions in the pancreatic head, while the transgastric approach is more appropriate for lesions in the pancreatic body and tail. Bang *et al*[29] proposed an algorithm for needle selection based on anatomical site; a 25 G needle for the trans-duodenal approach and a 22 G or 25 G for all other punctures.

Comparative trials of EUS-FNA *vs* EUS-FNB for pancreatic mass lesions focus mostly on safety, diagnostic accuracy, sample adequacy for diagnosis and further testing (Table 1).

Randomized controlled trials of pancreatic masses with EUS-FNA or EUS-FNB

Bang *et al*[9] performed the earliest randomized controlled trial (RCT) comparing EUS-FNA and EUS-FNB. The study randomized 56 patients to receive either EUS-FNA 22 G or EUS-FNB 22 G ProCore for pancreatic mass lesions, with the primary outcome being the number of passes required to establish a diagnosis with ROSE. They found no significant difference in the median number of passes required to establish on-site

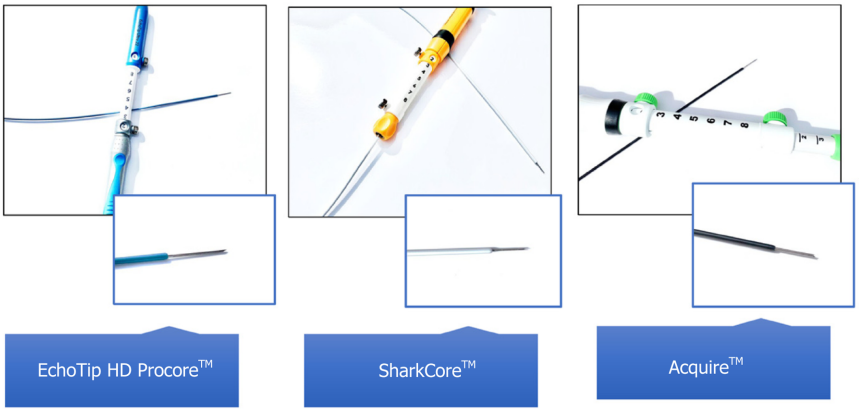

Figure 1 Fine needle biopsy needles.

diagnosis, and overall similar rates of diagnosis were achieved within 3 passes (100% EUS-FNA, 89% EUS-FNB). Incomplete diagnosis by EUS-FNB was due to diagnostic failure in two patients, and technical failure in 1 patient. Procedural complications among the two techniques were similar (one patient with post-procedural abdominal pain in the EUS-FNA cohort, and one patient with pancreatitis in the EUS-FNB cohort). With regard to secondary outcomes, EUS-FNA had a higher proportion of samples with histologic core tissue present (100% *vs* 88.3%, not-significant) but EUS-FNB had a higher percentage of histologic core tissue optimal for histochemical testing.

It is noteworthy that their technique varied from subsequent trials in several respects, and perhaps limited the study's generalizability. First, ROSE was carried out for all specimens, thereby possibly preferentially inflating the diagnostic ability of EUS-FNA. Additionally, they utilized an earlier model of FNB, the 22 G Echotip ProCore™ devise. Lastly, they utilized fewer needle movements for the EUS-FNB cohort (only 4 movements to and fro).

A subsequent larger RCT by Aadam *et al*[30] similarly showed no difference in diagnostic yield or specimen adequacy between EUS-FNA and EUS-FNB in patients with pancreatic lesions.

A 2018 RCT by Tian *et al*[31] of 36 patients similarly showed no superiority in diagnostic accuracy between EUS-FNA and EUS-FNB; although they did find a difference in the number of passes needed to make a diagnosis. Similar to Bang *et al*[9], patients were randomized to either EUS-FNA or EUS-FNB ProCore for solid pancreatic masses, although ROSE was not performed on any of the specimens. For the primary outcome of diagnostic yield, the authors found identical results (83%). However, among their secondary outcomes, EUS-FNB required fewer passes to make a diagnosis (1.11 *vs* 1.83, $P < 0.05$). It is noteworthy that a smaller percentage of their cohort were diagnosed with pancreatic adenocarcinoma (66.7%) compared to other trials. There were no complications in either cohort in their study.

Similar findings were also demonstrated in a larger, more recent RCT performed by Chen *et al*[32]. The authors randomized 235 patients with pancreatic mass lesions to EUS-FNA + ROSE (n = 120) *vs* EUS-FNB (22 G or 25 G Fork-tip needle, n = 115). For the primary outcome of diagnostic accuracy, the authors found no difference (92.2% *vs* 93.3%, respectively). However, among the secondary outcomes, EUS-FNB was associated with fewer needle passes to make a diagnosis compared to EUS-FNA + ROSE (2.3 *vs* 3.0) and decreased procedure time (19.3 min *vs* 22.7 min). There were no adverse events in the EUS-FNB cohort, and three adverse events in the EUS-FNA cohort (2 pancreatitis, 1 bleeding).

Crossover trials

In contradistinction to the aforementioned articles, in several trials patients underwent both EUS-FNA and EUS-FNB in a crossover study design, thereby allowing direct comparison between specimen procurement in the same patients and providing an internal control. Hedenstrom *et al*[33] randomized 68 patients with a pancreatic mass to receive either EUS-FNA (25 G) followed by EUS-FNB (22 G), 1 pass each, or vice versa. A reverse bevel EUS-FNB 22 G needle was used (Wilson-Cook Medical) and further passes were performed by alternating the two needles. They utilized similar

suction (10 cc) and fanning techniques for both EUS-FNA and EUS-FNB. ROSE was carried out for the majority of both EUS-FNA and EUS-FNB samples. The primary outcome of diagnostic accuracy was not significantly different between the two methods of tissue acquisition. No adverse events were recorded.

Utilizing a newer model of EUS-FNB Fork tip (SharkCore™ FNB Needle), Oppong *et al*[34] randomized 108 patients with pancreatic mass lesions to EUS-FNA and then EUS-FNB, 3 passes each, or vice versa. The primary endpoint was diagnostic performance for malignancy (malignant yes/no), compared to a gold standard of unequivocal malignant pathology obtained by EUS sampling, surgical resection, or alternative biopsy. For non-operated patients, clinical and radiological disease progression consistent with malignancy at 6-mo follow-up was required. The authors found increased sensitivity for the diagnosis of malignancy with EUS-FNB compared to EUS-FNA (82% *vs* 71%). The study was unique in that it also assessed procedural time and pathology viewing time, both of which were significantly shorter for EUS-FNB (710 s *vs* 759 s, $P = 0.001$; 188 s *vs* 332 s, $P < 0.001$, respectively). The authors performed a cost-analysis and found no significant difference; however, they analyzed only materials used and did not factor in operational/labor time. The authors reported four serious adverse events (2 cholangitis, 1 pancreatitis, 1 abdominal pain), but did not specify which cohorts the patients belonged to.

Other studies have utilized alternative endpoints to diagnostic accuracy or adequacy. As discussed previously, obtaining a diagnosis for pancreatic adenocarcinoma may still require further testing for personalized medicine, and therefore additional tissue may be required. Kandel *et al*[35] performed a RCT of 50 consecutive patients to assess adequacy for genomic profiling. In their study, they randomized patients to EUS-FNA followed by EUS-FNB (or vice versa) in a randomized order. They also utilized the SharkCore™ FNB needle. The first pass with each needle was used for histology, and subsequent passes were used to collect DNA. They found that EUS-FNB yielded significantly higher mean DNA concentrations compared to EUS-FNA (5.930 μg/mL *vs* 3.365 μg/mL, $P = 0.01$).

These findings have unclear clinical significance, since despite the quantitative difference in DNA acquired, both acquisition techniques yielded sufficient DNA for next generation sequencing (approximately 10 ng/μL). Furthermore, it is noteworthy that the EUS-FNA utilized a smaller needle (25 G) compared to both EUS-FNB needles (19 G or 22 G). This was likely done to maximize diagnostic accuracy, which was similar in both cohorts (100% final diagnosis in both), but may come at the expense of the DNA quantity acquired.

Systematic reviews and meta-analyses

Several systematic reviews and meta-analyses have attempted to summarize the conflicting data on pancreatic lesions. However, heterogeneity in the studies included and outcomes measured further perpetuate the confusion.

In 2017, Wang *et al*[26] performed a meta-analysis on 8 RCTs to determine diagnostic accuracy. Significant variability existed within needle size and suction technique between the trials. For diagnostic accuracy, they found no significant difference between EUS-FNA (84%) and EUS-FNB (88%, OR 0.72; 95%CI: 0.49-1.07). Among the 5 trials that reported specimen adequacy, and the four trials reporting the number of needle passes required, EUS-FNB demonstrated superiority (OR 0.57, 95%CI: 0.37-0.89; and OR 0.86, 95%CI: 0.45-1.26, respectively). Among the five studies that reported adverse events, the rates were low and not significantly different between the two groups (2/313 in the EUS-FNA group, and 4/311 in the EUS-FNB group), and specific complications were not mentioned.

One year later, in 2018, Li *et al*[27] performed a meta-analysis with the same 8 RCTs, and included an additional 3 RCTs, and yielded different results. They found that EUS-FNB had significantly better specimen adequacy (OR 1.83, 95%CI: 1.27-2.64), and higher diagnostic accuracy (OR 1.62, 95%CI: 1.17-2.26) than EUS-FNA, again with fewer needle passes (MD -0.69, 95%CI: -1.18 to -0.2). There was no difference in complications or technical success.

However, a larger 2019 meta-analysis by Facciorusso *et al*[20] of 27 RCTs found different results. They evaluated diagnostic accuracy, and found no significance difference between needle type (EUS-FNA or EUS-FNB) or needle size. The authors summarized the adverse events as rare among their studies; however, most studies did not itemize the etiology of the adverse events. The only studies that specifically reported bleeding episodes, all reported bleeding in the EUS-FNA cohort. Of note, the authors performed a network meta-analysis technique, thereby utilizing both direct RCT (EUS-FNA *vs* EUS-FNB) as well as indirect evidence (RCT of EUS-FNA *vs* EUS-FNA, or EUS-FNB *vs* EUS-FNB) and then extrapolated the data. Only 14 of the 27 trials

included were actually EUS-FNA *vs* EUS-FNB. As such, their results should be interpreted with caution.

Summary of pancreatic mass studies

Conflicting data exist among prospective studies evaluating the superiority of different EUS-TA techniques. Taken together, both methods provide overall high, and comparable, diagnostic accuracy and specimen adequacy for diagnosis. Adverse events, including bleeding, are rare in both techniques, with pancreatitis being the most common adverse event. Multiple trials have demonstrated that fewer passes are required for EUS-FNB compared to EUS-FNA. The ramifications of this, with the resulting decreased procedural time and likely fewer adverse events, may prove beneficial when applied broadly, but larger trials are required for further elucidation. Additionally, clinical benefit from the increased quantity of tissue obtained remains unclear, if standardized testing and next generation sequencing can be performed on all samples.

SUBEPITHELIAL LESIONS

Subepithelial lesions (SELs) of the GI tract are tumors that originate from the muscularis mucosa, submucosa, or muscularis propria[36]. Initial management of SELs focuses on proper diagnosis and determination of malignant potential, to guide further resection recommendations. EUS is the most accurate imaging method for evaluating SELs of the GI tract[37-39], because it can delineate the individual histologic layers and likely site of tumor origin. Certain SELs have a distinct endoscopic appearance, such as lipomas, duplication cysts, and ectopic pancreas, and endoscopic appearance may be considered diagnostic[36]. However, endoscopic appearance alone is not sufficient for diagnosis in many cases, such as hypoechoic and heterogeneous lesions from the submucosal and muscularis propria, and tissue acquisition is often required. Standard biopsy forceps and jumbo biopsy forceps (bite on bite technique) have low diagnostic yield[40,41].

EUS-FNA is the most widely used method for obtaining SEL tissue arising from the submucosal and muscularis propria layer[36]. However, the diagnostic accuracy of EUS-FNA is variable, ranging from 34% to 93%[39,42]. Additionally, the amount of cytological material obtained by EUS-FNA is often insufficient for the immunohistochemical staining required to differentiate different SELs[43].

Comparative trials

There are few prospective comparison trials of EUS-FNA and EUS-FNB focused solely on SELs, although several larger prospective trials contained cohorts of SELs (Table 2). We excluded trials that did not perform subgroup analysis on this SEL subgroup in isolation[30,44-46].

The first RCT focused solely on SELs was performed by Kim *et al*[47] in 2014. The authors randomized 22 patients with GI SELs of all types to either EUS-FNA (n = 10) or EUS-FNB (n = 12, ProCore). The patients did not receive both methods of tissue acquisition. The cohort was comprised of mostly gastric SELs (17/22), and mainly arising from the muscularis propria (20/22). The needle size was dependent on tumor diameter at the time of EUS, with a 22 G needle used if the tumor was estimated to be < 30 mm, and 19 G used if the tumor was > 30 mm. The authors utilized the unique endpoint of the number of passes required to obtain macroscopically optimal core samples. Since ROSE was not carried out at all sites, the endoscopist immediately inspected the material for the presence of tissue core, defined as whitish pieces of tissue with apparent bulk. If present, no further passes were obtained. However, if absent, the endoscopist proceeded with an additional pass with a maximum of 3 passes. If the sample still did not contain macroscopic tissue core, the number of passes was recorded at 4, and the patient crossed over to the other cohort. The authors found that the median number of needle passes required to obtain macroscopically optimal core sampled by EUS-FNB was significantly lower than that by EUS-FNA (2 *vs* 4, P = 0.025). Despite being macroscopically defined as optimal core samples, the core samples were suboptimal for microscopic analysis in three cases. Overall, the rates of obtaining macroscopically and histologically optimal core samples with EUS-FNB (92% and 75%, respectively) were superior to EUS-FNA (30% and 20%, respectively). No technical difficulties were encountered, and one patient in the entire cohort developed post-procedural bleeding which was managed conservatively. A limitation of the study design was lack of blinding of the endoscopist who assessed the primary

endpoint.

A follow-up study by Iwai *et al*[43] in 2017, focused solely on gastric SELs arising from the muscularis propria and randomized 24 patients to receive either EUS-FNA followed by EUS-FNB or vice versa. The two needles were used alternatively to puncture the same lesion with a total of four punctures per session. Similar to Kim *et al*[47], needle size was dependent on tumor size on EUS, and the ProCore needle was used for all EUS-FNB. The primary outcome was diagnostic yield. The authors found that the rate of correct diagnosis on immunohistochemical staining tended to be higher for EUS-FNB (91.3%) than for EUS-FNA (73.9%, P = 0.120), although this failed to reach statistical significance. When sub-characterized by tumor size, they found that EUS-FNB had significantly higher rates of positive histology among tumors 21-30 mm. The study was limited by sample size and was underpowered, as several of their findings trended towards significance.

A larger 2018 RCT performed by Hedenstrom *et al*[48] similarly found superiority of EUS-FNB to EUS-FNA for SELs, utilizing the reverse bevel ProCore EUS-FNB needle. The study randomized 70 patients with GI SELs to dual sampling with EUS-FNA and EUS-FNB in an alternating fashion until the yield was regarded as satisfactory by the cytotechnician, with a maximum of six passes. Similar to Iwai *et al*[43], in the absence of ROSE, gross examination was performed by the endoscopist. The cohort consisted of mostly gastric SELs (66/70). The study found significantly higher overall diagnostic accuracy for EUS-FNB than EUS-FNA (83% *vs* 49%, P < 0.001). A trend of lower sensitivity of EUS-FNA for extramural lesions compared to intramural lesions was also observed, a trend that did not exist for EUS-FNB. The authors hypothesized that this may be related to increased mobility of extramural lesions, preferentially affecting EUS-FNA diagnostic accuracy. The characterization of intramural and extramural was based on appearance at EUS. The authors reported few adverse events.

These findings are in contrast to Nagula *et al*[49] who found in the SELs cohort (n = 18) that there was no significant difference in diagnostic yield between EUS-FNB ProCore and EUS-FNB (EUS-FNB 75% *vs* EUS-FNA 83.3%, P = 0.754).

LYMPH NODES

Lymphadenopathy may arise from many different etiologies, ranging from benign inflammatory or infectious, to malignant etiologies. Evaluation of lymphadenopathy must include tissue sampling, as lymph node size has demonstrated poor specificity for differentiating malignant from benign lymphadenopathy[50,51]. Clarifying the malignant potential of lymphadenopathy is essential for clinical management[52].

The modality for sampling lymph nodes depends on anatomic location. For mediastinal lymph node sampling, EUS-TA is safer and less invasive compared to alternative techniques[10]. Additionally, for abdominal lymph nodes, EUS-sampling is successful in 92% of patients[53].

EUS-TA for lymph nodes is typically performed with EUS-FNA. However, the sensitivity of EUS-FNA for providing material for cytological evaluation is suboptimal, with reported rates of 88%-96%[54]. The suboptimal results are often attributed to damaged lymph node architecture[51,54]. This limitation of EUS-FNA is important in the evaluation of lymphadenopathy of unknown etiology, where the differential diagnosis includes lymphoma, metastasis, mycobacterial infection, and sarcoidosis, and core biopsy with preservation of lymph node architecture is particularly important for diagnostic purposes[53,55,56].

Comparative trials

We found no published prospective RCTs of EUS-FNA *vs* EUS-FNB for only lymph node biopsy. In the large RCT by Nagula *et al*[49] mentioned above, the subgroup of lymph node biopsies (n = 46) found no difference between EUS-FNA and EUS-FNB Procore in diagnostic yield (92.9% *vs* 94.4%) or number of passes needed to make a diagnosis (median 2, P = 0.43) (Table 3).

De Moura *et al*[52] performed a prospective study comparing EUS-FNA *vs* EUS-FNB exclusively for lymph node diagnosis. The authors performed an analysis on a prospectively collected database of 209 patients undergoing either EUS-FNA (n = 108) or EUS-FNB (n = 101) to evaluate lymph nodes. No predefined protocol was used in the study, and as such several different EUS-FNB needles were used including Acquire, SharkCore, and ProCore. The cohort consisted mostly of peri-hepatic lymph nodes (60%) followed by peri-pancreatic (10.4%) and mediastinal (10.4%), and were mostly accessed *via* a transgastric approach (45%). The pathology of most specimens

was benign (61%). Their primary outcome was diagnostic yield from cytological and histological analysis with and without immunohistochemical staining.

Overall, the authors found similar diagnostic accuracy between EUS-FNA and EUS-FNB (78.8% *vs* 83.2%, $P = 0.423$). However, the specificity for EUS-FNB demonstrated significant superiority (100% *vs* 93.62%, $P = 0.01$). In the subgroup analysis, EUS-FNB showed significantly higher sensitivity and specificity for abdominal lymph nodes. The diagnostic accuracy tended to be greater in the EUS-FNB cohort, but this failed to reach statistical significance. Following further analysis of lymph node location, EUS-FNB was associated with significantly higher sensitivity, specificity, and overall diagnostic accuracy for peri-hepatic lesions (88.9% *vs* 70.5%, $P = 0.038$).

Taken together, the study forms an important backdrop for further research, and argues for consideration of EUS-FNB over EUS-FNA for lymph node biopsy, specifically for peri-hepatic lesions.

UTILIZING BOTH TECHNIQUES

Additional studies have assessed the additive benefit of sampling lesions with both EUS-FNA and EUS-FNB. One such study by Hedenstrom *et al*[33] found that EUS-FNA/FNB compared to EUS-FNA alone had a higher diagnostic sensitivity for pancreatic tumors (89% *vs* 69%, $P = 0.02$), but not for pancreatic adenocarcinoma. However, compared to the diagnostic accuracy of EUS-FNB in isolation, Keswani *et al*[57] found no additional diagnostic accuracy by including EUS-FNA for pancreatic adenocarcinoma.

CONCLUSION

Endoscopic ultrasound tissue acquisition is routinely utilized in the evaluation of pancreatic mass lesions, subepithelial lesions, and lymph node biopsies. Ongoing confusion surrounds the ideal modality for EUS-TA, whether by EUS-FNA or EUS-FNB. While more robust comparative clinical trials exist for pancreatic lesions compared to subepithelial lesions and lymph nodes, the data continue to be mixed. Randomized controlled trials with homogenous populations and homogenous sampling protocols are needed in order to truly understand which needle is superior.

Based on the literature reviewed in this article, the authors conclude the following: EUS-FNA and EUS-FNB both provide high diagnostic accuracy, with low technical failure and adverse events, and thus either needle can be utilized for EUS-TA of pancreatic lesions, subepithelial lesions, and lymph nodes. In our experience we prefer FNB with a new generation needle as it allows us fewer passes of the needle, allows us to forgo ROSE which adds significant time and resources to a procedure, and gives a sample suitable for molecular testing. When increased quantity of DNA is desired for next generation sequencing, the utilization of EUS-FNB should be considered. For extramural subepithelial lesions, the utilization of EUS-FNB should be considered. Despite the dearth of prospective literature, we would recommend EUS-FNB for lymph node biopsy, specifically for peri-hepatic nodes.

REFERENCES

1 **Conti CB**, Cereatti F, Grassia R. Endoscopic ultrasound-guided sampling of solid pancreatic masses: the fine needle aspiration or fine needle biopsy dilemma. Is the best needle yet to come? *World J Gastrointest Endosc* 2019; **11**: 454-471 [PMID: 31523377 DOI: 10.4253/wjge.v11.i8.454]

2 **Brand B**, Pfaff T, Binmoeller KF, Sriram PV, Fritscher-Ravens A, Knöfel WT, Jäckle S, Soehendra N. Endoscopic ultrasound for differential diagnosis of focal pancreatic lesions, confirmed by surgery. *Scand J Gastroenterol* 2000; **35**: 1221-1228 [PMID: 11145297 DOI: 10.1080/003655200750056736]

3 **Delbeke D**, Pinson CW. Pancreatic tumors: role of imaging in the diagnosis, staging, and treatment. *J Hepatobiliary Pancreat Surg* 2004; **11**: 4-10 [PMID: 15747028 DOI: 10.1007/s00534-002-0775-x]

4 **Pannala R**, Hallberg-Wallace KM, Smith AL, Nassar A, Zhang J, Zarka M, Reynolds JP, Chen L. Endoscopic ultrasound-guided fine needle aspiration cytology of metastatic renal cell carcinoma to the pancreas: A multi-center experience. *Cytojournal* 2016; **13**: 24 [PMID: 27761149 DOI: 10.4103/1742-6413.192191]

5 **Rickes S**, Unkrodt K, Neye H, Ocran KW, Wermke W. Differentiation of pancreatic tumours by conventional ultrasound, unenhanced and echo-enhanced power Doppler sonography. *Scand J Gastroenterol* 2002; **37**: 1313-1320 [PMID: 12465731 DOI: 10.1080/003655202761020605]

6 **Wani S**, Muthusamy VR, McGrath CM, Sepulveda AR, Das A, Messersmith W, Kochman ML, Shah J. AGA White Paper: Optimizing Endoscopic Ultrasound-Guided Tissue Acquisition and Future Directions. *Clin Gastroenterol Hepatol* 2018; **16**: 318-327 [PMID: 29074447 DOI: 10.1016/j.cgh.2017.10.020]

7 **Hébert-Magee S**, Bae S, Varadarajulu S, Ramesh J, Frost AR, Eloubeidi MA, Eltoum IA. The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: a meta-analysis. *Cytopathology* 2013; **24**: 159-171 [PMID: 23711182 DOI: 10.1111/cyt.12071]

8 **Hikichi T**, Irisawa A, Bhutani MS, Takagi T, Shibukawa G, Yamamoto G, Wakatsuki T, Imamura H, Takahashi Y, Sato A, Sato M, Ikeda T, Hashimoto Y, Tasaki K, Watanabe K, Ohira H, Obara K. Endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic masses with rapid on-site cytological evaluation by endosonographers without attendance of cytopathologists. *J Gastroenterol* 2009; **44**: 322-328 [PMID: 19274426 DOI: 10.1007/s00535-009-0001-6]

9 **Bang JY**, Hebert-Magee S, Trevino J, Ramesh J, Varadarajulu S. Randomized trial comparing the 22-gauge aspiration and 22-gauge biopsy needles for EUS-guided sampling of solid pancreatic mass lesions. *Gastrointest Endosc* 2012; **76**: 321-327 [PMID: 22658389 DOI: 10.1016/j.gie.2012.03.1392]

10 **Dumonceau JM**, Deprez PH, Jenssen C, Iglesias-Garcia J, Larghi A, Vanbiervliet G, Aithal GP, Arcidiacono PG, Bastos P, Carrara S, Czakó L, Fernández-Esparrach G, Fockens P, Ginès À, Havre RF, Hassan C, Vilmann P, van Hooft JE, Polkowski M. Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline - Updated January 2017. *Endoscopy* 2017; **49**: 695-714 [PMID: 28511234 DOI: 10.1055/s-0043-109021]

11 **ASGE Standards of Practice Committee**, Eloubeidi MA, Decker GA, Chandrasekhara V, Chathadi KV, Early DS, Evans JA, Fanelli RD, Fisher DA, Foley K, Hwang JH, Jue TL, Lightdale JR, Pasha SF, Saltzman JR, Sharaf R, Shergill AK, Cash BD, DeWitt JM. The role of endoscopy in the evaluation and management of patients with solid pancreatic neoplasia. *Gastrointest Endosc* 2016; **83**: 17-28 [PMID: 26706297 DOI: 10.1016/j.gie.2015.09.009]

12 **Wang KX**, Ben QW, Jin ZD, Du YQ, Zou DW, Liao Z, Li ZS. Assessment of morbidity and mortality associated with EUS-guided FNA: a systematic review. *Gastrointest Endosc* 2011; **73**: 283-290 [PMID: 21295642 DOI: 10.1016/j.gie.2010.10.045]

13 **Bhutani MS**, Gress FG, Giovannini M, Erickson RA, Catalano MF, Chak A, Deprez PH, Faigel DO, Nguyen CC; No Endosonographic Detection of Tumor (NEST) Study. The No Endosonographic Detection of Tumor (NEST) Study: a case series of pancreatic cancers missed on endoscopic ultrasonography. *Endoscopy* 2004; **36**: 385-389 [PMID: 15100944 DOI: 10.1055/s-2004-814320]

14 **Jenssen C**, Dietrich CF. Endoscopic ultrasound-guided fine-needle aspiration biopsy and trucut biopsy in gastroenterology - An overview. *Best Pract Res Clin Gastroenterol* 2009; **23**: 743-759 [PMID: 19744637 DOI: 10.1016/j.bpg.2009.05.006]

15 **Levy MJ**. Endoscopic ultrasound-guided trucut biopsy of the pancreas: prospects and problems. *Pancreatology* 2007; **7**: 163-166 [PMID: 17592229 DOI: 10.1159/000104240]

16 **Levy MJ**, Wiersema MJ. EUS-guided Trucut biopsy. *Gastrointest Endosc* 2005; **62**: 417-426 [PMID: 16111962 DOI: 10.1016/j.gie.2005.04.044]

17 **Varadarajulu S**, Tamhane A, Eloubeidi MA. Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. *Gastrointest Endosc* 2005; **62**: 728-36; quiz 751, 753 [PMID: 16246688 DOI: 10.1016/j.gie.2005.06.051]

18 **Tempero MM**, M. Al-Hawary, M. Behrman, S. Benson III, Al. Cardin, D. CHa, C. Chiorean, E. Chung, V. Czito, B. Del Chiaro, M. Dillhoff, M. Donahue, T. Dotan, E. Ferrone, C. Fountzilas, C. Hardacre, J. Hawkins, W. Klute, K. Ko, A. LoConte, N. Lowy, A. Moravek, C. Nakakura, E. Narang, A. Obando, J. Polanco, P. Reddy, S. Reyngold, M. Scaife, C. Shen, J. Vollmer C. Wolff, R. Wolpin, B. NCCN Clinical Practice Guidelines in Oncology Pancreatic Adenocarcinoma Version 1.2021 2020. [cited 2021 January 1]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

19 **Larghi A**, Verna EC, Stavropoulos SN, Rotterdam H, Lightdale CJ, Stevens PD. EUS-guided trucut needle biopsies in patients with solid pancreatic masses: a prospective study. *Gastrointest Endosc* 2004; **59**: 185-190 [PMID: 14745390 DOI: 10.1016/S0016-5107(03)02538-0]

20 **Facciorusso A**, Wani S, Triantafyllou K, Tziatzios G, Cannizzaro R, Muscatiello N, Singh S. Comparative accuracy of needle sizes and designs for EUS tissue sampling of solid pancreatic masses: a network meta-analysis. *Gastrointest Endosc* 2019; **90**: 893-903. e7 [PMID: 31310744 DOI: 10.1016/j.gie.2019.07.009]

21 **Adler DG**, Muthusamy VR, Ehrlich DS, Parasher G, Thosani NC, Chen A, Buscaglia JM, Appannagari A, Quintero E, Aslanian H, Taylor LJ, Siddiqui A. A multicenter evaluation of a new EUS core biopsy needle: Experience in 200 patients. *Endosc Ultrasound* 2019; **8**: 99-104 [PMID: 29623911 DOI: 10.4103/eus.eus_53_17]

22 **DiMaio CJ**, Kolb JM, Benias PC, Shah H, Shah S, Haluszka O, Maranki J, Sharzehi K, Lam E, Gordon SR, Hyder SM, Kaimakliotis PZ, Allaparthi SB, Gress FG, Sethi A, Shah AR, Nieto J, Kaul V, Kothari S, Kothari TH, Ho S, Izzy MJ, Sharma NR, Watson RR, Muthusamy VR, Pleskow DK, Berzin TM, Sawhney M, Aljahdi E, Ryou M, Wong CK, Gupta P, Yang D, Gonzalez S, Adler DG. Initial experience with a novel EUS-guided core biopsy needle (SharkCore): results of a large North American multicenter study. *Endosc Int Open* 2016; **4**: E974-E979 [PMID: 27652304 DOI: 10.1055/s-0042-112581]

23 **Fabbri C**, Luigiano C, Maimone A, Tarantino I, Baccarini P, Fornelli A, Liotta R, Polifemo A, Barresi L, Traina M, Virgilio C, Cennamo V. Endoscopic ultrasound-guided fine-needle biopsy of small solid pancreatic lesions using a 22-gauge needle with side fenestration. *Surg Endosc* 2015; **29**: 1586-1590 [PMID: 25303907 DOI: 10.1007/s00464-014-3846-6]

24 **Iwashita T**, Nakai Y, Samarasena JB, Park DH, Zhang Z, Gu M, Lee JG, Chang KJ. High single-pass diagnostic yield of a new 25-gauge core biopsy needle for EUS-guided FNA biopsy in solid pancreatic lesions. *Gastrointest Endosc* 2013; **77**: 909-915 [PMID: 23433596 DOI: 10.1016/j.gie.2013.01.001]

25 **Larghi A**, Iglesias-Garcia J, Poley JW, Monges G, Petrone MC, Rindi G, Abdulkader I, Arcidiacono PG, Costamagna G, Biermann K, Bories E, Doglioni C, Dominguez-Muñoz JE, Hassan C, Bruno M, Giovannini M. Feasibility and yield of a novel 22-gauge histology EUS needle in patients with pancreatic masses: a multicenter prospective cohort study. *Surg Endosc* 2013; **27**: 3733-3738 [PMID: 23644834 DOI: 10.1007/s00464-013-2957-9]

26 **Wang J**, Zhao S, Chen Y, Jia R, Zhang X. Endoscopic ultrasound guided fine needle aspiration versus endoscopic ultrasound guided fine needle biopsy in sampling pancreatic masses: A meta-analysis. *Medicine (Baltimore)* 2017; **96**: e7452 [PMID: 28700483 DOI: 10.1097/MD.0000000000007452]

27 **Li H**, Li W, Zhou QY, Fan B. Fine needle biopsy is superior to fine needle aspiration in endoscopic ultrasound guided sampling of pancreatic masses: A meta-analysis of randomized controlled trials. *Medicine (Baltimore)* 2018; **97**: e0207 [PMID: 29595661 DOI: 10.1097/MD.0000000000010207]

28 **Guedes HG**, Moura DTH, Duarte RB, Cordero MAC, Santos MELD, Cheng S, Matuguma SE, Chaves DM, Bernardo WM, Moura EGH. A comparison of the efficiency of 22G versus 25G needles in EUS-FNA for solid pancreatic mass assessment: A systematic review and meta-analysis. *Clinics (Sao Paulo)* 2018; **73**: e261 [PMID: 29451621 DOI: 10.6061/clinics/2018/e261]

29 **Bang JY**, Ramesh J, Trevino J, Eloubeidi MA, Varadarajulu S. Objective assessment of an algorithmic approach to EUS-guided FNA and interventions. *Gastrointest Endosc* 2013; **77**: 739-744 [PMID: 23369651 DOI: 10.1016/j.gie.2012.11.029]

30 **Aadam AA**, Wani S, Amick A, Shah JN, Bhat YM, Hamerski CM, Klapman JB, Muthusamy VR, Watson RR, Rademaker AW, Keswani RN, Keefer L, Das A, Komanduri S. A randomized controlled cross-over trial and cost analysis comparing endoscopic ultrasound fine needle aspiration and fine needle biopsy. *Endosc Int Open* 2016; **4**: E497-E505 [PMID: 27227104 DOI: 10.1055/s-0042-106958]

31 **Tian L**, Tang AL, Zhang L, Liu XW, Li JB, Wang F, Shen SR, Wang XY. Evaluation of 22G fine-needle aspiration (FNA) versus fine-needle biopsy (FNB) for endoscopic ultrasound-guided sampling of pancreatic lesions: a prospective comparison study. *Surg Endosc* 2018; **32**: 3533-3539 [PMID: 29404729 DOI: 10.1007/s00464-018-6075-6]

32 **Chen YI**, Chatterjee A, Berger R, Kanber Y, Wyse J, Lam E, Gan I, Auger M, Kenshil S, Telford J, Donnellan F, Quinlan J, Lutzak G, Alshamsi F, Parent J, Waschke K, Alghamdi A, Barkun J, Metrakos P, Chaudhury P, Martel M, Dorreen A, Candido K, Miller C, Adam V, Barkun A, Zogopoulos G, Wong C. Endoscopic ultrasound (EUS)-guided fine needle biopsy alone vs. EUS-guided fine needle aspiration with rapid onsite evaluation in pancreatic lesions: a multicenter randomized trial. *Endoscopy* 2021 [PMID: 33506455 DOI: 10.1055/a-1375-9775]

33 **Hedenström P**, Demir A, Khodakaram K, Nilsson O, Sadik R. EUS-guided reverse bevel fine-needle biopsy sampling and open tip fine-needle aspiration in solid pancreatic lesions - a prospective, comparative study. *Scand J Gastroenterol* 2018; **53**: 231-237 [PMID: 29301477 DOI: 10.1080/00365521.2017.1421704]

34 **Oppong KW**, Bekkali NLH, Leeds JS, Johnson SJ, Nayar MK, Darné A, Egan M, Bassett P, Haugk B. Fork-tip needle biopsy versus fine-needle aspiration in endoscopic ultrasound-guided sampling of solid pancreatic masses: a randomized crossover study. *Endoscopy* 2020; **52**: 454-461 [PMID: 32162287 DOI: 10.1055/a-1114-5903]

35 **Kandel P**, Nassar A, Gomez V, Raimondo M, Woodward TA, Crook JE, Fares NS, Wallace MB. Comparison of endoscopic ultrasound-guided fine-needle biopsy versus fine-needle aspiration for genomic profiling and DNA yield in pancreatic cancer: a randomized crossover trial. *Endoscopy* 2021; **53**: 376-382 [PMID: 32767288 DOI: 10.1055/a-1223-2171]

36 **Standards of Practice Committee**, Faulx AL, Kothari S, Acosta RD, Agrawal D, Bruining DH, Chandrasekhara V, Eloubeidi MA, Fanelli RD, Gurudu SR, Khashab MA, Lightdale JR, Muthusamy VR, Shaukat A, Qumseya BJ, Wang A, Wani SB, Yang J, DeWitt JM. The role of endoscopy in subepithelial lesions of the GI tract. *Gastrointest Endosc* 2017; **85**: 1117-1132 [PMID: 28385194 DOI: 10.1016/j.gie.2017.02.022]

37 **Hwang JH**, Saunders MD, Rulyak SJ, Shaw S, Nietsch H, Kimmey MB. A prospective study comparing endoscopy and EUS in the evaluation of GI subepithelial masses. *Gastrointest Endosc* 2005; **62**: 202-208 [PMID: 16046979 DOI: 10.1016/S0016-5107(05)01567-1]

38 **Landi B**, Palazzo L. The role of endosonography in submucosal tumours. *Best Pract Res Clin Gastroenterol* 2009; **23**: 679-701 [PMID: 19744633 DOI: 10.1016/j.bpg.2009.05.009]

39 **Polkowski M**, Gerke W, Jarosz D, Nasierowska-Guttmejer A, Rutkowski P, Nowecki ZI, Ruka W, Regula J, Butruk E. Diagnostic yield and safety of endoscopic ultrasound-guided trucut [corrected] biopsy in patients with gastric submucosal tumors: a prospective study. *Endoscopy* 2009; **41**: 329-334 [PMID: 19340737 DOI: 10.1055/s-0029-1214447]

40 **Buscaglia JM**, Nagula S, Jayaraman V, Robbins DH, Vadada D, Gross SA, DiMaio CJ, Pais S, Patel

K, Sejpal DV, Kim MK. Diagnostic yield and safety of jumbo biopsy forceps in patients with subepithelial lesions of the upper and lower GI tract. *Gastrointest Endosc* 2012; **75**: 1147-1152 [PMID: 22425270 DOI: 10.1016/j.gie.2012.01.032]

41 **Ji JS**, Lee BI, Choi KY, Kim BW, Choi H, Huh M, Chung WC, Chae HS, Chung IS. Diagnostic yield of tissue sampling using a bite-on-bite technique for incidental subepithelial lesions. *Korean J Intern Med* 2009; **24**: 101-105 [PMID: 19543487 DOI: 10.3904/kjim.2009.24.2.101]

42 **Wani S**, Muthusamy VR, Komanduri S. EUS-guided tissue acquisition: an evidence-based approach (with videos). *Gastrointest Endosc* 2014; **80**: 939-59. e7 [PMID: 25434654 DOI: 10.1016/j.gie.2014.07.066]

43 **Iwai T**, Kida M, Imaizumi H, Miyazawa S, Okuwaki K, Yamauchi H, Kaneko T, Hasegawa R, Miyata E, Koizumi W. Randomized crossover trial comparing EUS-guided fine-needle aspiration with EUS-guided fine-needle biopsy for gastric subepithelial tumors. *Diagn Cytopathol* 2018; **46**: 228-233 [PMID: 29243411 DOI: 10.1002/dc.23872]

44 **Asokkumar R**, Yung Ka C, Loh T, Kah Ling L, Gek San T, Ying H, Tan D, Khor C, Lim T, Soetikno R. Comparison of tissue and molecular yield between fine-needle biopsy (FNB) and fine-needle aspiration (FNA): a randomized study. *Endosc Int Open* 2019; **7**: E955-E963 [PMID: 31367675 DOI: 10.1055/a-0903-2565]

45 **van Riet PA**, Erler NS, Bruno MJ, Cahen DL. Comparison of fine-needle aspiration and fine-needle biopsy devices for endoscopic ultrasound-guided sampling of solid lesions: a systemic review and meta-analysis. *Endoscopy* 2021; **53**: 411-423 [PMID: 32583392 DOI: 10.1055/a-1206-5552]

46 **van Riet PA**, Larghi A, Attili F, Rindi G, Nguyen NQ, Ruszkiewicz A, Kitano M, Chikugo T, Aslanian H, Farrell J, Robert M, Adeniran A, Van Der Merwe S, Roskams T, Chang K, Lin F, Lee JG, Arcidiacono PG, Petrone M, Doglioni C, Iglesias-Garcia J, Abdulkader I, Giovannini M, Bories E, Poizat F, Santo E, Scapa E, Marmor S, Bucobo JC, Buscaglia JM, Heimann A, Wu M, Baldaque-Silva F, Moro CF, Erler NS, Biermann K, Poley JW, Cahen DL, Bruno MJ. A multicenter randomized trial comparing a 25-gauge EUS fine-needle aspiration device with a 20-gauge EUS fine-needle biopsy device. *Gastrointest Endosc* 2019; **89**: 329-339 [PMID: 30367877 DOI: 10.1016/j.gie.2018.10.026]

47 **Kim GH**, Cho YK, Kim EY, Kim HK, Cho JW, Lee TH, Moon JS; Korean EUS Study Group. Comparison of 22-gauge aspiration needle with 22-gauge biopsy needle in endoscopic ultrasonography-guided subepithelial tumor sampling. *Scand J Gastroenterol* 2014; **49**: 347-354 [PMID: 24325591 DOI: 10.3109/00365521.2013.867361]

48 **Hedenström P**, Marschall HU, Nilsson B, Demir A, Lindkvist B, Nilsson O, Sadik R. High clinical impact and diagnostic accuracy of EUS-guided biopsy sampling of subepithelial lesions: a prospective, comparative study. *Surg Endosc* 2018; **32**: 1304-1313 [PMID: 28812151 DOI: 10.1007/s00464-017-5808-2]

49 **Nagula S**, Pourmand K, Aslanian H, Bucobo JC, Gonda TA, Gonzalez S, Goodman A, Gross SA, Ho S, DiMaio CJ, Kim MK, Pais S, Poneros JM, Robbins DH, Schnoll-Sussman F, Sethi A, Buscaglia JM; New York Endoscopic Research Outcomes Group (NYERO). Comparison of Endoscopic Ultrasound-Fine-Needle Aspiration and Endoscopic Ultrasound-Fine-Needle Biopsy for Solid Lesions in a Multicenter, Randomized Trial. *Clin Gastroenterol Hepatol* 2018; **16**: 1307-1313. e1 [PMID: 28624647 DOI: 10.1016/j.cgh.2017.06.013]

50 **Dietrich CF**, Jenssen C, Arcidiacono PG, Cui XW, Giovannini M, Hocke M, Iglesias-Garcia J, Saftoiu A, Sun S, Chiorean L. Endoscopic ultrasound: Elastographic lymph node evaluation. *Endosc Ultrasound* 2015; **4**: 176-190 [PMID: 26374575 DOI: 10.4103/2303-9027.162995]

51 **Lisotti A**, Ricci C, Serrani M, Calvanese C, Sferrazza S, Brighi N, Casadei R, Fusaroli P. Contrast-enhanced endoscopic ultrasound for the differential diagnosis between benign and malignant lymph nodes: a meta-analysis. *Endosc Int Open* 2019; **7**: E504-E513 [PMID: 31044153 DOI: 10.1055/a-0854-3785]

52 **de Moura DTH**, McCarty TR, Jirapinyo P, Ribeiro IB, Farias GFA, Ryou M, Lee LS, Thompson CC. Endoscopic Ultrasound Fine-Needle Aspiration versus Fine-Needle Biopsy for Lymph Node Diagnosis: A Large Multicenter Comparative Analysis. *Clin Endosc* 2020; **53**: 600-610 [PMID: 31794654 DOI: 10.5946/ce.2019.170]

53 **Puri R**, Mangla R, Eloubeidi M, Vilmann P, Thandassery R, Sud R. Diagnostic yield of EUS-guided FNA and cytology in suspected tubercular intra-abdominal lymphadenopathy. *Gastrointest Endosc* 2012; **75**: 1005-1010 [PMID: 22421494 DOI: 10.1016/j.gie.2011.12.032]

54 **Puli SR**, Batapati Krishna Reddy J, Bechtold ML, Ibdah JA, Antillon D, Singh S, Olyaee M, Antillon MR. Endoscopic ultrasound: it's accuracy in evaluating mediastinal lymphadenopathy? *World J Gastroenterol* 2008; **14**: 3028-3037 [PMID: 18494054 DOI: 10.3748/wjg.14.3028]

55 **García Sabater JF**, Amador Yscla A, Perales Obenich JA. [Classification and identification of the genus Mycobacterium]. *Rev Latinoam Microbiol* 1977; **19**: 7-15 [PMID: 279061 DOI: 10.1186/s13256-016-1121-2]

56 **Nieuwoudt M**, Lameris R, Corcoran C, Rossouw TM, Slavik T, Du Plessis J, Omoshoro-Jones JA, Stivaktas P, Potgieter F, Van der Merwe SW. Polymerase chain reaction amplifying mycobacterial DNA from aspirates obtained by endoscopic ultrasound allows accurate diagnosis of mycobacterial disease in HIV-positive patients with abdominal lymphadenopathy. *Ultrasound Med Biol* 2014; **40**: 2031-2038 [PMID: 25023118 DOI: 10.1016/j.ultrasmedbio.2014.04.006]

57 **Keswani RN**, Krishnan K, Wani S, Keefer L, Komanduri S. Addition of Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration and On-Site Cytology to EUS-Guided Fine Needle Biopsy

Increases Procedure Time but Not Diagnostic Accuracy. *Clin Endosc* 2014; **47**: 242-247 [PMID: 24944988 DOI: 10.5946/ce.2014.47.3.242]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4208-4220

DOI: 10.3748/wjg.v27.i26.4208

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Metal-organic framework IRMOFs coated with a temperature-sensitive gel delivering norcantharidin to treat liver cancer

Xiu-Yan Li, Qing-Xia Guan, Yu-Zhou Shang, Yan-Hong Wang, Shao-Wa Lv, Zhi-Xin Yang, Rui Wang, Yu-Fei Feng, Wei-Nan Li, Yong-Ji Li

ORCID number: Xiu-Yan Li 0000-0003-1060-8442; Qing-Xia Guan 0000-0002-4282-9154; Yu-Zhou Shang 0000-0001-6003-258X; Yan-Hong Wang 0000-0002-9247-1453; Shao-Wa Lv 0000-0002-1386-6132; Zhi-Xin Yang 0000-0002-4514-795X; Rui Wang 0000-0002-8486-4660; Yu-Fei Feng 0000-0002-9507-6950; Wei-Nan Li 0000-0002-9888-4806; Yong-Ji Li 0000-0003-0746-0360.

Author contributions: Li XY, Guan QX, Shang YZ, Wang YH, Lv SW, Yang ZX, Wang R, Feng YF, Li WN, and Li YJ performed the experiments and acquired and analyzed the data; Li XY, Guan QX, Shang YZ, and Wang YH wrote the manuscript; All authors approved the final version of the article.

Supported by National Natural Science Foundation of China, No. 82074025 and No. 82074271; the Heilongjiang Traditional Chinese Medicine Research Project, No. ZHY18-047; and Scientific Research Project of Heilongjiang Health Committee, No. 2020-293.

Institutional review board statement: This study was approved by Ethics Committee of Heilongjiang University of Chinese Medicine, Harbin, China.

Xiu-Yan Li, Qing-Xia Guan, Yu-Zhou Shang, Yan-Hong Wang, Shao-Wa Lv, Zhi-Xin Yang, Rui Wang, Yu-Fei Feng, Wei-Nan Li, Yong-Ji Li, College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin 150040, Heilongjiang Province, China

Corresponding author: Yong-Ji Li, PhD, Professor, College of Pharmacy, Heilongjiang University of Chinese Medicine, No. 24 Heping Road, Xiangfang District, Harbin 150040, Heilongjiang Province, China. liyongji2009@163.com

Abstract

BACKGROUND

Norcantharidin (NCTD) is suitable for the treatment of primary liver cancer, especially early and middle primary liver cancer. This compound can reduce tumors and improve immune function. However, the side effects of NCTD have limited its application. There is a marked need to reduce the side effects and increase the efficacy of NCTD.

AIM

To develop a nanomaterial carrier, NCTD-loaded metal-organic framework IRMOF-3 coated with a temperature-sensitive gel (NCTD-IRMOF-3-Gel), aiming to improve the anticancer activity of NCTD and reduce the drug dose.

METHODS

NCTD-IRMOF-3-Gel was obtained by a coordination reaction. The apparent characteristics and *in vitro* release of NCTD-IRMOF-3-Gel were investigated. Cell cytotoxicity assays, flow cytometry, and apoptosis experiments in mouse hepatoma (Hepa1-6) cells were used to determine the anti-liver cancer activity of NCTD-IRMOF-3-Gel in *in vitro* models.

RESULTS

The particle size of NCTD-IRMOF-3-Gel was 50-100 nm, and the particle size distribution was uniform. The release curve showed that NCTD-IRMOF-3-Gel had an obvious sustained-release effect. The cytotoxicity assays showed that the free drug NCTD and NCTD-IRMOF-3-Gel treatments markedly inhibited Hepa1-6 cell proliferation, and the inhibition rate increased with increasing drug concentration. By flow cytometry, NCTD-IRMOF-3-Gel was observed to block the Hepa1-6 cell cycle in the S and G2/M phases, and the thermosensitive gel

Conflict-of-interest statement: The authors report no conflicts of interest in this work.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: China

Peer-review report's scientific quality classification

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

Received: February 25, 2021
Peer-review started: February 25, 2021
First decision: April 18, 2021
Revised: April 27, 2021
Accepted: May 27, 2021
Article in press: May 27, 2021
Published online: July 14, 2021

P-Reviewer: Martinello M, Martins VH, Tabet P
S-Editor: Gong ZM
L-Editor: Filipodia
P-Editor: Liu JH

nanoparticles may inhibit cell proliferation by inducing cell cycle arrest. Apoptosis experiments showed that NCTD-IRMOF-3-Gel induced the apoptosis of Hepa1-6 cells.

CONCLUSION

Our results indicated that the NCTD-IRMOF-3-Gel may be beneficial for liver cancer disease treatment.

Key Words: Norcantharidin; Metal-organic frameworks; IRMOF-3; Temperature-sensitive gel; Drug delivery; Liver cancer

Core Tip: Norcantharidin (NCTD) is suitable for the treatment of primary liver cancer, especially early and middle primary liver cancer. However, the side effects of NCTD have limited its application. Therefore, we established a liver-targeting therapy in which NCTD is loaded into IRMOF-3 coated with a thermosensitive gel, which can be efficiently delivered to liver cancer cells and slowly released. The results demonstrate that this thermosensitive gel-encapsulated IRMOF-3 has great advantages as an antitumor drug carrier and provides some ideas for passive targeting therapy of tumors.

Citation: Li XY, Guan QX, Shang YZ, Wang YH, Lv SW, Yang ZX, Wang R, Feng YF, Li WN, Li YJ. Metal-organic framework IRMOFs coated with a temperature-sensitive gel delivering norcantharidin to treat liver cancer. *World J Gastroenterol* 2021; 27(26): 4208-4220
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4208.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4208

INTRODUCTION

Liver cancer has the characteristics of a high incidence, poor prognosis, and high mortality. The latest World Health Organization data show that the global incidence rate of liver cancer is ranked fifth among malignant tumors, and the incidence rate is ranked third. China is a country with a high incidence of liver cancer and hepatitis B[1,2]. At present, surgical resection[3,4], drug chemotherapy[5,6], nanotechnology[7], and interventional therapy[8] are the main treatment methods for liver or other cancers. Among many chemotherapeutic drugs, norcantharidin (NCTD) has strong antitumor activity and can inhibit a variety of tumors including gastrointestinal cancer[9], malignant lymphoma[10], lung cancer[11], and liver cancer[12].

NCTD was synthesized by removing the 1,2-methyl group from cantharidin, which was extracted from the cantharides of Coleoptera. Compared with cantharidin, NCTD exhibits not only significantly improved anticancer effect but also a great reduction in renal toxicity and strong irritation to the urinary system[13]. The clinical use of NCTD is mainly based on tablets and injections, and this drug has unique advantages in the treatment of cancer. However, the side effects of NCTD have limited its application [14]. First, compared to cantharidin, the toxicity of NCTD is reduced to a large extent but still has a certain degree of urinary system toxicity, and organ toxicity occurs with large doses or long-term use, so there is a strict limit on the maximum dosage of NCTD in the clinic[15]. Second, NCTD is rapidly distributed in various tissues after absorption when administered to mice by gavage. The concentration of NCTD peaks in liver and cancer tissues 15 min after administration. However, this concentration significantly decreases 6 h after administration. Most NCTD is excreted through the kidney within 24 h, with little accumulation in the body. The elimination speed of NCTD from the body is fast, which reduces the compliance of patients with medication[16]. In addition, NCTD is widely distributed in the body after oral administration, and is less distributed in the liver tissue due to its fast elimination speed, which not only reduces its efficacy but also increases the toxicity to other organs[17]. Third, most NCTD injections used in the clinic are sodium salt, with a pH value of approximately 9.0, which makes it highly irritating[18].

In recent years, a large number of studies have been carried out to reduce the side effects and increase the efficacy of NCTD[19-22]. This project aimed to develop a multifunctional metal-organic framework (IRMOF-3) that can play an important role in drug carrying and delivery. Because of the special topological structure of IRMOF-3, drugs can be loaded into the spatial structure to the maximum extent, and it plays great role in controlled release[23-26]. However, when NCTD-IRMOF-3 enters the body, burst release is caused due to endocytosis or gastrointestinal absorption. Therefore, we established a liver-targeting therapy in which NCTD is loaded into IRMOF-3 coated with a thermosensitive gel (NCTD-IRMOF-3-Gel), which can be efficiently delivered to liver cancer cells and slowly released. In this study, NCTD-IRMOF-3-Gel was prepared, and the *in vitro* targeting behavior was explored. It was shown that the combination of IRMOF-3 and the thermosensitive gel could decrease the toxicity and increase the bioavailability of NCTD, representing an effective method for the chemotherapy of liver cancer. This study lays a foundation for the liver-targeting ability of NCTD-IRMOF-3-Gel. The results demonstrate that this thermosensitive gel-encapsulated IRMOF-3 has great advantages as an antitumor drug carrier and provides some ideas for passive targeting therapy of tumors.

MATERIALS AND METHODS

Materials

All of the chemicals used were of analytical grade. N,N-Dimethylformamide (DMF), dichloromethane (CH_2Cl_2) (both Tianjin Fuyu Fine Chemical Co., Ltd., Tianjin, China), zinc acetate dihydrate ($Zn(OAc)_2.2H_2O$) (Kaitong Chemical Industry Co. Ltd., Tianjin, China), and 2-amino-terephthalic acid (NH_2-BDC, $C_8H_7NO_4$) (Henghua Technology Co., Ltd., Jinan, China) were used to prepare nanosized IRMOF-3.

Preparation of NCTD-IRMOF-3-Gel

First, $Zn(OAc)_2.2H_2O$ (4 mmol) and NH_2-BDC (1 mmol) were completely dissolved in 10 mL and 15 mL DMF, respectively. Then the zinc salt solution was quickly poured into the ligand solution at room temperature (25 °C) to form a milky white precipitate. After magnetic stirring for 1 min and centrifugation for 5 min (12000 r/min), the supernatant was removed and the precipitate was obtained. The deposit was washed three times with DMF (removing unreacted raw materials) and soaked for 3 d with CH_2Cl_2 (removing DMF), with the solvent replaced once per day. Next, the deposit was centrifugally filtered and dried under natural conditions. Then, the samples were activated under vacuum for 12 h at 100 °C, and IRMOF-3 was obtained. For the encapsulation studies, 30 mg NCTD and 10 mg nanoIRMOF-3 were accurately weighed in a 5 mL volumetric flask, and 80% alcohol solution was added. The suspension was stirred for 72 h at room temperature. NCTD-loaded nanoIRMOF-3 (NCTD-IRMOF-3) was then collected by centrifugation and vacuum-dried at room temperature. NCTD-IRMOF-3 (15 mg) was accurately weighed, 3 mL freeze-dried protective agent (4% mannitol and 2% poloxamer) was added, and the mixture was fully dissolved. A small amount of supernatant was collected, frozen in a vial at -40 °C for 24 h, and then frozen in a vacuum freeze dryer for 30 h to obtain the freeze-dried product. NCTD-IRMOF-3 was then dispersed into a thermosensitive gel solution at room temperature to form a dispersion of nanoparticles. When the NCTD-IRMOF-3 nanoparticle dispersion was injected into the body or heated to 37 °C, the NCTD-IRMOF-3-Gel gel could be formed.

Characterization

Physical characterization was performed by powder X-ray diffraction (PXRD) analysis (Phillips Xpert Pro MPD diffractometer with Cu Kα radiation λ = 1.5418 nm at 40 kV and 50 mA). Scanning electron microscopy (SEM) images were obtained using a Quanta 200F (FEI Sirion SEM), confirming the regular shape and nanosize of the particles. Nitrogen adsorption/desorption isotherms and pore size distributions were measured using an MFA-140 system (Beijing Builder Electronic Technology Co., Ltd., Beijing, China). The particle size distribution was measured by a Zetasizer Nano-ZS90 Laser particle size analyzer (Malvern Instruments Co., Ltd., Malvern, United Kingdom).

NCTD release assay

In vitro studies of the release of NCTD from NCTD, NCTD-IRMOF-3, and NCTD-

IRMOF-3-Gel were carried out using dialysis bags (Sigma, St. Louis, MO, United States) soaked in double-distilled water for 12 h. Freeze-dried NCTD, NCTD-IRMOF-3, and NCTD-IRMOF-3-Gel suspensions were added into a dialysis bag, which then was placed in 50 mL phosphate-buffered saline (pH 5.0) to maintain sink conditions and shaken at 100 rpm in a constant-temperature shaker (SHAB; Donglian Electric Technique Co. Ltd., Harbin, China) at 37 °C. Subsequently, 2 mL release medium was withdrawn at regular intervals, and fresh release medium was added to maintain a constant volume. Every trial was repeated three times. The samples were analyzed using high-performance liquid chromatography, and the control experiments were similarly performed using the same proportions to investigate drug release.

In vitro examination

MTT assay for cytotoxicity detection: The mouse hepatoma (Hepa1-6) cell line (Beijing Boyu Kangtai International Biological Technology Co., Ltd., Beijing, China) used in this experiment was maintained in Roswell Park Memorial Institute-1640 medium (HyClone; Thermo Fisher Scientific, Inc., Waltham, MA, United States) supplemented with 10% heat-inactivated fetal calf serum (Sijiqing Tianhang Biological Science and Technology Co., Ltd., Hangzhou, China), 105 U/L penicillin G, and 100 mg/L streptomycin in a CO_2 incubator at 5% CO_2 and 37 °C.

The cells were plated in 96-well cell culture plates, and different concentrations of NCTD (0-80 μg/mL) were added to the complete cell culture medium. Then, 10 concentrations of NCTD-IRMOF-3 and NCTD-IRMOF-3-Gel were prepared (0-80 μg/mL). After 24, 48, 72, and 96 h of incubation, chemosensitivity was evaluated using thiazolyl blue tetrazolium bromide (MTT reagent, 98%; Wuhan Baodu DE Co., Ltd., Wuhan, China) in complete cell culture medium (5 μg/mL). Then, 20 μL MTT reagent was added to each well and incubated for 4 h, and the mitochondrial aldehyde dehydrogenase from the viable cells subsequently reduced the yellow, water-soluble MTT reagent to water-insoluble blue formazan crystals, which were dissolved by adding 150 μL dimethyl sulfoxide to each well. The absorbance of the dissolved formazan blue dye was measured at 490 nm using a BioTex microplate reader (American Power Instruments Co., Ltd., Wilmington, MA, United States), and the cell viability calculations were performed.

Flow cytometry assay of the effects on the Hepa1-6 cell cycle: The cells were placed in 6-well cell plates and cultured for 24 h in a CO_2 incubator at 5% CO_2 and 37 °C. Then, NCTD-IRMOF-3-Gel at different concentrations (10-40 μg/mL) was added to each well and incubated in an incubator for 24 h. The cells were digested with trypsin (Shanghai Beyotime Biotechnology Co., Ltd., Shanghai, China) and centrifuged at 1500 rpm for 5 min. The supernatant was removed from the solution and washed twice with phosphate-buffered saline (PBS). Then the precooled 75% ethanol solution was added to each well and fixed at 4 °C for 12 h. The solution was centrifuged at 1500 rpm for 5 min to remove the supernatant and resuspended in PBS. Finally, the prepared solution (including 0.5 mL of PBS, 25 μL propidium iodide staining solution and 10 μL ribozyme A) was added to each well and incubated at 37 °C for 30 min. The cells were filtered using a 35 μm cell filter and detected by flow cytometry (Becton, Dickinson and Company, Franklin Lakes, NJ, United States).

Apoptosis experiment: Apoptosis was detected by Annexin V-FITC/PI double staining. K562 cells in the logarithmic growth phase were inoculated into 24-well culture plates at 1×10^5/well. NCTD-IRMOF-3-Gel, NCTD-IRMOF-3 and NCTD were added at two different concentrations (25 μg/mL and 50 μg/mL). After 48 h, the cells were washed with PBS three times, and 1 μL propidium iodide and 5 μL Annexin V-FITC (Shanghai Beyotime Biotechnology Co., Ltd., Shanghai, China) were added. The cells were incubated in the dark for 10 min and washed once with PBS. Then, 400 μL PBS was added to each tube. Apoptosis was detected by flow cytometry, and the apoptosis rate was calculated.

Statistical analyses

Statistical analyses were performed using analysis of variance with SPSS 24.0 software (version 24.0.0; Chicago, IL, United States). Statistical differences were defined as $^aP < 0.05$ and $^bP < 0.01$. The data are presented as the mean ± SD.

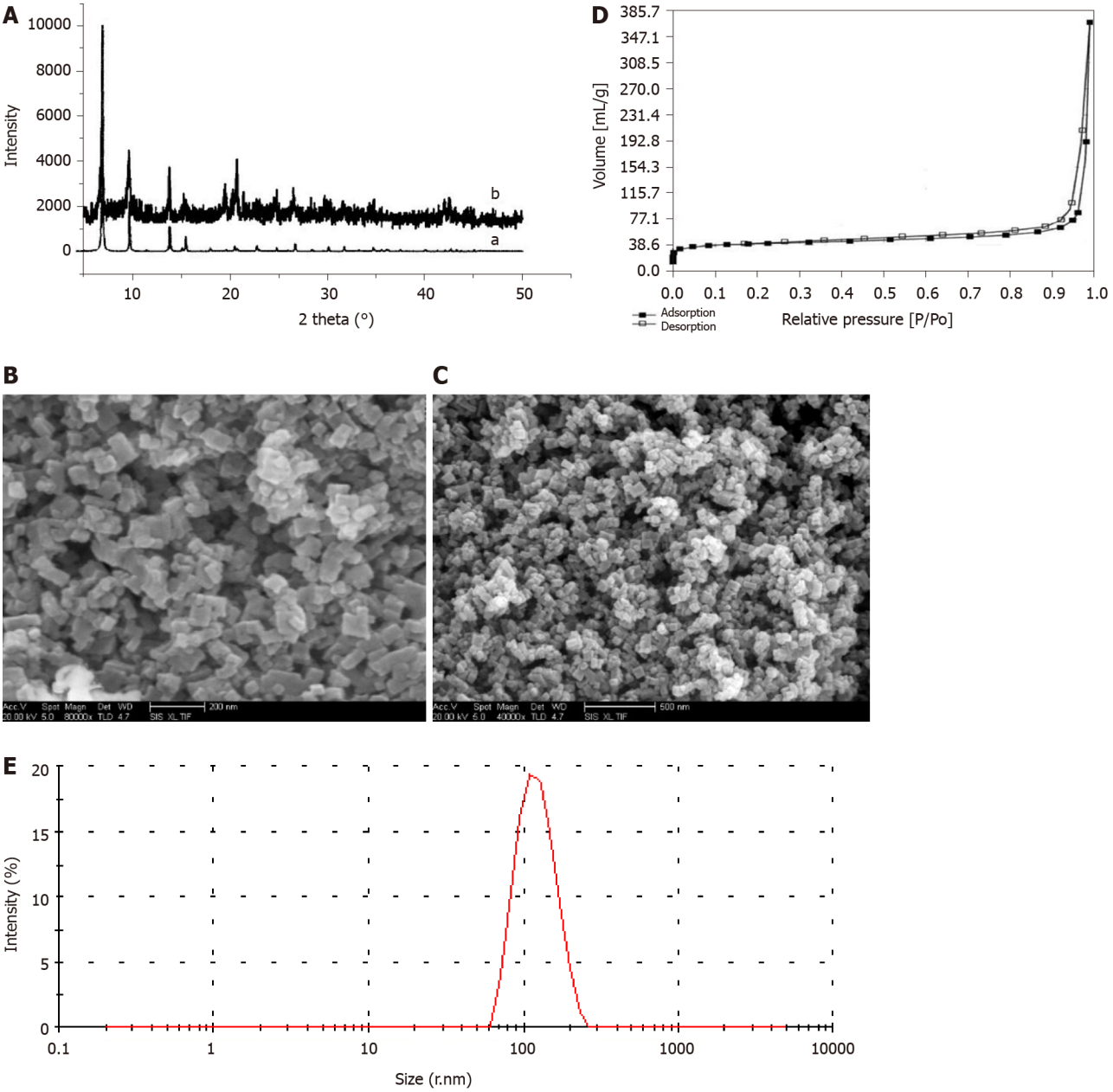

Figure 1 Structure and morphology of IRMOF-3 and norcantharidin-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel. A: X-ray diffraction (XRD) spectra of IRMOF-3. Note: XRD patterns of synthetic IRMOF-3 (b) and standard IRMOF-3 (a). XRD patterns of the sample show the same peaks as those of the standard, confirming the high purity of IRMOF-3. The peak patterns of the sample account for the rough appearance of the nanosized particles; B: Scanning electron microscopy (SEM) images of the morphology of IRMOF-3. SEM images of the morphology of IRMOF-3. The particles show a regular square, uniform distribution and a size of 50-100 nm. The single-particle surface is rough, indicating the existence of pores; C: SEM images of the morphology of norcantharidin (NCTD)-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (NCTD-IRMOF-3-Gel). SEM images of the morphology of NCTD-IRMOF-3-Gel showing the same size as that of IRMOF-3; D: Nitrogen adsorption–desorption isotherms of IRMOF-3. Nitrogen adsorption–desorption isotherms. A hysteresis loop phenomenon appears under relatively high pressure, indicating the existence of channels in the sample; E: Particle size distribution of NCTD-IRMOF-3-Gel. The particle size distribution of NCTD-IRMOF-3-Gel indicated that the average particle size was 100 nm.

RESULTS

Structure and morphology

The PXRD patterns shown in Figure 1A illustrate that the IRMOF-3 materials possessed three well-resolved peaks, similar to the standard patterns. The SEM images of IRMOF-3 and NCTD-IRMOF-3-Gel are presented in Figure 1B and Figure 1C which show that the sample consisted of square particles (50-100 nm). The single-particle surface was rough, indicating the existence of pores. SEM images show that the morphology of NCTD-IRMOF-3-Gel had similar size features as those of IRMOF-3. The nitrogen gas (N_2) adsorption–desorption isotherms of IRMOF-3 are shown in Figure 1D. The hysteresis loop phenomenon appeared in the range of relatively high

pressure, indicating the existence of channels in the sample (Figure 1D). The particle size distribution of NCTD-IRMOF-3-Gel indicated that the average particle size was 100 nm (Figure 1E).

NCTD release assay

In the simulated pH 5.0 environment of tumor cells, the drug release data were fitted by a zero-order dynamics equation, first-order kinetic equation, Higuchi equation and Weibull model. The NCTD regression equation obtained is shown in Table 1. The resulting correlation coefficients of the drug release kinetics show that the drug release conforms to the Weibull equation, and the R^2 value is 0.9508.

As shown in Figure 2, the release of NCTD is very fast and is completely finished at 5 h. However, the drug release of NCTD-IRMOF-3 was slower than that of NCTD. The first half of the drug release curve of NCTD was steep and showed a sudden release within 0.6 h. The reason was that the free drug molecules adsorbed on the surface of the nanoparticles diffused rapidly into the medium. Then the curve of NCTD-IRMOF-3 showed a steady slowly release process because the drug in the pores was slowly released. Approximately 5 h were necessary for 50% NCTD release from NCTD-IRMOF-3. After 36 h, the release rate was more than 70%, and the release was basically complete. The release rate of NCTD-IRMOF-3-Gel nanoparticles at 0.3 h was lower than that of the other formulations because NCTD was released gradually with the slow dissolution of poloxamer. After 10 h, the release rate was approximately 50%; after 36 h, the release rate was more than 65%.

In vitro examination

MTT assay: In contrast to that of NCTD- and NCTD-IRMOF-3-treated cells, the inhibition of Hepa1-6 cells treated with NCTD-IRMOF-3-Gel increased in a dose-dependent manner (Figure 3). When the half-maximal inhibitory concentration (IC_{50}) values of each group at different time periods were compared, it was found that these values were 30.59 μg/mL, 93.74 μg/mL, and 112.3 μg/mL with NCTD, NCTD-IRMOF-3, and NCTD-IRMOF-3-Gel, respectively. The inhibitory effects of NCTD-IRMOF-3-Gel on Hepa1-6 cells were stronger than those of NCTD and NCTD-IRMOF-3 and showed a certain sustained-release effect.

Flow cytometry assay of the effects on the Hepa1-6 cell cycle: Figure 4 and Table 2 show that the percentage of the total number of cells in S phase and G2/M phase increased significantly with increasing NCTD-IRMOF-3-Gel concentration, while the proportion of cells in G0/G1 phase decreased significantly. This result indicates that NCTD-IRMOF-3-Gel can block the cell cycle in the S and G2/M phases, and thermosensitive gel nanoparticles may inhibit cell proliferation by inducing cell cycle arrest.

Apoptosis experiment

It can be seen from the figure that the apoptosis rates of NCTD-IRMOF-3-Gel at the high concentration (C) and low concentration (F) were 32.11 μg/mL and 65.60 μg/mL, respectively. Compared with that in the NCTD control group, the apoptosis rate in the NCTD-IRMOF-3-Gel group was highest, which indicated that NCTD-IRMOF-3-Gel could induce the apoptosis of Hepa1-6 cells (Figures 5 and 6).

DISCUSSION

In recent years, with the wide application of medical polymer materials and increased clinical utilization[27,28], research on sustained-release and controlled-release preparations has increased and has become an important research direction. As a new type of drug formulation, sustained- and controlled-release preparations can increase efficacies and reduce side effects compared with traditional drugs. The thermosensitive gel has a hydrophilic three-dimensional network structure, which can be loaded in the liquid state to control drug release. In addition, thermosensitive gel has a stronger affinity, longer retention time and less stimulation in medically relevant locations than traditional gel, especially in mucosal tissue. This type of gel is suitable for all kinds of drug carriers and has now become a research hotspot in pharmaceutics [29,30].

The aim of this project was to prepare NCTD-IRMOF-3-Gel by using the porous material metal-organic framework IRMOF-3 as a drug carrier and NCTD as a model drug. Loading the drug and drug carrier within the thermosensitive gel not only delayed the action time of the drug but also compensated for sudden drug release

Table 1 Release equations and correlation coefficients of norcantharidin-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel

	Model	Equation	R^2
DM	Zero-order processes	Q = 1.2786 t + 36.322	0.5383
	First-order processes	In (100-Q) = -0.0268 t + 4.1375	0.667
	Higuchi	$Q = 9.6078\ t^{0.5} + 25.29$	0.7719
	Weibull	InIn (1/1-Q) = 0.3813 Int - 0.9163	0.9508

Table 2 Effects of norcantharidin-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel on the cell cycle (*n* = 3)

Groups (μg/mL)	G0/G1	S	G2/M
Blank group	88.1 ± 2.8	7.2 ± 1.9	4.7 ± 2.1
10	21.9 ± 1.6[b]	59.4 ± 2.2[b]	18.7 ± 1.8[a]
20	16.6 ± 1.2[b]	54.1 ± 2.4[b]	29.2 ± 1.9[a]
40	18.3 ± 2.4[b]	45.8 ± 3.1[b]	35.8 ± 2.7[a]

[a]$P < 0.05$.
[b]$P < 0.01$.

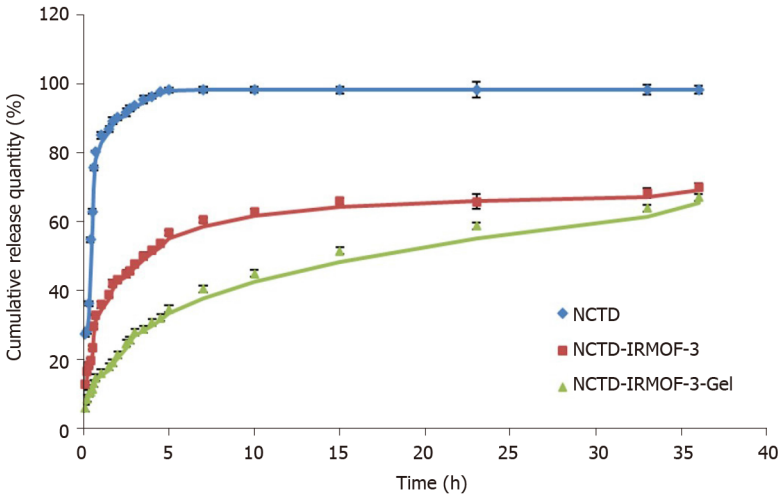

Figure 2 *In vitro* release curves of norcantharidin (NCTD) (blue line), NCTD-loaded metal-organic framework IRMOF-3 (red line), and NCTD-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (green line). After approximately 5 h, 90% of norcantharidin (NCTD) was found in the release medium, while only 50% of NCTD was released from NCTD-loaded metal-organic framework IRMOF-3 (NCTD-IRMOF-3), and 30% of NCTD was released from NCTD-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (NCTD-IRMOF-3-Gel). Every trial was repeated three times. All values are shown as the mean ± SD.

from the metal-organic framework carrier. The XRD pattern of the IRMOFs is consistent with that of the standard materials and the peak pattern is rough, which suggest that IRMOFs are nanoparticles with rough surfaces and high purity. The SEM images of the morphology of IRMOF-3 and NCTD-IRMOF-3-Gel showed that the IRMOFs were square and regular nanoparticles containing pores. NCTD-IRMOF-3-Gel was prepared using a poloxamer thermosensitive gel as the carrier, and its morphology did not change. This result shows that the thermosensitive gel has no effect on the original metal-organic framework structure, solves the problem of sudden drug release, and can reduce the toxicity caused by sudden drug release.

The Brunauer–Emmett–Teller surface areas and micropore volume of NCTD-IRMOF-3-Gel were determined using N_2 adsorption isotherms, which showed that it was a microporous material. The N_2 adsorption method is commonly used to

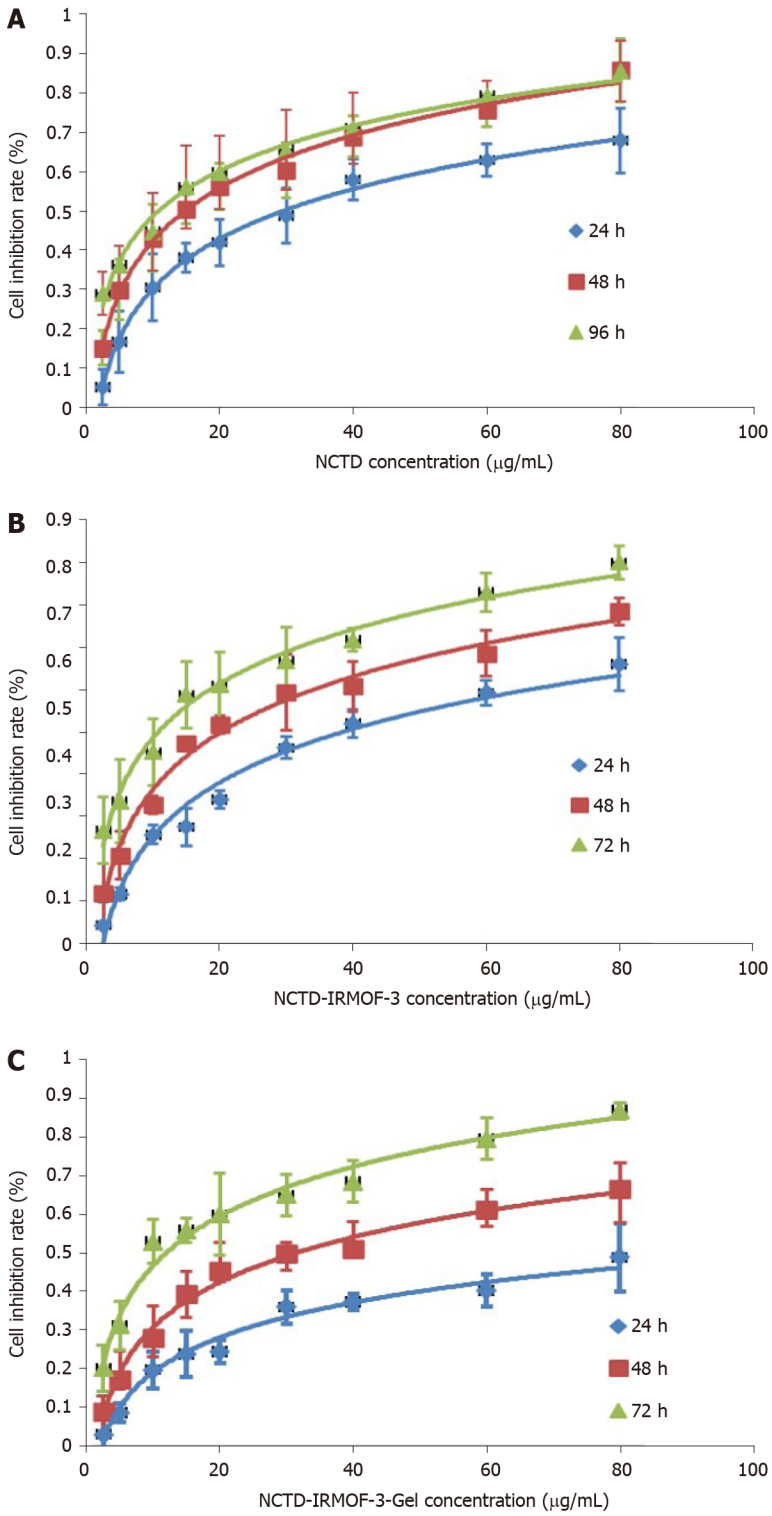

Figure 3 Comparison of the cytotoxicities of norcantharidin (NCTD), NCTD-loaded metal-organic framework IRMOF-3, and NCTD-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel. The inhibitory effect of norcantharidin (NCTD)-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (NCTD-IRMOF-3-Gel) (C) on Hepa1-6 cells was stronger than that of NCTD (A) and NCTD-loaded metal-organic framework IRMOF-3 (NCTD-IRMOF-3) (B) and showed a certain sustained-release effect.

determine the specific surface area and pore size of nanomaterials. The determination principle of N_2 adsorption is that the surface pores of porous materials will adsorb nitrogen at liquid nitrogen temperatures. The flat areas in the low-pressure section were caused by the nanopores. The N_2 adsorption quantity increased suddenly, which generated a hysteresis loop, indicating the existence of micropores, and this phenomenon was caused by capillary condensation. After zeta potential analysis of the particle size distribution, NCTD-IRMOF-3-Gel was shown to have a particle size of approximately 100 nm and good dispersibility.

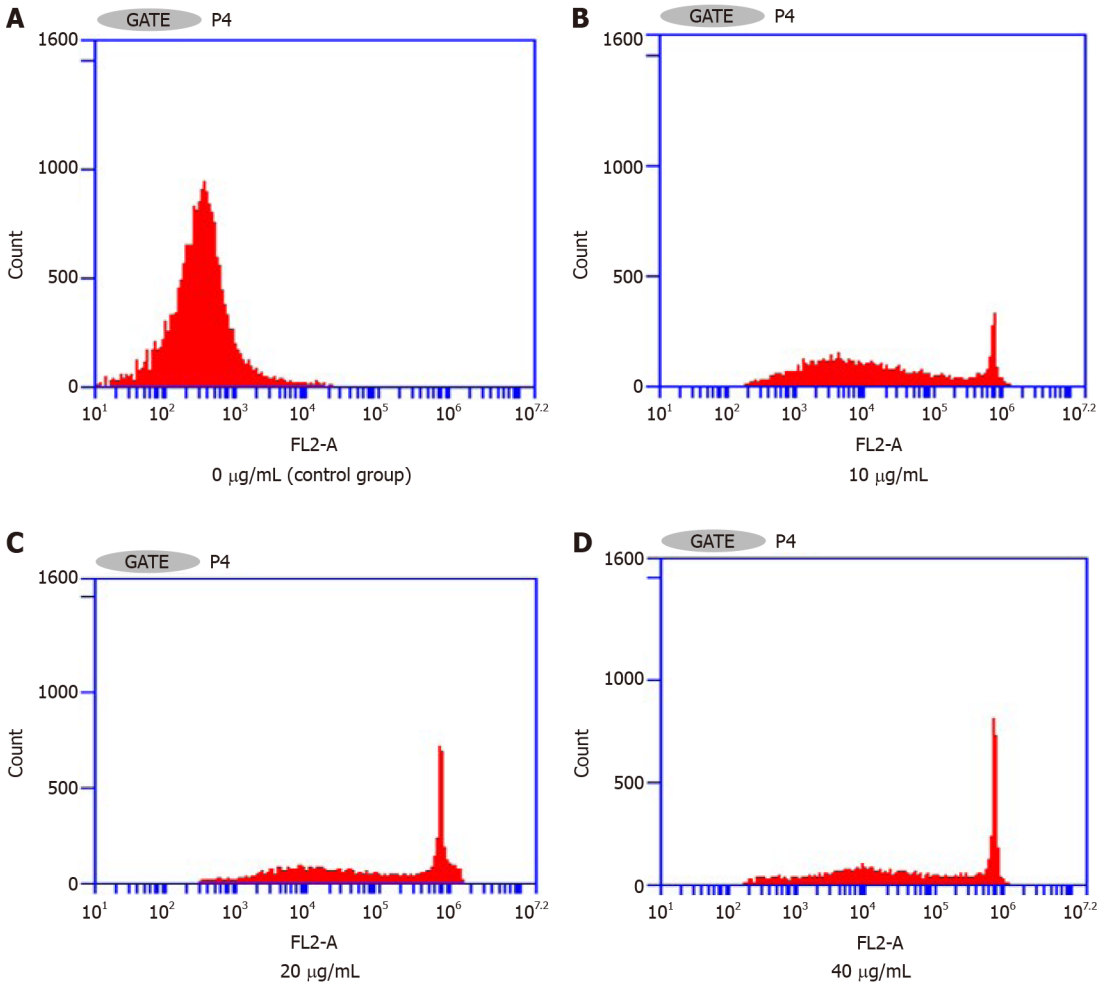

Figure 4 Effects of norcantharidin-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel on the cell cycle. The percentage of total cells in S and G2/M phases increased significantly with increasing norcantharidin (NCTD)-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (NCTD-IRMOF-3-Gel) concentration, and the proportion of cells in G0/G1 phase decreased significantly. A: Control group; B: 10 μg/mL; C: 20 μg/mL; D: 40 μg/mL. G1 phase: DNA presynthetic phase, where mitosis is complete before DNA replication begins; G2 phase: DNA synthesis replication phase, where DNA replication is complete before mitosis begins; M phase: Cell division phase; S phase: DNA synthesis replication phase.

The kinetics of *in vitro* drug release can effectively determine the profile of *in vitro* drug release and predict the conditions of *in vivo* drug release. From the drug release curve, NCTD was released quickly, with basically complete release at 5 h. NCTD-IRMOF-3 nanoparticles released NCTD more slowly than free NCTD treatment and showed sudden release within 0.6 h. After 36 h, the release rate was more than 70%, and the release was basically completed. NCTD-IRMOF-3-Gel nanoparticles showed a significantly slower release trend, and the degree of release at 0.3 h was lower than that in the other groups, which was due to the gradual release of NCTD with the slow dissolution of poloxamer. After 36 h, the release rate reached more than 65%. We speculated that NCTD-IRMOF-3-Gel nanoparticles had a certain sustained-release effect and could effectively improve the drug release process.

The MTT assay demonstrated the cytotoxicity of the NCTD-IRMOF-3-Gel nanoparticles. At the same concentration, the inhibition rate of each group of drugs acting on Hepa1-6 cells increased with the extension of time. The inhibition rate of the free drug group was slightly lower than that of the nanoparticle group, but it still had a killing effect on the cells. Compared with that of NCTD-IRMOF-3-Gel, the inhibition rate of NCTD-IRMOF-3 was slightly low, indicating that the thermosensitive gel-coated nanoparticles had a better inhibitory effect on cells. The inhibition rate of the nanoparticle group was low at 24 h and gradually increased after 48 h to the level of inhibition of the free drug, indicating that the drug-loaded nanoparticle-thermosensitive gel group presented an obvious sustained-release effect. Meanwhile, the cell cycle study using flow cytometry showed that NCTD-IRMOF-3-Gel could block the S phase and G2/M phase of the cell cycle, and thermosensitive gel-suspended nanoparticles may inhibit cell proliferation by blocking the cell cycle. The apoptosis

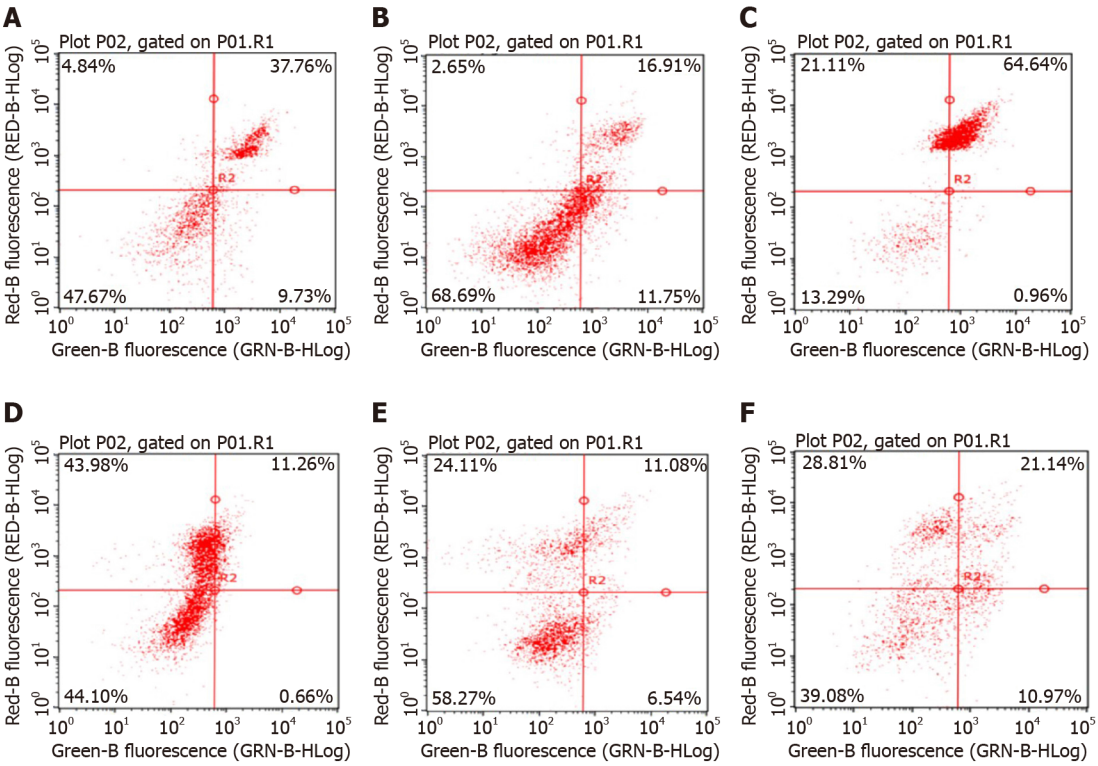

Figure 5 Apoptosis rates of Hepa1-6 cells after 48 h. Apoptosis rates of norcantharidin (NCTD)-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (NCTD-IRMOF-3-Gel) at the high concentration (C) and low concentration (F) were 32.11 μg/mL and 65.60 μg/mL, respectively. Compared with the NCTD control group, the apoptosis rate in the NCTD-IRMOF-3-Gel group was highest, which indicated that NCTD-IRMOF-3-Gel could induce the apoptosis of Hepa1-6 cells. A: 50 μg/mL NCTD-IRMOF-3; B: 50 μg/mL NCTD; C: 50 μg/mL NCTD-IRMOF-3-Gel; D: 25 μg/mL NCTD-IRMOF-3; E: 25 μg/mL NCTD; F: 25 μg/mL NCTD-IRMOF-3-Gel.

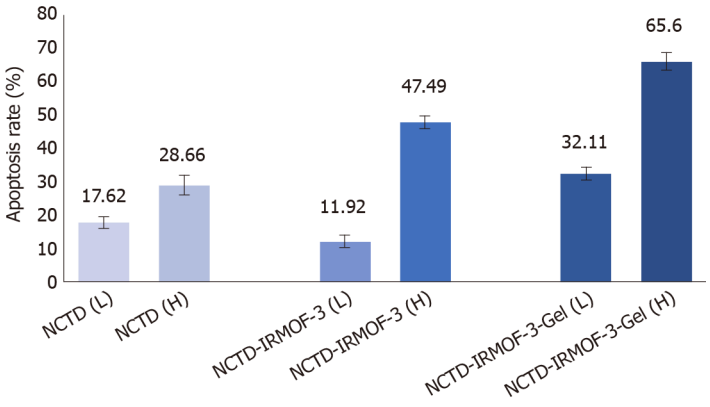

Figure 6 Apoptosis rates of norcantharidin (NCTD), NCTD-loaded metal-organic framework IRMOF-3, and NCTD-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel. Compared with the norcantharidin (NCTD) control group, the apoptosis rate in the NCTD-loaded metal-organic framework IRMOF-3 coated with temperature-sensitive gel (NCTD-IRMOF-3-Gel) group was highest, which indicated that NCTD-IRMOF-3-Gel can induce the apoptosis of Hepa1-6 cells.

rates of NCTD-IRMOF-3-Gel at high concentrations and low concentrations were determined, which indicated that NCTD-IRMOF-3-Gel could induce the apoptosis of Hepa1-6 cells.

CONCLUSION

Based on the results of this study, NCTD-loaded IRMOF-3 nanoparticles incorporated into a thermosensitive gel appeared to be a useful tool for cancer treatment because of the enhanced inhibition rate of cancer cells and controlled release of drugs from these

nanocarriers. Our future studies will focus on elucidating the activity of the drug delivery system and its effects on the mechanism of action of the encapsulated anticancer drug.

ARTICLE HIGHLIGHTS

Research background

Norcantharidin (NCTD) is suitable for the treatment of primary liver cancer, especially early and middle primary liver cancer. As a new type of drug formulation, sustained- and controlled-release preparations can increase the efficacy and reduce the side effects compared with traditional drugs. Metal-organic frameworks (MOFs) have potential applications in drug carriers. The thermosensitive gel has a hydrophilic three-dimensional network structure, which can be loaded in the liquid state to control drug release.

Research motivation

The side effects of NCTD have limited its application in liver cancer, which has prompted the development of sustained- and controlled-release preparations.

Research objectives

This study established a liver-targeting therapy in which NCTD is loaded into IRMOF-3 coated with a thermosensitive gel (NCTD-IRMOF-3-Gel), which can be efficiently delivered to liver cancer cells and slowly released.

Research methods

NCTD-loaded IRMOF-3 coated with a temperature-sensitive gel (NCTD-IRMOF-3-Gel) was obtained by a coordination reaction. The apparent characteristics and *in vitro* release of NCTD-IRMOF-3-Gel were investigated. Cell cytotoxicity assays, flow cytometry and apoptosis experiments on mouse hepatoma (Hepa1-6.) cells were used to determine the anti-liver cancer activity of NCTD-IRMOF-3-Gel in *in vitro* models.

Research results

The particle size of NCTD-IRMOF-3-Gel was 50-100 nm, and the particle size distribution was uniform. The release curve showed that NCTD-IRMOF-3-Gel had an obvious sustained-release effect. The cytotoxicity assays showed that the free drug NCTD and NCTD-IRMOF-3-Gel treatments markedly inhibited Hepa1-6 cell proliferation, and with increasing drug concentrations, the inhibition rate increased. By flow cytometry, NCTD-IRMOF-3-Gel was observed to block the Hepa1-6 cell cycle in the S and G2/M phases, and the thermosensitive gel nanoparticles may inhibit cell proliferation by inducing cell cycle arrest. Apoptosis experiments showed that NCTD-IRMOF-3-Gel induced the apoptosis of Hepa1-6 cells.

Research conclusions

NCTD-loaded IRMOF-3 nanoparticles incorporated into a thermosensitive gel appeared to be a useful tool for cancer treatment because of the enhanced inhibition rate of cancer cells and controlled release of drugs from these nanocarriers.

Research perspectives

Thermosensitive gel-encapsulated IRMOF-3 has great advantages as an antitumor drug carrier and provides some ideas for passive targeting therapy of tumors.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Zhang X, Dong S and Xie GL for skillful technical assistance.

REFERENCES

1 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID:

29307467 DOI: 10.1016/S0140-6736(18)30010-2]
2 **Gao Q**, Zhu H, Dong L, Shi W, Chen R, Song Z, Huang C, Li J, Dong X, Zhou Y, Liu Q, Ma L, Wang X, Zhou J, Liu Y, Boja E, Robles AI, Ma W, Wang P, Li Y, Ding L, Wen B, Zhang B, Rodriguez H, Gao D, Zhou H, Fan J. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. *Cell* 2019; **179**: 561-577. e22 [PMID: 31585088 DOI: 10.1016/j.cell.2019.08.052]
3 **Orcutt ST**, Anaya DA. Liver Resection and Surgical Strategies for Management of Primary Liver Cancer. *Cancer Control* 2018; **25**: 1073274817744621 [PMID: 29327594 DOI: 10.1177/1073274817744621]
4 **Zachos I**, Zachou K, Dalekos GN, Tzortzis V. Management of Patients with Liver Cirrhosis and Invasive Bladder Cancer: A Case-series. *J Transl Int Med* 2019; **7**: 29-33 [PMID: 30997354 DOI: 10.2478/jtim-2019-0006]
5 **Power DG**, Kemeny NE. Chemotherapy for the conversion of unresectable colorectal cancer liver metastases to resection. *Crit Rev Oncol Hematol* 2011; **79**: 251-264 [PMID: 20970353 DOI: 10.1016/j.critrevonc.2010.08.001]
6 **Mohri J**, Katada C, Ueda M, Sugawara M, Yamashita K, Moriya H, Komori S, Hayakawa K, Koizumi W, Atsuda K. Predisposing Factors for Chemotherapy-induced Nephrotoxicity in Patients with Advanced Esophageal Cancer Who Received Combination Chemotherapy with Docetaxel, Cisplatin, and 5-fluorouracil. *J Transl Int Med* 2018; **6**: 32-37 [PMID: 29607302 DOI: 10.2478/jtim-2018-0007]
7 **Michael JS**, Lee BS, Zhang M, Yu JS. Nanotechnology for Treatment of Glioblastoma Multiforme. *J Transl Int Med* 2018; **6**: 128-133 [PMID: 30425948 DOI: 10.2478/jtim-2018-0025]
8 **Andrašina T**, Rohan T, Hustý J, Válek V. Interventional radiology therapies for liver cancer. *Cas Lek Cesk* 2018; **157**: 195-202 [PMID: 30189743]
9 **Chi J**, Jiang Z, Qiao J, Zhang W, Peng Y, Liu W, Han B. Antitumor evaluation of carboxymethyl chitosan based norcantharidin conjugates against gastric cancer as novel polymer therapeutics. *Int J Biol Macromol* 2019; **136**: 1-12 [PMID: 31158420 DOI: 10.1016/j.ijbiomac.2019.05.216]
10 **Lv H**, Li Y, Du H, Fang J, Song X, Zhang J. The Synthetic Compound Norcantharidin Induced Apoptosis in Mantle Cell Lymphoma In Vivo and In Vitro through the PI3K-Akt-NF- κ B Signaling Pathway. *Evid Based Complement Alternat Med* 2013; **2013**: 461487 [PMID: 23935664 DOI: 10.1155/2013/461487]
11 **Jin D**, Wu Y, Shao C, Gao Y, Wang D, Guo J. Norcantharidin reverses cisplatin resistance and inhibits the epithelial mesenchymal transition of human nonsmall lung cancer cells by regulating the YAP pathway. *Oncol Rep* 2018; **40**: 609-620 [PMID: 29901163 DOI: 10.3892/or.2018.6486]
12 **Zhang Z**, Yang L, Hou J, Xia X, Wang J, Ning Q, Jiang S. Promising positive liver targeting delivery system based on arabinogalactan-anchored polymeric micelles of norcantharidin. *Artif Cells Nanomed Biotechnol* 2018; **46**: S630-S640 [PMID: 30449176 DOI: 10.1080/21691401.2018.1505742]
13 **Zhou J**, Ren Y, Tan L, Song X, Wang M, Li Y, Cao Z, Guo C. Norcantharidin: research advances in pharmaceutical activities and derivatives in recent years. *Biomed Pharmacother* 2020; **131**: 110755 [PMID: 33152920 DOI: 10.1016/j.biopha.2020.110755]
14 **Deng L**, Tang S. Norcantharidin analogues: a patent review (2006 - 2010). *Expert Opin Ther Pat* 2011; **21**: 1743-1753 [PMID: 22017412 DOI: 10.1517/13543776.2011.629190]
15 **Mo L**, Zhang X, Shi X, Wei L, Zheng D, Li H, Gao J, Li J, Hu Z. Norcantharidin enhances antitumor immunity of GM-CSF prostate cancer cells vaccine by inducing apoptosis of regulatory T cells. *Cancer Sci* 2018; **109**: 2109-2118 [PMID: 29770533 DOI: 10.1111/cas.13639]
16 **Wei CM**, Wang BJ, Ma Y, Sun ZP, Li XL, Guo RC. [Pharmacokinetics and biodistribution of 3H-norcantharidin in mice]. *Yao Xue Xue Bao* 2007; **42**: 516-519 [PMID: 17703775]
17 **Zhang R**, Wang J, Yuan G, Wei C, Liu X, Wang B, Gao H, Guo R. Determination of norcantharidin in mouse tissues by liquid chromatography coupled to tandem mass spectrometry and its tissue distribution study. *Arzneimittelforschung* 2012; **62**: 290-294 [PMID: 22473525 DOI: 10.1055/s-0032-1308980]
18 **Chen YC**, Chang SC, Wu MH, Chuang KA, Wu JY, Tsai WJ, Kuo YC. Norcantharidin reduced cyclins and cytokines production in human peripheral blood mononuclear cells. *Life Sci* 2009; **84**: 218-226 [PMID: 19100750 DOI: 10.1016/j.lfs.2008.11.020]
19 **Zhang J**, Shen D, Jia M, Zhao H, Tang Y. The targeting effect of Hm^2E8b-NCTD-liposomes on B-lineage leukaemia stem cells is associated with the HLF-SLUG axis. *J Drug Target* 2018; **26**: 55-65 [PMID: 28627280 DOI: 10.1080/1061186X.2017.1339193]
20 **Zeng L**, Zhang Y. Development, optimization and *in vitro* evaluation of norcantharidin loadedself-nanoemulsifying drug delivery systems (NCTD-SNEDDS). *Pharm Dev Technol* 2017; **22**: 399-408 [PMID: 27487261 DOI: 10.1080/10837450.2016.1219915]
21 **Zeng L**, Liu Y, Pan J, Liu X. Formulation and evaluation of norcanthridin nanoemulsions against the Plutella xylostella (Lepidotera: Plutellidae). *BMC Biotechnol* 2019; **19**: 16 [PMID: 30871528 DOI: 10.1186/s12896-019-0508-8]
22 **Zhu J**, Zhang W, Wang D, Li S, Wu W. Preparation and characterization of norcantharidin liposomes modified with stearyl glycyrrhetinate. *Exp Ther Med* 2018; **16**: 1639-1646 [PMID: 30186382 DOI: 10.3892/etm.2018.6416]
23 **Li Y**, Li X, Guan Q, Zhang C, Xu T, Dong Y, Bai X, Zhang W. Strategy for chemotherapeutic delivery using a nanosized porous metal-organic framework with a central composite design. *Int J Nanomedicine* 2017; **12**: 1465-1474 [PMID: 28260892 DOI: 10.2147/IJN.S119115]

24 **Zhao H**, Hou S, Zhao X, Liu D. Adsorption and pH-responsive release of tinidazole on metal–organic framework CAU-1. *Chem Eng Data* 2019; **64**: 1851-1858 [DOI: 10.1021/acs.jced.9b00106]

25 **Lin SX**, Pan WL, Niu RJ, Liu Y, Chen JX, Zhang WH, Lang JP, Young DJ. Effective loading of cisplatin into a nanoscale UiO-66 metal-organic framework with preformed defects. *Dalton Trans* 2019; **48**: 5308-5314 [PMID: 30938739 DOI: 10.1039/c9dt00719a]

26 **Nezhad-Mokhtari P**, Arsalani N, Javanbakht S, Shaabani A. Development of gelatin microsphere encapsulated Cu-based metal-organic framework nanohybrid for the methotrexate delivery. *J Drug Deliv Sci Technol* 2019; **50**: 174-180 [DOI: 10.1016/j.jddst.2019.01.020]

27 **Nasrollahi P**, Khajeh K, Tamjid E, Taleb M, Soleimani M, Nie G. Sustained release of sodium deoxycholate from PLGA-PEG-PLGA thermosensitive polymer. *Artif Cells Nanomed Biotechnol* 2018; **46**: 1170-1177 [PMID: 29989444 DOI: 10.1080/21691401.2018.1481861]

28 **Abulateefeh SR**, Alkawareek MY, Alkilany AM. Tunable sustained release drug delivery system based on mononuclear aqueous core-polymer shell microcapsules. *Int J Pharm* 2019; **558**: 291-298 [PMID: 30641178 DOI: 10.1016/j.ijpharm.2019.01.006]

29 **Svirskis D**, Chandramouli K, Bhusal P, Wu Z, Alphonso J, Chow J, Patel D, Ramakrishna R, Yeo SJ, Stowers R, Hill A, Munro J, Young SW, Sharma M. Injectable thermosensitive gelling delivery system for the sustained release of lidocaine. *Ther Deliv* 2016; **7**: 359-368 [PMID: 27250538 DOI: 10.4155/tde-2016-0014]

30 **Liu Y**, Wang X, Liu Y, Di X. Thermosensitive In Situ Gel Based on Solid Dispersion for Rectal Delivery of Ibuprofen. *AAPS PharmSciTech* 2018; **19**: 338-347 [PMID: 28733828 DOI: 10.1208/s12249-017-0839-5]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4221-4235

DOI: 10.3748/wjg.v27.i26.4221

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Ubiquitin-specific protease 15 contributes to gastric cancer progression by regulating the Wnt/β-catenin signaling pathway

Min Zhong, Ling Zhou, Zhi Fang, Yang-Yang Yao, Jian-Ping Zou, Jian-Ping Xiong, Xiao-Jun Xiang, Jun Deng

ORCID number: Min Zhong 0000-0001-5423-0918; Ling Zhou 0000-0002-2131-1751; Zhi Fang 0000-0002-5612-0963; Yang-Yang Yao 0000-0001-6165-2879; Jian-Ping Zou 0000-0003-4151-4633; Jian-Ping Xiong 0000-0003-0398-6090; Xiao-Jun Xiang 0000-0002-8734-584X; Jun Deng 0000-0002-7644-5853.

Author contributions: Zhong M, Zhou L, and Fang Z contributed equally to this work; Zhong M performed data acquisition and drafted the manuscript; Zhou L and Fang Z completed the statistical analyses; Yao YY and Zou JP were involved in critically revising the manuscript for important intellectual content; Deng J, Xiong JP, and Xiang XJ conceived and designed the study.

Supported by National Natural Science Foundation of China, No. 81760432; Science and Technology Department of Jiangxi Province, No. 20202BBGL73036; and Jiangxi Provincial Outstanding Young Talents Projects, No. 20204BCJ23016.

Institutional review board statement: The study was reviewed and approved by the Medical Research Ethics Committee of the First Affiliated Hospital of Nanchang University.

Min Zhong, Ling Zhou, Zhi Fang, Jian-Ping Zou, Jun Deng, Department of Jiangxi Key Laboratory for Individualized Cancer Therapy, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

Yang-Yang Yao, Jian-Ping Xiong, Xiao-Jun Xiang, Department of Cancer Center, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

Corresponding author: Jun Deng, PhD, Assistant Professor, Department of Jiangxi Key Laboratory for Individualized Cancer Therapy, The First Affiliated Hospital of Nanchang University, No. 17 Yongwai Street, Nanchang 330006, Jiangxi Province, China.
dengjun19871106@ncu.edu.cn

Abstract

BACKGROUND

Ubiquitin-specific protease 15 (USP15) is an important member of the ubiquitin-specific protease family, the largest deubiquitinase subfamily, whose expression is dysregulated in many types of cancer. However, the biological function and the underlying mechanisms of USP15 in gastric cancer (GC) progression have not been elucidated.

AIM

To explore the biological role and underlying mechanisms of USP15 in GC progression.

METHODS

Bioinformatics databases and western blot analysis were utilized to determine the expression of USP15 in GC. Immunohistochemistry was performed to evaluate the correlation between USP15 expression and clinicopathological characteristics of patients with GC. A loss- and gain-of-function experiment was used to investigate the biological effects of USP15 on GC carcinogenesis. RNA sequencing, immunofluorescence, and western blotting were performed to explore the potential mechanism by which USP15 exerts its oncogenic functions.

RESULTS

USP15 was up-regulated in GC tissue and cell lines. The expression level of USP15 was positively correlated with clinical characteristics (tumor size, depth of invasion, lymph node involvement, tumor-node-metastasis stage, perineural invasion, and vascular invasion), and was related to poor prognosis. USP15

Institutional animal care and use committee statement: All animal experiments conformed to the internationally accepted principles for the care and use of laboratory animals [license No. SYXK (GAN) 2015-0001, Laboratory Animal Science Center of Nanchang University; protocol No. 2020-130, The Medical Research Ethics Committee of the First Affiliated Hospital of Nanchang University].

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

Data sharing statement: No additional data are available.

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

Manuscript source: Unsolicited manuscript

Specialty type: Oncology

Country/Territory of origin: China

Peer-review report's scientific quality classification

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

Received: March 8, 2021
Peer-review started: March 8, 2021
First decision: April 17, 2021

knockdown significantly inhibited cell proliferation, invasion and epithelial-mesenchymal transition (EMT) of GC *in vitro*, while overexpression of USP15 promoted these processes. Knockdown of USP15 inhibited tumor growth *in vivo*. Mechanistically, RNA sequencing analysis showed that USP15 regulated the Wnt signaling pathway in GC. Western blotting confirmed that USP15 silencing led to significant down-regulation of β-catenin and Wnt/β-catenin downstream genes (c-myc and cyclin D1), while overexpression of USP15 yielded an opposite result and USP15 mutation had no change. Immunofluorescence indicated that USP15 promoted nuclear translocation of β-catenin, suggesting activation of the Wnt/β-catenin signaling pathway, which may be the critical mechanism promoting GC progression. Finally, rescue experiments showed that the effect of USP15 on gastric cancer progression was dependent on Wnt/β-catenin pathway.

CONCLUSION

USP15 promotes cell proliferation, invasion and EMT progression of GC *via* regulating the Wnt/β-catenin pathway, which suggests that USP15 is a novel potential therapeutic target for GC.

Key Words: Ubiquitin-specific protease 15; Gastric cancer; Wnt/β-catenin; Cell proliferation; Cell invasion; Epithelial-mesenchymal transition

Core Tip: Ubiquitin-specific protease 15 (USP15) was upregulated in gastric cancer (GC) cells and tissues, and was associated with a poor prognosis in patients with GC. USP15 promoted cell proliferation, invasion, and epithelial-mesenchymal transition of GC cells *in vitro* and tumor growth *in vivo*. Mechanistic studies showed that USP15 functioned as a tumor promoter in GC by regulating the Wnt/β-catenin signaling pathway. Thus, USP15 is expected to be a novel potential target for GC therapy.

Citation: Zhong M, Zhou L, Fang Z, Yao YY, Zou JP, Xiong JP, Xiang XJ, Deng J. Ubiquitin-specific protease 15 contributes to gastric cancer progression by regulating the Wnt/β-catenin signaling pathway. *World J Gastroenterol* 2021; 27(26): 4221-4235
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4221.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4221

INTRODUCTION

Gastric cancer (GC) has a high incidence worldwide and is one of the main causes of cancer-related deaths, especially in China[1,2]. Although there have been great advances in surgical procedures and targeted chemotherapy in recent years, the results are still not satisfactory and the survival rate is low, with median overall survival (OS) less than 12 mo[3-5]. Therefore, identifying novel potential targets for GC diagnosis and therapy and elucidating the underlying mechanisms of disease progression are essential for the prevention and treatment of GC.

In recent years, increasing evidence has shown that ubiquitin-specific proteases (USPs), the largest deubiquitinase subfamily, plays an important role in GC. For example, USP14[6], USP42[7], and USP44[8] are upregulated in GC and can be used as independent prognostic markers in GC patients. USP15, one of the most important members of the USP family, has been found to have some amplifications in many tumors. The N terminus of the protein encoding USP15 includes a ubiquitin-specific protease (DUSP) domain and two ubiquitin-like (UBL) domains, which can specifically remove the substrate protein by monoubiquitination and polyubiquitination modification[9]. The active site of the USP15 protein is located at Cys-269, and mutation of Cys269 to Ser (USP15 C269S) can inhibit enzyme activity[10]. Previous studies have reported that USP15 is upregulated in the liver and pancreatic cancer, and is associated with poor prognosis[11,12]. Mechanistically, USP15 can activate the transforming growth factor β (TGF-β) signaling pathway and promote the progression of advanced malignant glioma by combining the SMAD-specific E3 ubiquitin protein ligase 2

Revised: April 27, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 14, 2021

P-Reviewer: Appiah-Kubi K, Nakajima N
S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Xing YX

complex and deubiquitinating and thus stabilizing the TGF b type I receptor[13]. In addition, USP15 can negatively regulate the function of p53 through affecting de-ubiquitination and stabilizing MDM2. Interestingly, inhibiting the activity of USP15 can induce tumor apoptosis and improve the antitumor T-cell response[14]. However, the role of USP15 in GC and its potential mechanisms have not been identified.

The Wnt/β-catenin signaling pathway is involved in many cellular processes such as tumor growth, differentiation and invasion, and tumorigenesis[15]. It is often activated in many types of cancer, and the nuclear accumulation of β-catenin is an important sign of Wnt signaling activation[16]. The activation of β-catenin can activate many oncogenes including c-myc and cyclin D1, and regulate cell proliferation, cell cycle progression and apoptosis during tumorigenesis[17-19]. However, the mechanisms of Wnt/β-catenin activation in GC have not been fully elucidated.

We found that USP15 was upregulated in GC cells and tissues, and was associated with a poor prognosis in GC patients. USP15 promoted cell proliferation, invasion, and epithelial-mesenchymal transition (EMT) of GC cells *in vitro* and tumor growth *in vivo*. Mechanistic studies showed that USP15 functioned as a tumor promoter in GC by regulating the Wnt/β-catenin signaling pathway. Thus, USP15 is expected to be a novel potential target for GC therapy.

MATERIALS AND METHODS

Tissue samples

Paraffin-embedded GC samples, including cancerous tissues (n = 115) and adjacent tissues (n = 30), from May 2011 and May 2013, were obtained from the First Affiliated Hospital of Nanchang University (Nanchang, China). The clinicopathological characteristics of these patients are shown in Table 1. The fresh GC tissues (n = 8) and corresponding adjacent noncancerous tissues were stored in liquid nitrogen until use. This study obtained ethical approval from the Human Research Ethics Committee of the First Affiliated Hospital of Nanchang University.

Cell lines and culture

Human GC cell lines (SGC-7901, HGC-27, MKN-45, MGC-803, BGC-823, and AGS) and the human immortalized gastric epithelial cell line (GES-1) were purchased from the Beijing Beina Chuanglian Institute of Biotechnology (Beijing, China). The cells were cultured in (RPMI-1640) or Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS; HyClone, Logan, UT, United States) in an incubator with 5% CO_2 at 37 °C.

Immunohistochemistry

Immunostaining of USP15 proteins in 115 clinical GC samples followed previously described methods[20]. A primary antibody against USP15 (1:100, #66310; Cell Signaling Technology, Danvers, MA, United States) was used to detect the expression of USP15. All staining scores were evaluated blindly by two pathologists based on staining intensity and positive staining ratio. The grading standard of immunohistochemistry was carried out as previously described [20].

Cell Counting Kit-8 assay and colony formation assay

At 48 h after transfection, 2000 GC cells per well were seeded into a 96-well plate for the Cell Counting Kit-8 (CCK-8) assay and 1000 GC cells per well were seeded into a 6-well plate for the colony formation assay as previously described[21].

Wound healing assay

At 48 h after transfection, 5 × 10^5 GC cells per well were seeded into a 6-well plate, and the cells were starved for 24 h until complete fusion. Straight lines were drawn with a sterile 10-μL pipette tip to form wounds. Then the cells were carefully washed with phosphate-buffered saline (PBS) and cultured in serum-free medium. Images were captured at 0, 24, and 48 h to assess wound closure.

Transwell assay

The transwell assay was performed *via* using 8-μm transwell chambers (Merck KGaA, Darmstadt, Germany) with or without 60 μL Matrigel gel (BD Biosciences, Hercules, CA, United States), and then the chambers were put in each well of a 24-well plate. Cells of each group (5 × 10^4) were placed in 200 μL serum-free medium for 48 h after transfection, and subsequently transferred to the upper compartment of the above

Table 1 Ubiquitin-specific protease 15 expression and clinicopathological characteristics of gastric cancer patients

Parameters	*n*	USP15 expression		*P* value
		low	High	
Gender				
Male	62	20	42	0.218
Female	53	23	30	
Age in year				
≤ 60	53	18	35	0.482
> 60	62	25	37	
Differentiation				
Poor	69	25	44	0.753
Moderate/well	46	18	28	
Tumor size in cm				
≤ 4	66	32	34	0.004
> 4	49	11	38	
TNM stage				
I + II	58	28	30	0.015
III + IV	57	15	42	
Depth of invasion				
T1 + T2	54	27	27	0.009
T3 + T4	61	16	45	
LNI				
N0	43	24	19	0.002
N1 + N2+N3	72	19	53	
Perineural invasion				
No	51	25	26	0.021
Yes	64	18	46	
Vascular invasion				
No	50	27	23	0.001
Yes	65	16	49	
Total	115	43	72	

GC: Gastric cancer; LNI: Lymph node involvement; TNM: Tumor-node-metastasis; USP15: Ubiquitin-specific protease 15.

chambers. The lower chamber contained RPMI-1640 with 10% FBS. After 36 h of incubation, the cells that had migrated or invaded to the bottom side of the chamber were fixed with methanol, and then stained with crystal violet.

Immunofluorescence

We dipped the coverslip into the culture medium to allow the cells to attach and grow, and then washed the cells three times with PBS. At room temperature, the cells were fixed on a coverslip with 4% tetraformaldehyde for 20 min, and then were washed again three times with PBS. After a 10 min incubation with 0.5% Triton X-100, the cells were blocked in 5% bovine serum albumin for 2 h and then were incubated with anti-β-catenin antibody (1:200 dilution; Cell Signaling Technology) at 4 °C. After washing three times with PBS, cells were incubated with secondary antibody (1:50 dilution, ab150077; Abcam, Cambridge, MA, United States) for 1 h at room temperature. The coverslips were subsequently washed three times with PBS and then were stained with 4′,6-diamidino-2-phenylindole (DAPI). Fluorescence images were captured *via*

laser confocal microscopy.

Western blotting

Western blotting was performed following as previously described[22]. The following primary antibodies were used: USP15 (1:2000, #66310; Cell Signaling Technology), E-cadherin (1:1000, ab1416; Abcam), N-cadherin (1:1000, ab18203; Abcam), vimentin (1:1500, ab8978; Abcam), c-Myc (1:1500, #5605; Cell Signaling Technology), β-catenin (1:2000, #8480; Cell Signaling Technology), cyclin D1 (1:1000, #2978; Cell Signaling Technology), and GAPDH (1:2000, #60004-1-Ig; Proteintech, Rosemont, IL, United States).

Plasmid construction and cell transfection

To knock down the expression of USP15, three different small interfering RNAs (siRNAs) and a negative control (NC) were designed as followed: USP15-Homo-249, 5′-GGAACACCUUAUUGAUGAATT-3′; USP15-Homo-1150, 5′-GCAGAUGGAAGGC-CAGAUATT-3′; USP15-HoMo-1382, 5′-CCAAACCUAUGCAGUACAATT-3′; and a NC siRNA, 5′-UUCUCCGAACGUGUCACGUTT-3′. USP15 overexpression plasmid (USP15: NM_006313.2) and USP15 mutated plasmid (USP15-C269S) were based on pcDNA3.1 plasmid. The above siRNA and plasmid were synthesized by GenePharma (Suzhou, China). Cells were grown to 50%–60% confluency and transfected using TurboFect transfection reagent (R0532; Thermo Scientific Scientific, Waltham, MA, United States).

RNA sequencing analysis

The isolated USP15 knockdown and control BGC-823 cells were used for cDNA amplification and RNA sequencing (RNA-seq) library preparation. RNA-seq was performed by Beijing Novel Bioinformatics Co. Ltd. (Beijing, China). Genes with a false discovery rate < 5% and a fold change > 2.0 that met the established threshold criteria were considered to be significantly differentially expressed.

Nude mouse tumor cell xenograft assay

Short hairpin RNAs (shRNAs) targeting USP15 or scramble shRNAs were subcloned into the lentiviral expression vector (Jikai Co. Shanghai, China). BGC□823 cells transfected with LV-shUSP15 or LV-scramble shRNA was stably expressed and screened by puromycin. The stably expressed strain was amplified and inoculated at a rate of 5×10^6 cells per animal into 5- to 6-wk-old BALB/c-nu mice. Tumor volume was measured every 3 d and calculated according to the formula: volume (mm^3) = (length × $width^2$)/2. Mice were sacrificed after 28 d and xenograft tumors were measured and weighed. Proteins were extracted from tumors and USP15 and β-catenin expression was detected by western blotting.

Statistical analyses

We statistically analyzed the data using SPSS version 26.0 software (Chicago, IL, United States). The relationship between clinical characteristics and USP15 expression was evaluated by the χ^2 test. The Kaplan–Meier method was performed to determine the OS curve of all enrolled GC patients. Student's *t*-test was used to determine the mean difference between two groups. $P < 0.05$ was considered statistically significant.

RESULTS

USP15 is upregulated in GC cells and tissues and is associated with a poor prognosis in patients with GC

First, an online database, cBioPortal for Cancer Genomics (http://www.cbioportal.org/)[23], showed that USP15 was amplified in many types of tumor including GC (Figure 1A). TIMER (https://cistrome.shinyapps.io/timer/)[24] and UALCAN database (http://ualcan.path.uab.edu/)[25], based on The Cancer Genome Atlas (TCGA) database, indicated that the mRNA levels of USP15 in GC tissues were higher than those in normal tissues (Figure 1B and C). To confirm the protein expression level of USP15 in GC, western blotting was conducted on GC cell lines and tissues. Most GC cell lines expressed a higher level of USP15 than the gastric epithelial cell line GES-1 (Figure 1D). USP15 was elevated in most of the eight pairs of clinical GC tissues and their adjacent normal tissues (Figure 1E).

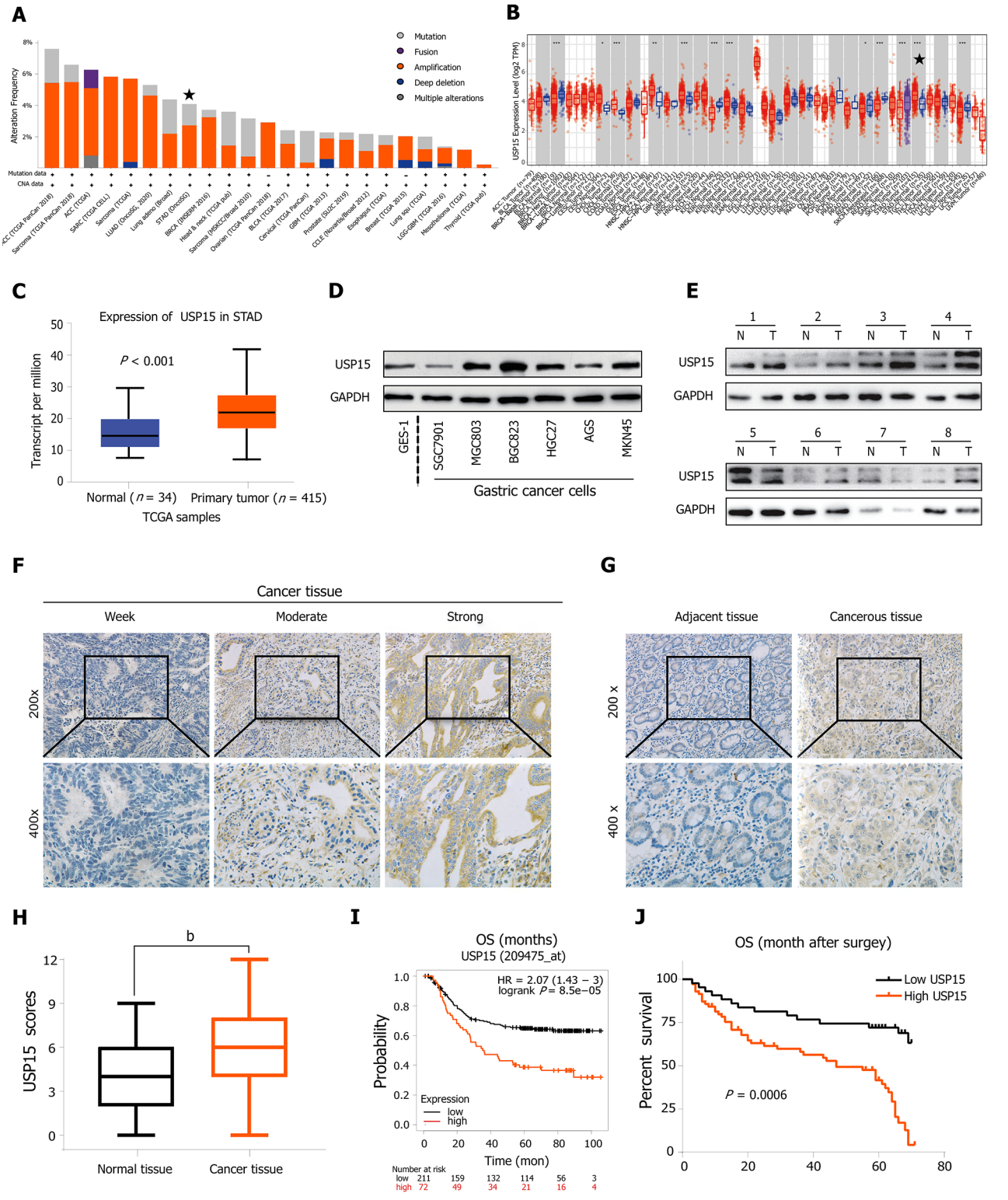

Figure 1 Ubiquitin-specific protease 15 is upregulated in gastric cancer cells and tissues, and is associated with a poor prognosis in gastric cancer patients. A: Frequency of ubiquitin-specific protease 15 (USP15) in various cancers, including gastric cancer (GC). The data were derived from the cBioPortal for Cancer Genomics; B, C: USP15 mRNA expression level of cancer tissue and normal tissue in GC. Analysis of The Cancer Genome Atlas data from TIMER and UALCAN databases; D: Protein expression of USP15 in GES-1 cell line and GC cell lines; E: Protein expression of USP15 in GC tissue and adjacent noncancerous tissues (n = 8); F, G: Representative images of immunohistochemical staining of USP15 in human GC tissues and adjacent noncancerous tissues; H: USP15 staining scores of GC and normal tissues; I: Correlation between USP15 expression level and overall survival (OS) in GC patients obtained from Kaplan–Meier Plotter databases (209475_at, HR = 2.07 (1.43-3), log rank $P = 8.5e^{-05}$); J: Kaplan–Meier curve stratified by USP15 expression in 115 GC patients (log-rank test, P = 0.0006). $^{b}P < 0.01$, data were expressed as the mean ± standard error of the mean.

As USP15 was found to be upregulated in GC, we confirmed its clinical significance *via* using immunohistochemistry. The staining of USP15 protein ranged from weak to strong and located in the cytoplasm (Figure 1F), which showed that USP15 was markedly increased in GC tissue sections, whereas USP15 staining was weak or negative in noncancerous tissue sections (Figure 1G). The staining scores of USP15 in adjacent tissues were significantly lower than those in GC tissues, which were considered significantly different (Figure 1H).

Subsequently, we evaluated the correlation between the staining score of USP15 and the clinicopathological characteristics of patients. There was no significant difference among patient gender, age, differentiation, and USP15 expression; however, tumor size (P = 0.004), tumor-node-metastasis (TNM) stage (P = 0.015), depth of invasion (P = 0.009), lymph node involvement (LNI) (P = 0.002), perineural invasion (P = 0.021), and vascular invasion (P = 0.001) were significantly associated with USP15 expression in GC. Consistent with the results obtained from Kaplan–Meier Plotter Database Analysis[26] (http://kmplot.com/analysis/) , the Kaplan–Meier curve stratified by USP15 expression in these 115 GC patients showed that patients with lower USP15 expression had longer OS (Figure 1I and J).

Knockdown of USP15 inhibits cell proliferation, invasion, and EMT progression of GC in vitro

As shown above, BGC-823 and MKN-45 cells had high expression of USP15. siRNA-mediated knockdown of USP15 expression in BGC-823 and MKN-45 cells was used to detect the function of USP15 *in vitro*. Western blotting was used to confirm the silencing efficiency of USP15 in GC cells (Figure 2A). The results of CCK-8 and colony formation assays showed that the proliferation rate and colony formation ability were markedly decreased in the USP15-siRNA-1/2 group compared to the NC group (Figure 2B and C). Based on correlation of USP15 expression and lymph node status, perineural and vascular invasion, wound healing and transwell assays were used to evaluate the role of USP15 in tumor cell migration and invasion. As shown in Figure 2D and Figure 2E, USP15 silencing suppressed GC cell migration and invasion. In addition, knockdown of USP15 upregulated E-cadherin and downregulated N-cadherin and vimentin (Figure 2F).

USP15 overexpression promotes cell proliferation, invasion, and EMT progression in GC

We explored the cellular behavioral changes caused by overexpression of USP15. A stably transfected cell line with USP15 overexpression plasmid, USP15 mutant plasmid (USP15-C269S), and a NC (empty-vector) cell line were established in SGC7901 cells. Western blotting confirmed the transfection efficiency (Figure 3A). Compared with the empty vector group, proliferation of the USP15 group was significantly enhanced, while the USP15-C269S group had no changes (Figure 3B and C). Overexpression of USP15 promoted GC cell migration and invasion, while USP15-C269S did not (Figure 3D and E). Western blotting analysis showed that overexpression of USP15 upregulated vimentin and N-cadherin but downregulated E-cadherin (Figure 3F). Collectively, these data demonstrated that USP15 overexpression promoted GC proliferation, invasion, and EMT progression.

USP15 regulates the Wnt/β-catenin pathway in GC cells

To explore the potential molecular mechanism responsible for the effects of USP15 on GC progression, the whole transcriptome profiles of BGC-823 cells with USP15 knockdown or NC were analyzed by RNA-seq. The transfection efficiency was confirmed by western blotting (Figure 4A). The most differentially expressed genes (DEGs) (29829) were displayed on the heat map (Figure 4B). Among the 2343 significant DEGs (adjusted $P < 0.05$), transcripts of 1134 genes were upregulated and transcripts of 1209 were downregulated in USP15 knockdown groups compared to the control groups (Figure 4C). Gene Ontology enrichment analyses showed that the difference in Wnt signaling pathway was the most obvious (Figure 4D) among the enriched pathways.

As one of the most classic Wnt signaling pathways, the Wnt/β-catenin pathway has been involved in multiple physiological processes of GC progression. To confirm the role of the Wnt/β-catenin signaling pathway in the malignant biological behavior in GC mediated by USP15, western blotting was performed to investigate expression of β-catenin and Wnt/β-catenin downstream genes (including c-myc and cyclin D1). USP15 knockdown resulted in downregulation of the protein level of β-catenin, c-Myc and cyclin D1, while USP15 overexpression yielded opposite results and there was no

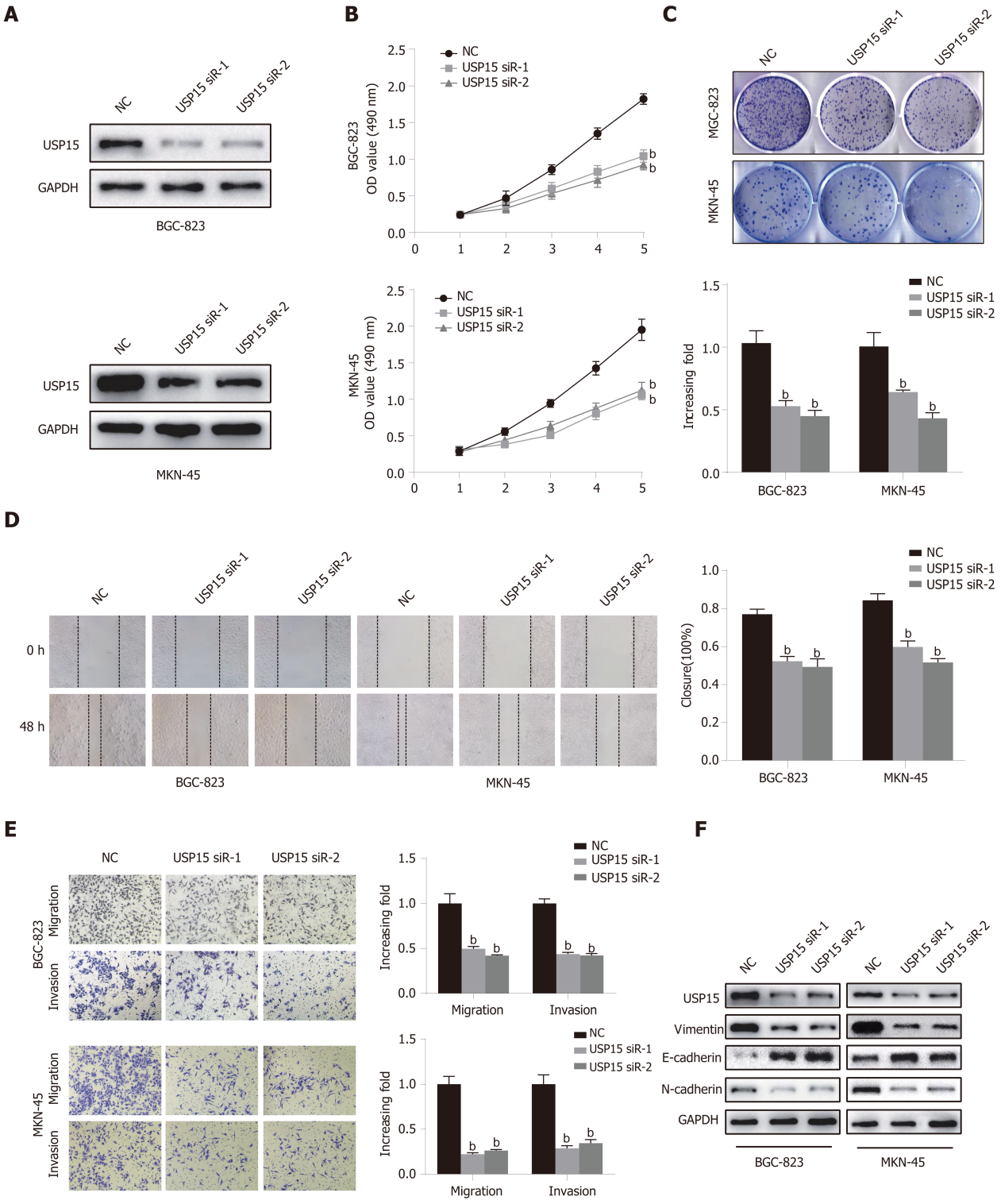

Figure 2 Knockdown of ubiquitin-specific protease 15 inhibits cell proliferation, invasion, and epithelial-mesenchymal transition progression of gastric cancer *in vitro*. A: BGC-823 and MKN-45 cells were transfected with ubiquitin-specific protease 15 (USP15) small interfering RNA or negative control for 48 h, and the efficiency was detected by Western blotting; B and C: Cell Counting Kit-8 assay and colony formation assay evaluated cell proliferation ability; D and E: Wound healing assay and transwell assays evaluated migration and invasion; F: Western blotting detected epithelial-mesenchymal transition markers (E-cadherin, vimentin, and N-cadherin). $^{b}P < 0.01$, data were expressed as mean ± standard error of the mean.

change in USP15 C269S group (Figure 4E). In addition, immunofluorescence assay showed that USP15 knockdown significantly reduced nuclear β-catenin accumulation compared with the control groups, while USP15 overexpression yielded opposite results, and there was no change in the USP15 C269S group (Figure 4F).

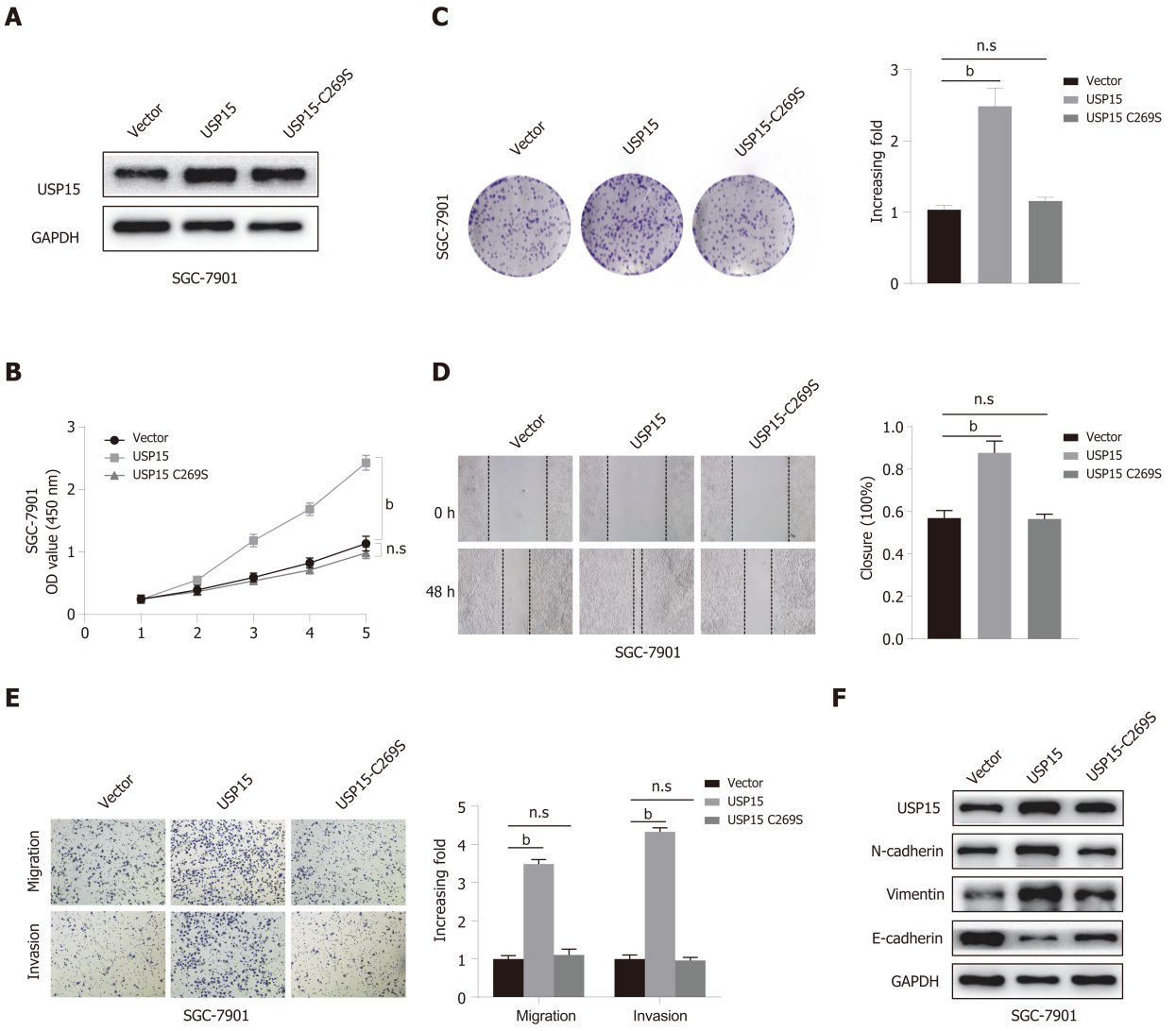

Figure 3 Overexpression of ubiquitin-specific protease 15 promotes proliferation, invasion, and epithelial–mesenchymal transition of gastric cancer cells. A: SGC-7901 cells were transfected with ubiquitin-specific protease 15 (USP15) overexpression plasmid, USP15-C269S plasmid (USP15 mutant) or empty vector for 48 h, and the efficiency was detected by Western blotting; B, C: Cell Counting Kit-8 assay and colony formation assay evaluated cell proliferation ability; D, E: Wound healing assay and transwell assay evaluated migration and invasion; F: Western blotting detected epithelial–mesenchymal transition markers (E-cadherin, vimentin, and N-cadherin). $^{b}P < 0.01$, data are expressed as the mean ± standard error of the mean. n.s: Not significant.

Lithium chloride partly reversed the effects of USP15 knockdown on GC progression

To further clarify whether the function of USP15 in GC was mediated by the Wnt/β-catenin pathway, we performed a rescue experiment using lithium chloride (LiCl) (Wnt/β-catenin pathway activator). The cell proliferation ability of BGC-823 and MKN-45 cells transfected with USP15 siRNA-1 was significantly elevated after treatment with LiCl compared to the untreated group (Figure 5A and B). Furthermore, the inhibition of invasion by USP15 knockdown can also be partly reversed by LiCl (Figure 5C). In addition, LiCl-treatment induced upregulation of β-catenin, c-myc, and cyclin D1 (Figure 5D). The above findings suggest that the function of USP15 on GC progression is dependent on Wnt/β-catenin pathway.

USP15 knockdown inhibits tumor growth in vivo

We investigated the function of USP15 *in vivo*. BGC-823 cells transfected with LV-shUSP15 or LV-scramble shRNA were subcutaneously injected into nude mice to establish a xenograft mouse model. After the mice were sacrificed on day 29, we obtained tumor images (Figure 6A). Compared with the scramble shRNA group, USP15 knockdown reduced tumor volume and weight (Figure 6B and C). In addition, USP15 knockdown significantly reduced the protein levels of β-catenin, c-myc, and cyclin D1 in tumor tissue of nude mouse, consistent with the *in vitro* results

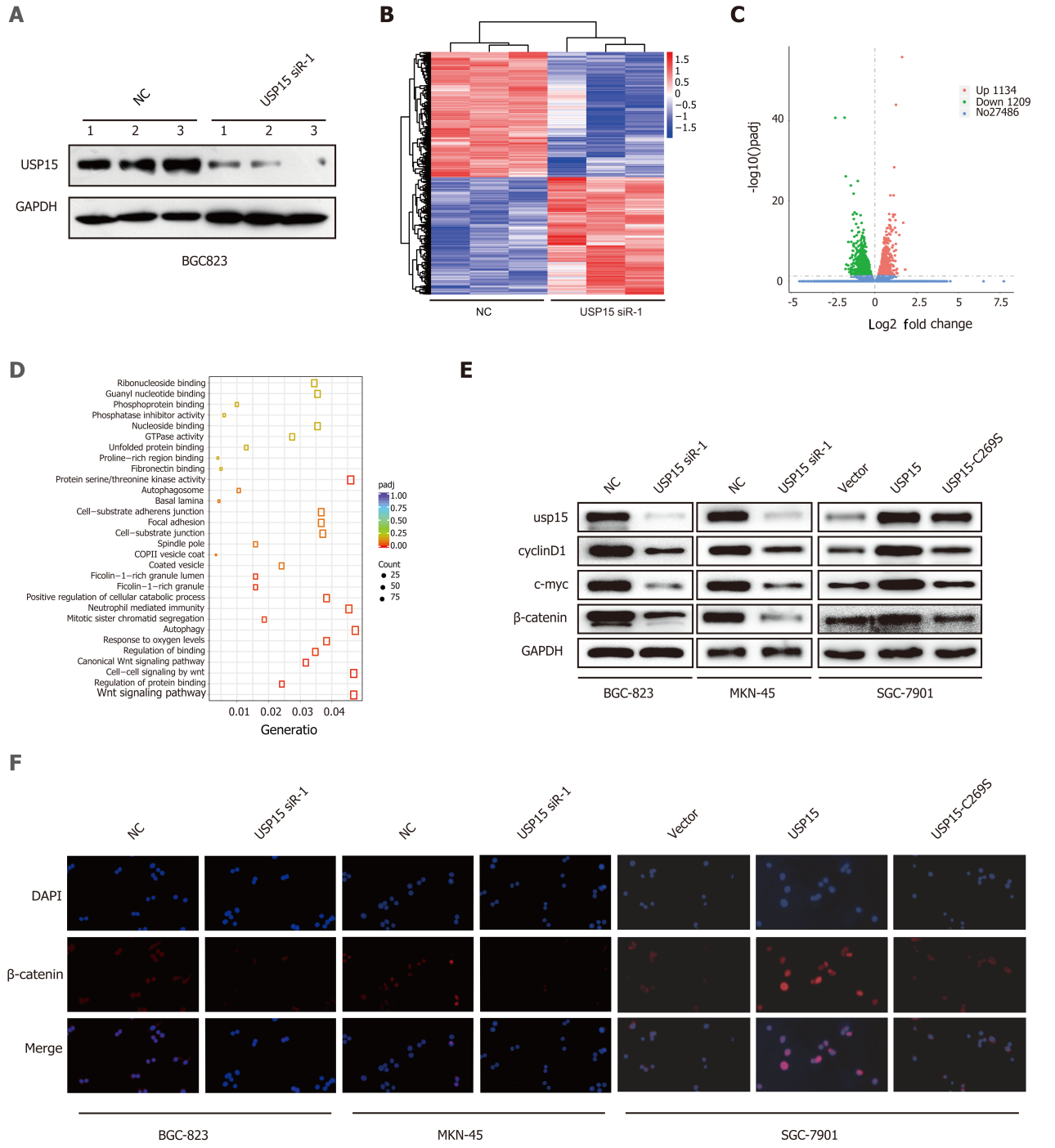

Figure 4 Ubiquitin-specific protease 15 regulates the Wnt/β-catenin pathway in gastric cancer. A: Western blotting confirmed the transfection efficiency of ubiquitin-specific protease 15 (USP15) knockdown in BGC-823 cell lines before RNA sequencing analysis; B: Heat map and hierarchical clustering based on the most differentially expressed genes (5739); C: Volcano plot illustrated differentially regulated gene expression. 1134 upregulated genes (red) and 1209 downregulated genes (green); D: Gene Ontology enrichment analyses of targets associated with USP15 for biological process, cellular component, and molecular function (top 10 most significantly affected categories are shown); E: Expression levels of USP15, β-catenin, c-Myc, and cyclin D1 in BGC-823 and MKN-45 cell lines transfected with USP15 knockdown or SGC-7901 cell lines transfected with USP15 overexpression; F: Immunofluorescence staining of β-catenin in BGC-823 and MKN-45 cell lines transfected with USP15 knockdown. Blue: DAPI; Red: β-catenin. Bar = 50 μm.

(Figure 6D).

DISCUSSION

In recent years, an increasing number of USP proteins have been reported to be critical to human cancers. For example, high expression of USP28 is related to the OS of patients with non-small cell lung cancer[27], while the expression of USP22 and USP11

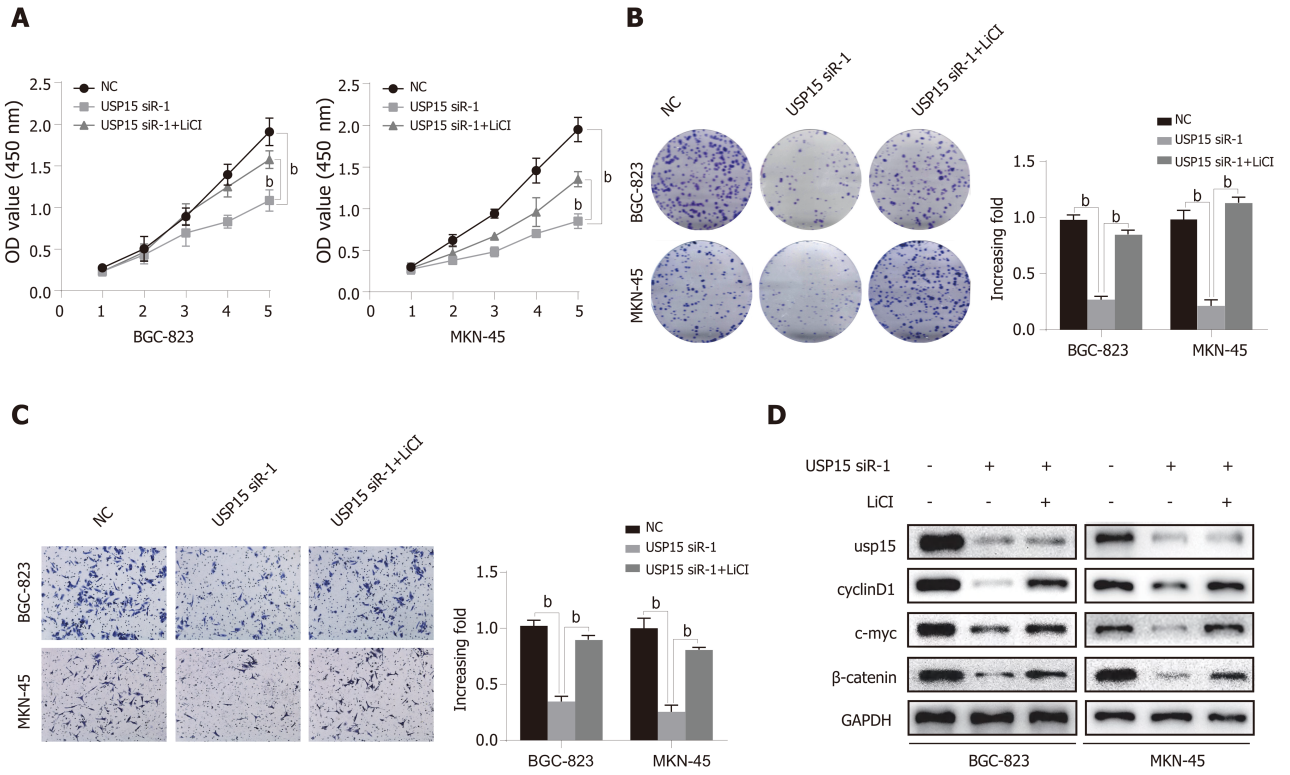

Figure 5 Lithium chloride partly reverses the effects of ubiquitin-specific protease 15 knockdown on gastric cancer progression. BGC-823 and MKN-45 cells transfected with ubiquitin-specific protease 15 (USP15) small interfering RNA-1 or negative control were incubated with or without lithium chloride (LiCl) (20 mmol/L). A, B: The Cell Counting Kit-8 (CCK-8) assay and colony formation assay evaluated cell proliferation ability; C: Transwell assays evaluated cell invasion ability; D: Western blotting detected the protein expression of β-catenin, c-Myc, and cyclin D1. $^{b}P < 0.01$, data are expressed as the mean ± standard error of the mean.

is related to the poor prognosis of breast cancer[28,29]. Two recent studies have shown that USP15 is upregulated in liver cancer and pancreatic ductal cell carcinoma[11,12]. In this study, IHC analyses showed that the high expression of USP15 was closely related to the depth of invasion, LNI, TNM stage, which indicated that USP15 acted as an oncogene, thereby promoting GC invasion, metastasis, and progression. In addition, the high expression of USP15 was related to the poor survival rate of GC patients, suggesting that USP15 is very important in the pathogenesis and development of GC, and could be used as a prognostic biomarker.

Similar to previous results[11,12], our study confirmed that USP15 was significantly associated with tumor cell proliferation *in vitro*. In addition, we also found that USP15 could participate in the tumor growth *in vivo*. Subsequently, we further found that USP15 can significantly promote the migration and invasion of GC cells *in vitro*. Migration and invasion, as the basic characteristics of malignant tumors, are the main reasons for the short survival time of cancer patients[30,31]. Mounting evidence has shown that tumor cells after EMT has high motility and aggressiveness, among which E-cadherin, N-cadherin, and vimentin are important molecular markers[32]. In addition, the epithelial marker E-cadherin is downregulated, while the mesenchymal markers vimentin and N-cadherin are upregulated during EMT[32]. As shown in our results, knockdown of USP15 resulted in upregulation of E-cadherin and downregulation of N-cadherin and vimentin, while overexpression of USP15 had the opposite effects, suggesting that USP15 can induce EMT in GC cells. The above findings indicated that USP15 may promote cell proliferation, migration, invasion and EMT process to become an oncogene of GC.

USP15 was related to a variety of cell signaling events, including transforming growth factor β (TGF-β)[13,33], constitutive photomorphogenesis 9 (COP9) signaling body[34], p53 signaling pathway[14], and nuclear factor kappa B (NF-kB)[35]. For example, USP15 promotes the stabilization of TGF-β receptor and its downstream signal transducers, thereby resulting in enhanced TGF-β signaling[13,36]. USP15 can protect the constituent subunits of cullin-RING ubiquitin ligase from self-ubiquitination and degradation *via* a stable cooperation with COP9-signalosome[34,37]. USP15 can stabilize MDM2 and negatively regulate the protein level of p53, and inactivation of USP15 can induce tumor apoptosis and improve the antitumor T-cell

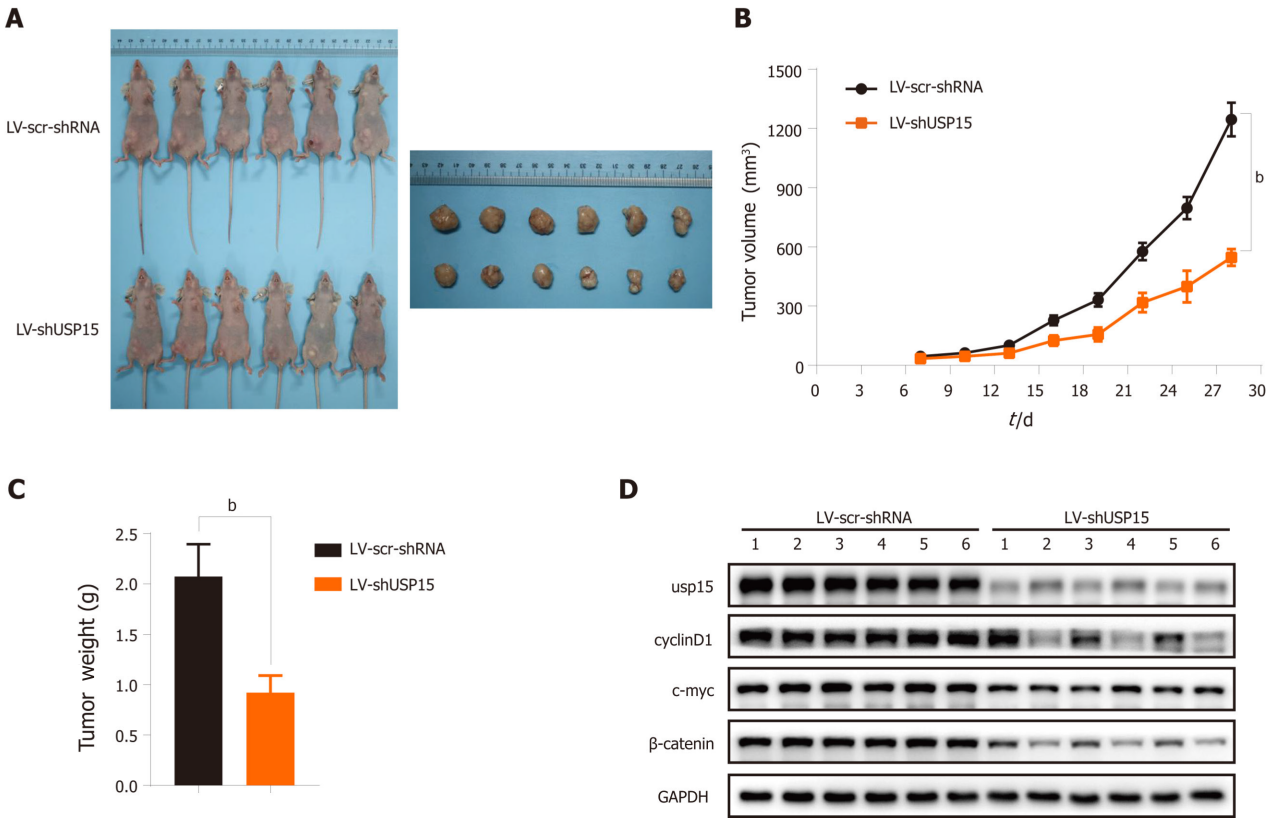

Figure 6 Ubiquitin-specific protease 15 silencing inhibits tumor growth *in vivo*. A: Cervical dislocation was used to sacrifice nude mice on day 29 and representative images of xenograft mouse samples were obtained (n = 6); B: Tumor volume was measured every 3 d and a growth curve was drawn; C: Tumor weight was measured when mice were sacrificed; D: Western blotting detected the protein expression of ubiquitin-specific protease 15, β-catenin, c-Myc, and cyclin D1 in tumor samples of two groups. $^{b}P < 0.01$, data are expressed as the mean ± standard error of the mean. USP15: Ubiquitin-specific protease 15.

response[14]. Another recent study showed that USP15 can effectively activate NF-κB by maintaining the stability of TAB2/3 differentially[38]. In our study, GO enrichment analysis based on RNA-Seq indicated that USP15 regulated the Wnt/β-catenin signaling pathway in GC. Previous studies have shown that abnormal activation of the Wnt/β-catenin pathway could promote the malignant progression of a variety of cancers, including GC[39,40]. Increased nuclear expression of β-catenin is an important sign of Wnt/β-catenin signaling pathway activation, which mainly depends on the transport of cytoplasmic β-catenin to the nucleus[39,40]. In our study, knockdown of USP15 significantly reduced the nuclear expression of β-catenin and downregulation of Wnt/β-catenin downstream genes in GC cells, while USP15 overexpression yielded opposite results, and there was no change in the USP15 C269S group (USP15 mutant), indicating that USP15 acted as a Wnt/β-catenin pathway activator. A rescue experiment by using LiCl (a Wnt/β-catenin pathway activator) showed that the effect of USP15 on GC progression was dependent on Wnt/β-catenin pathway. All of these findings suggest that USP15 contributes to GC progression by regulating the Wnt/β-catenin signaling pathway.

To the best of our knowledge, this study is the first to explore the clinical significance and molecular function of USP15 in GC. However, our research had some limitations. This was a retrospective study that included a small number of GC patients from a single center in our hospital, so there may have been a degree of bias. In the future, a large multicenter study should be conducted to verify our results. In addition, although GeneMANIA[41], a protein interaction bioinformatics website, predicts that USP15 can interact with some upstream proteins (CTNNB1, NUSAP1) of the Wnt/β-catenin pathway, the specific molecular mechanism problems need to be resolved in future research.

CONCLUSION

In conclusion, the results presented in our study demonstrated that USP15 was

upregulated in GC cells and tissues, and was associated with a poor prognosis in patients with GC. Furthermore, USP15 promoted cell proliferation, invasion, and EMT progression *via* the Wnt/β-catenin signaling pathway *in vitro* and promoted the growth of GC cells *in vivo*. All of our findings shed light on USP15 as a novel promising therapeutic target for understanding the pathogenesis of GC, providing new insights into the development of novel strategies for diagnosis and treatment from the bench to clinic.

ARTICLE HIGHLIGHTS

Research background

Ubiquitin-specific protease 15 (USP15) is an important member of the ubiquitin-specific protease (USP) family, whose expression is dysregulated in many types of cancer. However, the function role and the underlying mechanism of USP15 in gastric cancer (GC) progression have not yet been elucidated.

Research motivation

To explore the underlying mechanisms of GC development and discover biomarkers for the treatment of GC.

Research objectives

To investigate the role and potential mechanism of USP15 in GC.

Research methods

Bioinformatics databases and western blot analysis were utilized to determine the expression of USP15 in GC. Immunohistochemistry was performed to evaluate the correlation between expression of USP15 and clinicopathological characteristics of GC patients. A loss- and gain-of-function experiment was used to investigate the biological effects of USP15 on GC carcinogenesis. RNA sequencing analysis, immunofluorescence, and western blotting were performed to explore the potential mechanism by which USP15 exerted its oncogenic functions.

Research results

USP15 was upregulated in GC tissue and cell lines. The expression level of USP15 was positively correlated with clinical characteristics (tumor size, depth of invasion, lymph node involvement (LNI), tumor-node-metastasis (TNM) stage, perineural invasion, and vascular invasion), and was related to poor prognosis. USP15 knockdown significantly inhibited cell proliferation, invasion and epithelial-mesenchymal transition of GC *in vitro*, while overexpression of USP15 promoted these processes. Knockdown of USP15 inhibited tumor growth *in vivo*. Mechanistically, RNA-seq analysis showed that USP15 regulated the Wnt signaling pathway in GC. Western blotting confirmed that USP15 silencing led to significant downregulation of β-catenin and Wnt/β-catenin downstream genes (c-myc and cyclin D1), while overexpression of USP15 yielded the opposite results and USP15 mutation showed no change. Immunofluorescence indicated that USP15 promoted the nuclear translocation of β-catenin, suggesting activation of the Wnt/β-catenin signaling pathway, which may be the critical mechanism promoting GC progression. Finally, rescue experiments showed that the effects of USP15 on gastric cancer progression were dependent on the Wnt/β-catenin pathway.

Research conclusions

USP15 promotes cell proliferation, invasion, and EMT progression of GC *via* regulating the Wnt/β-catenin pathway.

Research perspectives

USP15 is expected to be a novel potential therapeutic target for GC.

REFERENCES

1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

2 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]
3 **Nagini S**. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. *World J Gastrointest Oncol* 2012; **4**: 156-169 [PMID: 22844547 DOI: 10.4251/wjgo.v4.i7.156]
4 **Sasako M**. Optimizing adjuvant therapies for the treatment of gastric cancer: with a special focus on Asia. *Expert Rev Anticancer Ther* 2019; **19**: 939-945 [PMID: 31661989 DOI: 10.1080/14737140.2019.1685877]
5 **Yoshikawa T**, Muro K, Shitara K, Oh DY, Kang YK, Chung HC, Kudo T, Chin K, Kadowaki S, Hamamoto Y, Hironaka S, Yoshida K, Yen CJ, Omuro Y, Bai LY, Maeda K, Ozeki A, Yoshikawa R, Kitagawa Y. Effect of First-line S-1 Plus Oxaliplatin With or Without Ramucirumab Followed by Paclitaxel Plus Ramucirumab on Advanced Gastric Cancer in East Asia: The Phase 2 RAINSTORM Randomized Clinical Trial. *JAMA Netw Open* 2019; **2**: e198243 [PMID: 31373648 DOI: 10.1001/jamanetworkopen.2019.8243]
6 **Fu Y**, Ma G, Liu G, Li B, Li H, Hao X, Liu L. USP14 as a novel prognostic marker promotes cisplatin resistance *via* Akt/ERK signaling pathways in gastric cancer. *Cancer Med* 2018; **7**: 5577-5588 [PMID: 30296012 DOI: 10.1002/cam4.1770]
7 **Hou K**, Zhu Z, Wang Y, Zhang C, Yu S, Zhu Q, Yan B. Overexpression and Biological Function of Ubiquitin-Specific Protease 42 in Gastric Cancer. *PLoS One* 2016; **11**: e0152997 [PMID: 27030989 DOI: 10.1371/journal.pone.0152997]
8 **Nishimura S**, Oki E, Ando K, Iimori M, Nakaji Y, Nakashima Y, Saeki H, Oda Y, Maehara Y. High ubiquitin-specific protease 44 expression induces DNA aneuploidy and provides independent prognostic information in gastric cancer. *Cancer Med* 2017; **6**: 1453-1464 [PMID: 28544703 DOI: 10.1002/cam4.1090]
9 **Teyra J**, Singer AU, Schmitges FW, Jaynes P, Kit Leng Lui S, Polyak MJ, Fodil N, Krieger JR, Tong J, Schwerdtfeger C, Brasher BB, Ceccarelli DFJ, Moffat J, Sicheri F, Moran MF, Gros P, Eichhorn PJA, Lenter M, Boehmelt G, Sidhu SS. Structural and Functional Characterization of Ubiquitin Variant Inhibitors of USP15. *Structure* 2019; **27**: 590-605. e5 [PMID: 30713027 DOI: 10.1016/j.str.2019.01.002]
10 **Peng Y**, Liao Q, Tan W, Peng C, Hu Z, Chen Y, Li Z, Li J, Zhen B, Zhu W, Li X, Yao Y, Song Q, Liu C, Qi X, He F, Pei H. The deubiquitylating enzyme USP15 regulates homologous recombination repair and cancer cell response to PARP inhibitors. *Nat Commun* 2019; **10**: 1224 [PMID: 30874560 DOI: 10.1038/s41467-019-09232-8]
11 **Yao XQ**, Li L, Piao LZ, Zhang GJ, Huang XZ, Wang Y, Liang ZL. Overexpression of Ubiquitin-Specific Protease15 (USP15) Promotes Tumor Growth and Inhibits Apoptosis and Correlated With Poor Disease-Free Survival in Hepatocellular Carcinoma. *Technol Cancer Res Treat* 2020; **19**: 1533033820967455 [PMID: 33267707 DOI: 10.1177/1533033820967455]
12 **Jiang B**, Zhou L, Lu J, Wang Y, Liu C, Liang Z, Zhou W, You L, Guo J. Clinicopathological and prognostic significance of ubiquitin-specific peptidase 15 and its relationship with transforming growth factor-β receptors in patients with pancreatic ductal adenocarcinoma. *J Gastroenterol Hepatol* 2021; **36**: 507-515 [PMID: 32875609 DOI: 10.1111/jgh.15244]
13 **Eichhorn PJ**, Rodón L, Gonzàlez-Juncà A, Dirac A, Gili M, Martínez-Sáez E, Aura C, Barba I, Peg V, Prat A, Cuartas I, Jimenez J, García-Dorado D, Sahuquillo J, Bernards R, Baselga J, Seoane J. USP15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. *Nat Med* 2012; **18**: 429-435 [PMID: 22344298 DOI: 10.1038/nm.2619]
14 **Zou Q**, Jin J, Hu H, Li HS, Romano S, Xiao Y, Nakaya M, Zhou X, Cheng X, Yang P, Lozano G, Zhu C, Watowich SS, Ullrich SE, Sun SC. USP15 stabilizes MDM2 to mediate cancer-cell survival and inhibit antitumor T cell responses. *Nat Immunol* 2014; **15**: 562-570 [PMID: 24777531 DOI: 10.1038/ni.2885]
15 **Clevers H**, Nusse R. Wnt/β-catenin signaling and disease. *Cell* 2012; **149**: 1192-1205 [PMID: 22682243 DOI: 10.1016/j.cell.2012.05.012]
16 **Ogasawara N**, Tsukamoto T, Mizoshita T, Inada K, Cao X, Takenaka Y, Joh T, Tatematsu M. Mutations and nuclear accumulation of beta-catenin correlate with intestinal phenotypic expression in human gastric cancer. *Histopathology* 2006; **49**: 612-621 [PMID: 17163846 DOI: 10.1111/j.1365-2559.2006.02560.x]
17 **Dihlmann S**, von Knebel Doeberitz M. Wnt/beta-catenin-pathway as a molecular target for future anti-cancer therapeutics. *Int J Cancer* 2005; **113**: 515-524 [PMID: 15472907 DOI: 10.1002/ijc.20609]
18 **He TC**, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. *Science* 1998; **281**: 1509-1512 [PMID: 9727977 DOI: 10.1126/science.281.5382.1509]
19 **Tetsu O**, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999; **398**: 422-426 [PMID: 10201372 DOI: 10.1038/18884]
20 **Fang Z**, Deng J, Zhang L, Xiang X, Yu F, Chen J, Feng M, Xiong J. TRIM24 promotes the aggression of gastric cancer *via* the Wnt/β-catenin signaling pathway. *Oncol Lett* 2017; **13**: 1797-1806 [PMID: 28454326 DOI: 10.3892/ol.2017.5604]
21 **Huang S**, Cao Y, Guo H, Yao Y, Li L, Chen J, Li J, Xiang X, Deng J, Xiong J. Up-regulated acylglycerol kinase (AGK) expression associates with gastric cancer progression through the formation of a novel YAP1-AGK-positive loop. *J Cell Mol Med* 2020; **24**: 11133-11145 [PMID: 32827244 DOI: 10.1111/jcmm.15613]

22 **Li L**, Zhao J, Huang S, Wang Y, Zhu L, Cao Y, Xiong J, Deng J. MiR-93-5p promotes gastric cancer-cell progression *via* inactivation of the Hippo signaling pathway. *Gene* 2018; **641**: 240-247 [PMID: 29045821 DOI: 10.1016/j.gene.2017.09.071]

23 **Gao J**, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013; **6**: pl1 [PMID: 23550210 DOI: 10.1126/scisignal.2004088]

24 **Li T**, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* 2020; **48**: W509-W514 [PMID: 32442275 DOI: 10.1093/nar/gkaa407]

25 **Chandrashekar DS**, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, Varambally S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 2017; **19**: 649-658 [PMID: 28732212 DOI: 10.1016/j.neo.2017.05.002]

26 **Szász AM**, Lánczky A, Nagy Á, Förster S, Hark K, Green JE, Boussioutas A, Busuttil R, Szabó A, Győrffy B. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. *Oncotarget* 2016; **7**: 49322-49333 [PMID: 27384994 DOI: 10.18632/oncotarget.10337]

27 **Zhang L**, Xu B, Qiang Y, Huang H, Wang C, Li D, Qian J. Overexpression of deubiquitinating enzyme USP28 promoted non-small cell lung cancer growth. *J Cell Mol Med* 2015; **19**: 799-805 [PMID: 25656529 DOI: 10.1111/jcmm.12426]

28 **Zhang Y**, Yao L, Zhang X, Ji H, Wang L, Sun S, Pang D. Elevated expression of USP22 in correlation with poor prognosis in patients with invasive breast cancer. *J Cancer Res Clin Oncol* 2011; **137**: 1245-1253 [PMID: 21691749 DOI: 10.1007/s00432-011-0998-9]

29 **Bayraktar S**, Gutierrez Barrera AM, Liu D, Pusztai L, Litton J, Valero V, Hunt K, Hortobagyi GN, Wu Y, Symmans F, Arun B. USP-11 as a predictive and prognostic factor following neoadjuvant therapy in women with breast cancer. *Cancer J* 2013; **19**: 10-17 [PMID: 23337751 DOI: 10.1097/PPO.0b013e3182801b3a]

30 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

31 **Jiang WG**, Sanders AJ, Katoh M, Ungefroren H, Gieseler F, Prince M, Thompson SK, Zollo M, Spano D, Dhawan P, Sliva D, Subbarayan PR, Sarkar M, Honoki K, Fujii H, Georgakilas AG, Amedei A, Niccolai E, Amin A, Ashraf SS, Ye L, Helferich WG, Yang X, Boosani CS, Guha G, Ciriolo MR, Aquilano K, Chen S, Azmi AS, Keith WN, Bilsland A, Bhakta D, Halicka D, Nowsheen S, Pantano F, Santini D. Tissue invasion and metastasis: Molecular, biological and clinical perspectives. *Semin Cancer Biol* 2015; **35** Suppl: S244-S275 [PMID: 25865774 DOI: 10.1016/j.semcancer.2015.03.008]

32 **Zeisberg M**, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest* 2009; **119**: 1429-1437 [PMID: 19487819 DOI: 10.1172/JCI36183]

33 **Iyengar PV**, Jaynes P, Rodon L, Lama D, Law KP, Lim YP, Verma C, Seoane J, Eichhorn PJ. USP15 regulates SMURF2 kinetics through C-lobe mediated deubiquitination. *Sci Rep* 2015; **5**: 14733 [PMID: 26435193 DOI: 10.1038/srep14733]

34 **Hetfeld BK**, Helfrich A, Kapelari B, Scheel H, Hofmann K, Guterman A, Glickman M, Schade R, Kloetzel PM, Dubiel W. The zinc finger of the CSN-associated deubiquitinating enzyme USP15 is essential to rescue the E3 Ligase Rbx1. *Curr Biol* 2005; **15**: 1217-1221 [PMID: 16005295 DOI: 10.1016/j.cub.2005.05.059]

35 **Schweitzer K**, Bozko PM, Dubiel W, Naumann M. CSN controls NF-kappaB by deubiquitinylation of IkappaBalpha. *EMBO J* 2007; **26**: 1532-1541 [PMID: 17318178 DOI: 10.1038/sj.emboj.7601600]

36 **Inui M**, Manfrin A, Mamidi A, Martello G, Morsut L, Soligo S, Enzo E, Moro S, Polo S, Dupont S, Cordenonsi M, Piccolo S. USP15 is a deubiquitylating enzyme for receptor-activated SMADs. *Nat Cell Biol* 2011; **13**: 1368-1375 [PMID: 21947082 DOI: 10.1038/ncb2346]

37 **Huang X**, Langelotz C, Hetfeld-Pechoc BK, Schwenk W, Dubiel W. The COP9 signalosome mediates beta-catenin degradation by deneddylation and blocks adenomatous polyposis coli destruction *via* USP15. *J Mol Biol* 2009; **391**: 691-702 [PMID: 19576224 DOI: 10.1016/j.jmb.2009.06.066]

38 **Zhou Q**, Cheng C, Wei Y, Yang J, Zhou W, Song Q, Ke M, Yan W, Zheng L, Zhang Y, Huang K. USP15 potentiates NF-κB activation by differentially stabilizing TAB2 and TAB3. *FEBS J* 2020; **287**: 3165-3183 [PMID: 31903660 DOI: 10.1111/febs.15202]

39 **Nusse R**, Clevers H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* 2017; **169**: 985-999 [PMID: 28575679 DOI: 10.1016/j.cell.2017.05.016]

40 **Duñach M**, Del Valle-Pérez B, García de Herreros A. p120-catenin in canonical Wnt signaling. *Crit Rev Biochem Mol Biol* 2017; **52**: 327-339 [PMID: 28276699 DOI: 10.1080/10409238.2017.1295920]

41 **Warde-Farley D**, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD, Morris Q. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 2010; **38**: W214-W220 [PMID: 20576703 DOI: 10.1093/nar/gkq537]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4236-4245

DOI: 10.3748/wjg.v27.i26.4236

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Feasibility of totally laparoscopic gastrectomy without prophylactic drains in gastric cancer patients

Hao Liu, Peng Jin, Xu Quan, Yi-Bin Xie, Fu-Hai Ma, Shuai Ma, Yang Li, Wen-Zhe Kang, Yan-Tao Tian

ORCID number: Hao Liu 0000-0001-5809-6824; Peng Jin 0000-0001-8179-6191; Xu Quan 0000-0001-6177-9503; Yi-Bin Xie 0000-0002-0255-3018; Fu-Hai Ma 0000-0003-2437-6881; Shuai Ma 0000-0003-1738-6651; Yang Li 0000-0002-4549-7087; Wen-Zhe Kang 0000-0001-9965-8109; Yan-Tao Tian 0000-0001-6479-7547.

Author contributions: Liu H and Jin P contributed equally to this work; Xie YB and Xu Q designed the research; Liu H, Jin P, and Ma FH analyzed the data and wrote the main manuscript text; Li Y, Kang WZ, and Ma S collected the patients' clinical data; Tian YT was involved in study conception and design, data interpretation, manuscript revision, and discussion.

Supported by National Natural Science Foundation of China, No. 81772642; and Capital's Funds for Health Improvement and Research, No. CFH 2018-2-4022.

Institutional review board statement: This study was approved by the Institutional Review Board of the National Cancer Center Hospital.

Informed consent statement: Patients were not required to give informed consent to the study

Hao Liu, Peng Jin, Xu Quan, Yi-Bin Xie, Fu-Hai Ma, Shuai Ma, Yang Li, Wen-Zhe Kang, Yan-Tao Tian, Department of Pancreatic and Gastric Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Hao Liu, Department of Gastrointestinal Surgery, The Second Hospital, Jilin University, Changchun 130041, Jilin Province, China

Corresponding author: Yan-Tao Tian, MD, Professor, Surgeon, Department of Pancreatic and Gastric Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 17 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China. tianyantao@cicams.ac.cn

Abstract

BACKGROUND

Prophylactic drains have been used to remove intraperitoneal collections and detect complications early in open surgery. In the last decades, minimally invasive gastric cancer surgery has been performed worldwide. However, reports on routine prophylactic abdominal drainage after totally laparoscopic distal gastrectomy are few.

AIM

To evaluate the feasibility performing totally laparoscopic distal gastrectomy without prophylactic drains in selected patients.

METHODS

Data of patients with distal gastric cancer who underwent totally laparoscopic distal gastrectomy with and without prophylactic drainage at China National Cancer Center/Cancer Hospital from February 2018 to August 2019 were reviewed. The outcomes between patients with and without prophylactic drainage were compared.

RESULTS

A total of 457 patients who underwent surgery for gastric cancer were identified. Of these, 125 patients who underwent totally laparoscopic distal gastrectomy were included. After propensity score matching, data of 42 pairs were extracted. The incidence of concurrent illness was higher in the drain group (42.9% *vs* 31.0%, $P = 0.258$). The overall postoperative complication rates were 19.5% and 10.6% in

the drain (n = 76) and no-drain groups (n = 49), respectively; there were no significant differences between the two groups (P > 0.05). The difference between the two groups based on the need for percutaneous catheter drainage was also not significant (9.8% *vs* 6.4%, P = 0.700). However, patients with a larger body mass index (≥ 29 kg/m^2) were prone to postoperative complications (P = 0.042). In addition, the number of days from surgery until the first flatus (4.33 ± 1.24 d *vs* 3.57 ± 1.85 d, P = 0.029) was greater in the drain group.

CONCLUSION

Omitting prophylactic drainage may reduce surgery time and result in faster recovery. Routine prophylactic drains are not necessary in selected patients. A prophylactic drain may be useful in high-risk patients.

Key Words: Gastric cancer; Prophylactic drainage; Totally laparoscopic gastrectomy; Enhanced recovery after surgery; Minimally invasive surgery; Early gastric cancer

Core Tip: We reviewed the outcomes of 125 consecutive patients with distal gastric cancer who underwent totally laparoscopic distal gastrectomy with and without prophylactic drainage at China National Cancer Center/Cancer Hospital from February 2018 to August 2019. We found that performing totally laparoscopic gastrectomy without prophylactic drains in selected patients is possible. It significantly improved postoperative comfort and did not increase the risk of postoperative complications.

Citation: Liu H, Jin P, Quan X, Xie YB, Ma FH, Ma S, Li Y, Kang WZ, Tian YT. Feasibility of totally laparoscopic gastrectomy without prophylactic drains in gastric cancer patients. *World J Gastroenterol* 2021; 27(26): 4236-4245
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4236.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4236

INTRODUCTION

In the last decade, gastric cancer has been one of the most frequently occurring malignancies worldwide, with about one million new cases of gastric cancer in 2017. It is the fifth most common malignancy and the third highest malignant tumor, with an estimated 783000 deaths[1]. In China, there were approximately 677000 new gastric cancer cases in 2015. This accounted for half of the new gastric cancer cases worldwide [2].

In 1994, Kitano *et al*[3] reported the first case of laparoscopic assisted distal gastrectomy (LADG) with D2 lymphadenectomy[3]. A recent multi-center clinical study in South Korea also confirmed that the operation was safe and effective[4]. With the development of surgical instruments and technology, early minimally invasive gastric cancer surgery has been widely performed worldwide. Meanwhile, the interim results of a class 01 clinical trial led by China's Southern Hospital showed that the efficacy of laparoscopic surgery for advanced distal gastric cancer was comparable to that of open surgery[5].

The development of laparoscopic gastric cancer surgery has led to its emergence as a treatment modality for distal gastric cancer. Compared with laparoscopic assisted surgery, totally laparoscopic distal gastrectomy (TLDG) is an intra-cavitary anastomosis, which does not require an auxiliary small incision. The reconstruction of TLDG anastomosis is safer, regardless of tumor location, with a lower incidence of incision problems than LADG. Moreover, it can be performed more effectively in obese patients[6,7].

Prophylactic drains have been used to remove intraperitoneal collections and detect complications early. However, numerous trials have failed to demonstrate a reduction in postoperative complications by routine drainage in gastrointestinal surgery[8]. Several studies performed after open gastrectomy or LADG concluded that the prophylactic use of drains did not significantly improve postoperative outcomes.

because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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

Data sharing statement: Some or all data and code generated or used during the study are available from the corresponding author by request

Manuscript source: Unsolicited manuscript

Specialty type: Surgery

Country/Territory of origin: China

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

Received: November 10, 2020
Peer-review started: November 10, 2020
First decision: November 30, 2020
Revised: December 16, 2020
Accepted: March 29, 2021
Article in press: March 29, 2021
Published online: July 14, 2021

P-Reviewer: Amin S, Chen Y, Park J, Tsegmed U
S-Editor: Zhang L
L-Editor: Wang TQ
P-Editor: Wang LL

However, there are few studies on routine prophylactic drainage after TLDG.

In the current retrospective study, we compared the outcomes of patients who underwent TLDG with and without drainage to clarify the value of routine prophylactic drainage in uncomplicated TLDG procedures for distal gastric cancer.

MATERIALS AND METHODS

Patients

We reviewed the outcomes of 457 consecutive patients with distal gastric cancer who underwent TLDG with and without prophylactic drainage at China National Cancer Center/Cancer Hospital from February 2018 to August 2019. Among them, 145 patients who underwent proximal gastrectomy or total gastrectomy, 159 patients who underwent laparoscopic assisted surgery, 23 who underwent open gastrectomy (including four cases converted from laparoscopic surgery), and five who underwent simultaneous surgery for other diseases such as choledocholithiasis (n = 1), ovarian tumor (n = 1), and pancreatic tail (n = 3) were excluded. Finally, a total of 125 patients were included in this study. They were assigned to a drain or no-drain group according to their operation records. The drain group comprised 76 patients who underwent TLDG with routine prophylactic drainage, and the no-drain group comprised 49 patients who underwent TLDG without routine prophylactic drainage (Figure 1).

Totally laparoscopic distal gastrectomy

The extent of gastrectomy and lymph node dissection were determined based on the Japanese gastric cancer treatment guidelines[9]. The surgeon was on the left side of the patient to finish laparoscopic ligation and division, and the first assistant was positioned on the opposite side. A cameraman stood between the patient' s legs. A five-port system (*i.e.*, two 5 mm and three 12 mm ports) was used for each totally laparoscopic distal gastrectomy. Ten-millimeter flexible laparoscopes were used, with CO_2 pressure maintained at 13-15 mmHg.

The operator was on the left side of the patient to perform Billroth-I reconstruction using a modified delta-shaped anastomosis[10] or overlap anastomosis[8]. Billroth-II or Roux-en-Y reconstruction was performed on the right side of the patients.

Postoperative management

Patients in both groups were administered prophylactic antibiotics 30 min before surgery. The decision of whether to use a prophylactic drain was made by the surgeon. Oral intake of water was initiated on the first day after surgery. A soft diet was initiated after the patient could tolerate liquid meals, and postoperative upper gastrointestinal contrast confirmed the absence of anastomotic leakage.

Outcome assessment

The clinical, operative, and pathological variables were compared between the two groups based on the information obtained from our prospectively collected surgical database. Early postoperative complications (occurring on postoperative days 0-30) were graded using the Clavien–Dindo classification. Early postoperative complications requiring medical, radiological, or surgical interventions (grade 2 or higher) were regarded as events. The risk for the occurrence of postoperative complications was also assessed.

Statistical analyses

All values are expressed as the mean ± SD. The χ^2 test and Student's t test were used to compare the categorical and continuous variables, respectively. For categorical data, the chi-squared test or Fisher's exact test was performed. A P value of < 0.05 was considered significant. Statistical analyses were performed using Statistic Package for Social Science. 20.

Propensity score matching

Multiple factor logistic regression models were used to calculate the propensity score for each patient to balance the following covariates: Age, sex, body mass index (BMI), abdominal operation history, smoking history, drinking history, concurrent illness, American Society of Anesthesiologists classification, operation time, estimated blood loss, primary tumor stage, regional lymph node stage, tumor size, and number of

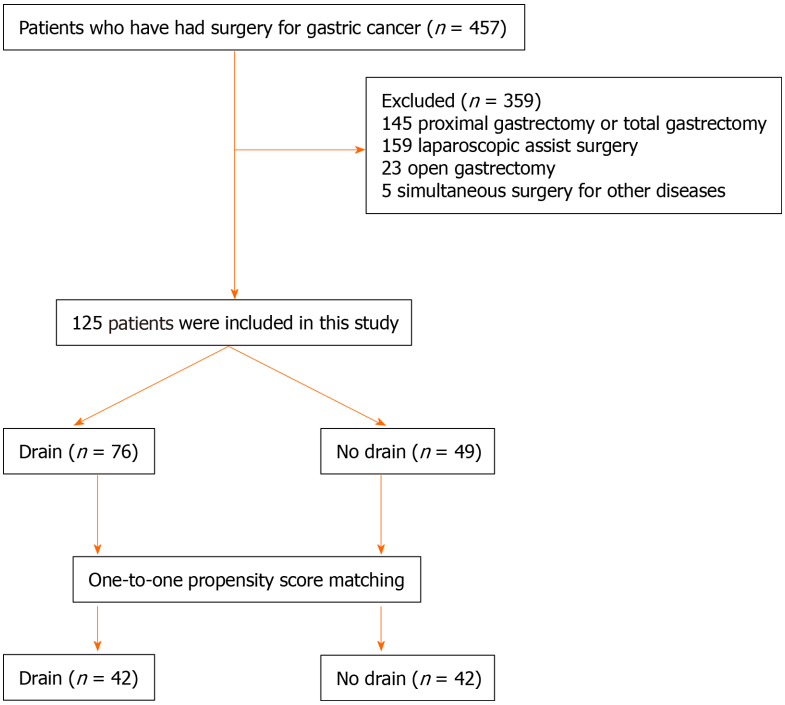

Figure 1 Flow chart of the patients assessed in this study.

retrieved lymph nodes. We imposed a caliper width of 0.1 of the standard deviation of the logistic propensity score.

RESULTS

Patient characteristics

Table 1 shows the clinical characteristics of patients undergoing TLDG with or without a prophylactic drain. No significant differences were observed in patient sex, age, BMI, American Society of Anesthesiologists classification, abdominal operation history, smoking history, drinking history, concurrent illness primary tumor stage, or regional lymph node stage between the two groups after propensity score matching (PSM).

Operative findings

The operative outcomes are summarized in Table 2. The drain group had a longer operating time than the no-drain group (198.4 ± 41.0 min *vs* 164.0 ± 37.0 min, $P < 0.001$). Mean estimated blood loss and intraoperative blood transfusion were similar between the two groups. There were no significant differences between the two groups in terms of the number of retrieved lymph nodes and tumor size ($P > 0.05$). After PSM, no significant differences were noted in operating time between the drain and no-drain groups.

Recovery

The recovery outcomes are listed in Table 3. The number of days from surgery to the initiation of soft diet (5.34 ± 2.27 d *vs* 4.17 ± 2.13 d, $P = 0.036$) and to first flatus (4.29 ± 1.45 d *vs* 3.55 ± 1.83 d, $P = 0.041$) were greater in the drain group. There were no significant differences in the time to ambulation or length of postoperative hospital stay (8.15 ± 2.9 d *vs* 6.77 ± 2.3 d, $P = 0.219$) between the two groups. Postoperative C-reactive protein levels (8.24 ± 4.47 mg/L *vs* 8.67 ± 5.97 mg/L, $P > 0.05$) and postoperative maximum body temperature (Tmax) (37.6 ± 0.6 ℃ *vs* 37.5 ± 0.4 ℃, $P > 0.05$) were similar between the two groups. After PSM, only the number of days from surgery to first flatus (4.33 ± 1.24 d *vs* 3.57 ± 1.85 d, $P = 0.029$) was greater in the drain group.

Short-time outcomes

Postoperative patient complications are listed in Table 4. No mortality was recorded in

Table 1 Characteristics of patients who underwent totally laparoscopic distal gastrectomy with or without prophylactic drain

	ALL patients			Propensity-matched patients		
Characteristic	Drain (*n* = 76)	No drain (*n* = 49)	*P* value	Drain (*n* = 42)	No drain (*n* = 42)	*P* value
Sex (M/F)	54/22	33/16	0.660	31/11	29/13	0.629
Age	57.58 ± 9.90	54.14 ± 12.63	0.092	57.4 ± 9.9	58.1 ± 10.8	0.739
BMI (kg/m^2)	24.71 ± 3.76	24.64 ± 3.72	0.915	24.3 ± 3.5	24.4 ± 2.7	0.879
ASA (1/2/3), *n* (%)			0.562			0.565
1	1 (1.3)	0 (0.0)		1 (2.4)	0 (0.0)	
2	70 (92.1)	44 (89.8)		38 (90.5)	38 (90.5)	
3	5 (6.6)	5 (10.2)		3 (7.1)	4 (9.5)	
pT stage, *n* (%)			0.605			0.805
T1	39 (52.0)	24 (49.0)		20 (47.6)	20 (47.6)	
T2	10 (13.3)	11 (22.4)		6 (14.3)	10 (23.8)	
T3	9 (12)	5 (10.2)		6 (14.3)	4 (9.5)	
T4a	17 (22.7)	9 (18.4)		10 (23.8)	8 (19)	
pN stage, *n* (%)			0.888			0.760
N0	34 (44.7)	20 (40.8)		16 (38.1)	18 (42.9)	
N1	16 (21.1)	12 (24.5)		9 (21.4)	11 (26.2)	
N2	14 (18.4)	7 (14.3)		9 (21.4)	5 (11.9)	
N3	12 (15.8)	10 (20.4)		8 (19)	8 (19)	
Previous abdominal operation, *n* (%)	13 (17.1)	13 (26.5)	0.205	6 (14.3)	10 (23.8)	0.266
Neoadjuvant chemotherapy, *n* (%)	5 (6.6)	2 (4.1)	0.704	4 (9.5)	2 (4.8)	0.676
Concurrent illness, *n* (%)	34 (44.7)	14 (28.6)	0.070	18 (42.9)	13 (31.0)	0.258

ASA: American Society of Anesthesiologists; pT: Primary tumor; pN: Regional lymph node; BMI: Body mass index.

Table 2 Operative findings

Variable	All patients			Propensity-matched patients		
	Drain (*n* = 76)	No drain (*n* = 49)	*P* value	Drain (*n* = 42)	No drain (*n* = 42)	*P* value
Operation time (min)	198.4 ± 41.0	164.0 ± 37.0	< 0.001	180.2 ± 33.4	168.0 ± 36.7	0.113
Estimated blood loss (mL)	85.3 ± 80.7	70.82 ± 51.5	0.267	72.9 ± 45.8	73.8 ± 54.4	0.931
Intraoperative blood transfusion, *n* (%)	2 (2.6)	2 (4.1)	0.645	1 (2.4)	2 (4.8)	1.000
Tumor size (cm)	3.5 ± 1.6	3.6 ± 1.5	0.664	3.6 ± 1.7	3.5 ± 1.4	0.839
No. of retrieved lymph nodes	36.7 ± 13.7	39.1 ± 14.2	0.346	40.0 ± 11.2	40.0 ± 15.1	0.923

either group. The overall postoperative complication rates were 15.8% and 10.2% in the drain and no-drain groups, respectively ($P > 0.05$). No anastomotic bleeding, anastomotic leakage, lymph leakage, ileus, or pancreatic fistula occurred in either group. Clavien-Dindo grade 3 complications included duodenal stump leakage ($n = 2$), anastomotic leakage ($n = 2$), intra-abdominal abscess ($n = 2$), and intra-abdominal bleeding ($n = 1$) in the drainage group. The need for percutaneous catheter drainage (PCD) was not significantly different between the groups (9.8% *vs* 6.4%, $P = 0.700$). After PSM, no significant differences were noted in the complications between the drain and no-drain groups.

Risk assessment for the occurrence of postoperative complication

Postoperative complication risk factors are listed in Table 5. Between the two groups,

Table 3 Recovery

Variable	All patients			Propensity-matched patients		
	Drain (*n* = 76)	No drain (*n* = 49)	*P* value	Drain (*n* = 42)	No drain (*n* = 42)	*P* value
Time to ambulation, POD	2.51 ± 1.34	2.98 ± 1.39	0.064	2.90 ± 1.54	3.07 ± 1.44	0.610
Time to first flatus, POD	3.97 ± 1.24	3.55 ± 1.79	0.122	4.33 ± 1.24	3.57 ± 1.85	0.029
Time to first eating of soft diet, POD	4.70 ± 2.17	4.14 ± 2.09	0.159	5.02 ± 1.88	4.17 ± 2.20	0.058
Postoperative hospital stay	7.88 ± 3.96	6.73 ± 5.13	0.164	7.93 ± 4.98	6.81 ± 5.50	0.331
CRP	7.54 ± 4.38	8.53 ± 5.91	0.286	7.66 ± 3.89	8.71 ± 5.95	0.339
Tmax	37.6 ± 0.5	37.5 ± 0.4	0.239	37.60 ± 0.60	37.48 ± 0.40	0.300

POD: Postoperative days.

Table 4 Postoperative complications

Complication, *n*	All patients			Propensity-matched patients		
	Drain (*n* = 76), *n* (%)	No drain (*n* = 49), *n* (%)	*P* value	Drain (*n* = 42), *n* (%)	No drain (*n* = 42), *n* (%)	*P* value
Total	12(15.8)	5 (10.2)	0.374	8 (19.0)	4 (9.5)	0.212
Clavien–Dindo grade II	4 (5.2)	2 (4.0)		3 (7.2)	1 (2.4)	
Incision	1 (1.3)	1 (2.0)		0 (0.0)	0 (0.0)	
System complications	1 (1.3)	1 (2.0)		1 (2.4)	1 (2.4)	
Abdominal effusion	2 (2.6)	0 (0.0)		2 (4.8)	0 (0.0)	
Clavien–Dindo grade III	8 (10.6)	3 (6.0)		5 (12)	3 (7.2)	
Duodenal stump leakage	2 (2.6)	0 (0.0)		2 (2.4)	0 (0.0)	
Anastomotic Leakage	2 (2.6)	0 (0.0)		0 (0.0)	0 (0.0)	
Intra-abdominal bleeding	1 (1.3)	0 (0.0)		1 (2.4)	0 (0.0)	
Intra-abdominal abscess	3 (3.9)	2 (4.0)		2 (2.4)	2 (2.4)	
Pleural effusion	0 (0.0)	1 (2.0)		0 (0.0)	1 (2.4)	
Mortality	0 (0.0)	0 (0.0)	1	0 (0.0)	0 (0.0)	1

PCD: Percutaneous catheter drainage.

no significant differences were observed in most variables. However, the patients with a larger BMI had a higher possibility of postoperative complications (27.44 ± 3.92 *vs* 24.25 ± 3.53, $P = 0.01$). In addition, we identified that patients with a BMI ≥ 29 kg/m^2 were prone to postoperative complications ($P = 0.042$). A prophylactic drain may be useful in patients with a higher risk, larger BMI, or more concurrent illness. Prophylactic drains was not an independent risk factor for postoperative complications.

DISCUSSION

Since 2015, totally laparoscopic surgery has been widely used in clinical practice, although there are few reports on whether totally laparoscopic surgery requires prophylactic drains[10,11]. Most studies on prophylactic drains were based on open gastrectomy. Cochrane review included four single-institution, randomized controlled trials that sought to evaluate the role of prophylactic drain placement in gastric resection for gastric cancer[12-14]. In this study, we reviewed the clinicopathological data of patients with gastric cancer during the past 2 years and found that routine prophylactic drains were not necessary in selected patients. To minimize the risk of confounding variables, PSM was used. Routine prophylactic drains are not necessary in all patients. A prophylactic drain may be useful in patients at higher risk.

Table 5 Risk assessment for the occurrence of postoperative complication

Variable	Postoperative complications (+) (*n* = 17)	Postoperative complications (-) (*n* = 108)	*P* value
Sex			0.584
Male	13	74	
Female	4	34	
Age	59.59 ± 9.62	55.70 ± 11.30	0.182
BMI (kg/m^2), *n* (%)	27.44 ± 3.92	24.25 ± 3.53	0.001
≥ 29	5 (38.5)	10 (13.3)	0.042
< 29	8 (61.5)	65 (86.7)	
ASA (1/2/3), *n* (%)			0.769
1	0 (0.0)	1 (100.0)	
2	15 (13.2)	99 (86.8)	
3	2 (20.0)	8 (80.0)	
Preoperative ALB (g)	39.62 ± 4.65	40.18 ± 5.86	0.709
Preoperative HGB (g/L)	136.59 ± 17.77	135.26 ± 19.36	0.791
pT stage, *n* (%)			0.776
T1	7 (38.5)	56 (49.3)	
T2	4 (23.1)	17 (20.0)	
T3	3 (15.4)	11 (8.0)	
T4a	3 (23.1)	23 (22.7)	
pN stage, *n* (%)			0.872
N0	8 (38.5)	46 (45.3)	
N1	3 (15.4)	25 (22.7)	
N2	3 (23.1)	18 (13.3)	
N3	3 (23.1)	19 (18.7)	
Previous abdominal operation			0.103
Yes	1	25	
No	16	83	
Neoadjuvant chemotherapy			0.234
Yes	2	5	
No	15	103	
Concurrent illness			0.800
Yes	7	41	
No	10	67	
Drain, *n* (%)	12 (15.8)	64 (84.2)	0.374
No drain, *n* (%)	5 (10.2)	44 (89.8)	
Type of reconstruction, *n* (%)			0.357
Billroth I	4 (30.8)	32 (36.0)	
Billroth II	10 (61.5)	69 (64.0)	
Roux-en-Y	3 (7.7)	7 (0.0)	
Operative time (min)	195.82 ± 49.12	183.16 ± 41.69	0.258
Blood loss (mL)	62.94 ± 42.54	82.22 ± 74.07	0.298

ASA: American Society of Anesthesiologists; BMI: Body mass index; ALB: Albumin; HGB: Hemoglobin; pT: Primar tumor; pN: Regional lymph node.

Prophylactic drains have been used to enhance early detection of complications, prevent collection of fluid, reduce morbidity and mortality, and decrease the duration of hospital stay[15,16]. The present study results showed that there was no significant difference between the two groups in terms of postoperative hospital stay. The length of the postoperative hospital stay in the no-drain group was shorter than that in the drain group (7.93 ± 4.98 d *vs* 6.81 ± 5.50 d, $P > 0.05$). Among the 17 patients who experienced postoperative complications, there was also no significant difference between the two groups in terms of postoperative hospital stay. This result was different from that of Hirahara *et al*[10] study. In addition, omitting prophylactic drainage significantly improved the postoperative comfort of patients due to an earlier flatus (4.33 ± 1.24 d *vs* 3.57 ± 1.85 d, $P < 0.05$).

Moreover, the application of prophylactic drains did not reduce the incidence of complications, and the rate of complications was even higher in the drain group. However, there was no statistically significant difference between the two groups (19.0% *vs* 9.5%, $P > 0.05$). Through risk assessment, we identified that patients with a BMI ≥ 29 kg/m^2 are prone to postoperative complications ($P = 0.042$). More visceral fat may make surgery more difficult. Thus, prophylactic drain is recommended for patients with a BMI > 29 kg/m^2.

For patients with mild symptoms, administration of broad-spectrum antibiotics may be a good conservative management strategy. However, patients with severe symptoms need PCD. In the current study, postoperative complications were recognized in approximately 15% of patients. Two cases of duodenal stump leakage and two cases of intra-abdominal abscess occurred in the drain group, all of which required PCD. In the no-drain group, two cases of intra-abdominal abscess and one case of pleural effusion needed PCD. There was no significant difference between the two groups. Prophylactic drains do not alter the rates of secondary drainage procedures. Thus, omitting prophylactic drains during gastric cancer surgery did not increase the risk of PCD postoperatively. Similarly, in a study by Lee *et al*[16], omitting prophylactic drains did not increase the risk of PCD postoperatively, while male sex, older age, and longer operative time were identified as independent risk factors for postoperative PCD in patients without prophylactic drains.

CONCLUSION

In conclusion, omitting the use of prophylactic drains in selected patients during surgery for gastric cancer is feasible. It can significantly improve the postoperative comfort of patients and does not increase the risk of postoperative complications.

ARTICLE HIGHLIGHTS

Research background

Prophylactic drains have been used to remove intraperitoneal collections and detect complications early in open surgery. In the last decades, minimally invasive gastric cancer surgery has been performed worldwide. However, reports on routine prophylactic abdominal drainage after totally laparoscopic distal gastrectomy are few.

Research motivation

To evaluate the feasibility of performing totally laparoscopic distal gastrectomy without prophylactic drains in selected patients.

Research objectives

To evaluate the feasibility of performing totally laparoscopic distal gastrectomy without prophylactic drains in selected patients.

Research methods

Data of patients with distal gastric cancer who underwent totally laparoscopic distal gastrectomy with and without prophylactic drainage at China National Cancer

Center/Cancer Hospital from February 2018 to August 2019 were reviewed.

Research results

After PSM, data of 42 pairs were extracted. The incidence of concurrent illness was higher in the drain group (42.9% *vs* 31.0%, P = 0.258). The overall postoperative complication rates were 19.5% and 10.6% in the drain (n = 76) and no-drain groups (n = 49), respectively; there were no significant differences between the two groups (P > 0.05). The difference between the two groups based on the need for percutaneous catheter drainage was also not significant (9.8% *vs* 6.4%, P = 0.700). However, patients with a larger body mass index (≥ 29 kg/m^2) were prone to postoperative complications (P = 0.042). In addition, the number of days from surgery until the first flatus (4.33 ± 1.24 d *vs* 3.57 ± 1.85 d, P = 0.029) was greater in the drain group.

Research conclusions

Omitting prophylactic drainage may reduce surgery time and result in faster recovery. Routine prophylactic drains are not necessary in selected patients. A prophylactic drain may be useful in high-risk patients.

Research perspectives

Omitting the use of prophylactic drains can significantly improve the postoperative comfort of patients and does not increase the risk of postoperative complications.

REFERENCES

1 **Smith RJ**, Bryant RG. Metal substitutions incarbonic anhydrase: a halide ion probe study. *Biochem Biophys Res Commun* 1975; **66**: 1281-1286 [DOI: 10.1016/0006-291x(75)90498-2]

2 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]

3 **Kitano S**, Iso Y, Moriyama M, Sugimachi K. Laparoscopy-assisted Billroth I gastrectomy. *Surg Laparosc Endosc* 1994; **4**: 146-148 [PMID: 8180768]

4 **Kim HH**, Hyung WJ, Cho GS, Kim MC, Han SU, Kim W, Ryu SW, Lee HJ, Song KY. Morbidity and mortality of laparoscopic gastrectomy *vs* open gastrectomy for gastric cancer: an interim report--a phase III multicenter, prospective, randomized Trial (KLASS Trial). *Ann Surg* 2010; **251**: 417-420 [PMID: 20160637 DOI: 10.1097/SLA.0b013e3181cc8f6b]

5 **Yu J**, Huang C, Sun Y, Su X, Cao H, Hu J, Wang K, Suo J, Tao K, He X, Wei H, Ying M, Hu W, Du X, Hu Y, Liu H, Zheng C, Li P, Xie J, Liu F, Li Z, Zhao G, Yang K, Liu C, Li H, Chen P, Ji J, Li G; Chinese Laparoscopic Gastrointestinal Surgery Study (CLASS) Group. Effect of Laparoscopic *vs* Open Distal Gastrectomy on 3-Year Disease-Free Survival in Patients With Locally Advanced Gastric Cancer: The CLASS-01 Randomized Clinical Trial. *JAMA* 2019; **321**: 1983-1992 [PMID: 31135850 DOI: 10.1001/jama.2019.5359]

6 **Kim MG**, Kawada H, Kim BS, Kim TH, Kim KC, Yook JH. A totally laparoscopic distal gastrectomy with gastroduodenostomy (TLDG) for improvement of the early surgical outcomes in high BMI patients. *Surg Endosc* 2011; **25**: 1076-1082 [PMID: 20835726 DOI: 10.1007/s00464-010-1319-0]

7 **Kim MG**, Kim KC, Kim BS, Kim TH, Kim HS, Yook JH. A totally laparoscopic distal gastrectomy can be an effective way of performing laparoscopic gastrectomy in obese patients (body mass index≥30). *World J Surg* 2011; **35**: 1327-1332 [PMID: 21424875 DOI: 10.1007/s00268-011-1034-6]

8 **Ishikawa K**, Matsumata T, Kishihara F, Fukuyama Y, Masuda H. Laparoscopy-assisted distal gastrectomy for early gastric cancer with *vs* without prophylactic drainage. *Surg Today* 2011; **41**: 1049-1053 [PMID: 21773892 DOI: 10.1007/s00595-010-4448-0]

9 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer* 2017; **20**: 1-19 [PMID: 27342689 DOI: 10.1007/s10120-016-0622-4]

10 **Hirahara N**, Matsubara T, Hayashi H, Takai K, Fujii Y, Tajima Y. Significance of prophylactic intra-abdominal drain placement after laparoscopic distal gastrectomy for gastric cancer. *World J Surg Oncol* 2015; **13**: 181 [PMID: 25962503 DOI: 10.1186/s12957-015-0591-9]

11 **Shimoike N**, Akagawa S, Yagi D, Sakaguchi M, Tokoro Y, Nakao E, Tamura T, Fujii Y, Mochida Y, Umemoto Y, Yoshimoto H, Kanaya S. Laparoscopic gastrectomy with and without prophylactic drains in gastric cancer: a propensity score-matched analysis. *World J Surg Oncol* 2019; **17**: 144 [PMID: 31420062 DOI: 10.1186/s12957-019-1690-9]

12 **Alvarez Uslar R**, Molina H, Torres O, Cancino A. Total gastrectomy with or without abdominal drains. A prospective randomized trial. *Rev Esp Enferm Dig* 2005; **97**: 562-569 [PMID: 16266223 DOI: 10.4321/s1130-01082005000800004]

13 **Kumar M**, Yang SB, Jaiswal VK, Shah JN, Shreshtha M, Gongal R. Is prophylactic placement of drains necessary after subtotal gastrectomy? *World J Gastroenterol* 2007; **13**: 3738-3741 [PMID: 17659736 DOI: 10.3748/wjg.v13.i27.3738]

14 **Kim J**, Lee J, Hyung WJ, Cheong JH, Chen J, Choi SH, Noh SH. Gastric cancer surgery without drains: a prospective randomized trial. *J Gastrointest Surg* 2004; **8**: 727-732 [PMID: 15358335 DOI: 10.1016/j.gassur.2004.05.018]

15 **Messager M**, Sabbagh C, Denost Q, Regimbeau JM, Laurent C, Rullier E, Sa Cunha A, Mariette C. Is there still a need for prophylactic intra-abdominal drainage in elective major gastro-intestinal surgery? *J Visc Surg* 2015; **152**: 305-313 [PMID: 26481067 DOI: 10.1016/j.jviscsurg.2015.09.008]

16 **Lee J**, Choi YY, An JY, Seo SH, Kim DW, Seo YB, Nakagawa M, Li S, Cheong JH, Hyung WJ, Noh SH. Do All Patients Require Prophylactic Drainage After Gastrectomy for Gastric Cancer? *Ann Surg Oncol* 2015; **22**: 3929-3937 [PMID: 25845430 DOI: 10.1245/s10434-015-4521-4]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4246-4247

DOI: 10.3748/wjg.v27.i26.4246

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CORRECTION

Correction to "Downregulation of FoxM1 inhibits the viability and invasion of gallbladder carcinoma cells, partially dependent on the induction of cellular senescence"

Jie Tao, Xin-Sen Xu, Yan-Zhou Song, Chang Liu

ORCID number: Jie Tao 0000-0002-9330-1077; Xin-Sen Xu 0000-0002-3339-9324; Yan-Zhou Song 0000-0001-9681-7194; Chang Liu 0000-0002-7318-1004.

Author contributions: Tao J and Xu XS revised the manuscript; Song YZ provided the raw data; Liu C designed the study.

Conflict-of-interest statement: There's no conflict of interest.

Manuscript source: Unsolicited manuscript

Specialty type: Oncology

Country/Territory of origin: China

Jie Tao, Xin-Sen Xu, Chang Liu, Department of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China

Yan-Zhou Song, Department of General Surgery, Lianyungang First People's Hospital, Lianyungang 222002, Jiangsu Province, China

Corresponding author: Chang Liu, MD, Chief Doctor, Department of Hepatobiliary Surgery, the First Affiliated Hospital of Medical College, Xi'an Jiaotong University, No. 277 West Yanta Road, Xi'an 710061, Shaanxi Province, China. liuchangdoctor@163.com

Abstract

We corrected the mistake of Figure 3, and replaced the incorrect images with the correct ones. The "adenovirus" was a typographical error in writing, and should be revised to "lentivirus".

Key Words: Correction; Unintentional; Mistake; Error; Sorting; Figure

Core Tip: We corrected the mistake of Figure 3 and manuscript text.

Citation: Tao J, Xu XS, Song YZ, Liu C. Correction to "Downregulation of FoxM1 inhibits the viability and invasion of gallbladder carcinoma cells, partially dependent on the induction of cellular senescence". *World J Gastroenterol* 2021; 27(26): 4246-4247

URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4246.htm

DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4246

TO THE EDITOR

We found a mistake in Figure 3[1]. This is an unintentional error that occurred when sorting through the images. We have replaced the incorrect images with the correct

Peer-review report's scientific quality classification

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

Received: March 30, 2021
Peer-review started: March 30, 2021
First decision: May 28, 2021
Revised: June 2, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 14, 2021

P-Reviewer: Chou YC, Fujita-Yoshigaki J
S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Liu JH

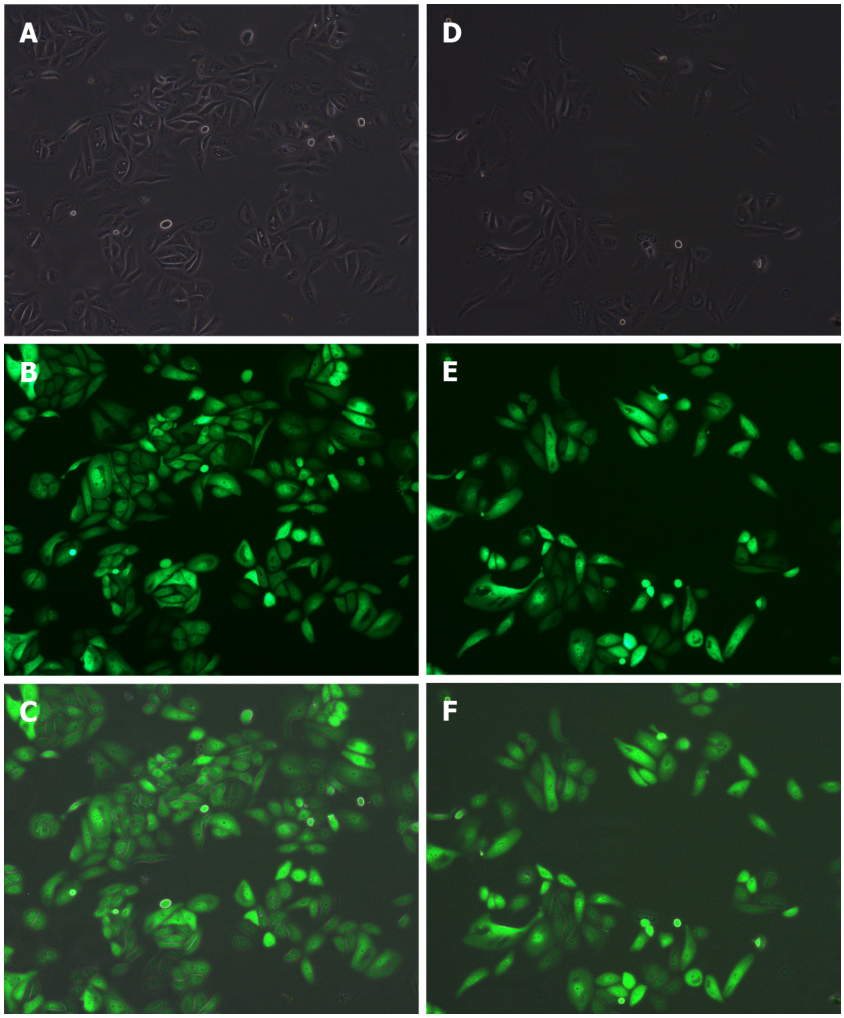

Figure 1 Representative photograph (100 ×) showing recombinant lentivirus transfection efficiency evaluated by fluorescence microscopy (transfected with the negative control, top; transfected with the shF1822, bottom). A and D: Light microscopy; B and E: Fluorescence microscopy; C and F: Superimposed image of the two images.

Figure 1. This technical error does not change the meaning of the picture or the conclusion of the manuscript. On the other hand, we only used lentivirus in the experiment. The "adenovirus" was a typographical error in writing. We mostly used "lentivirus" in the manuscript. "Adenovirus" should be revised to "lentivirus" in P9497 right column, line 11 and line 27; P9498 right column, line 26; P9499 right column, line 47; Figure 3 and Figure 4 captions. We apologize for our unintentional mistakes, which caused great inconvenience.

REFERENCES

1 **Tao J**, Xu XS, Song YZ, Qu K, Wu QF, Wang RT, Meng FD, Wei JC, Dong SB, Zhang YL, Tai MH, Dong YF, Wang L, Liu C. Down-regulation of FoxM1 inhibits viability and invasion of gallbladder carcinoma cells, partially dependent on inducement of cellular senescence. *World J Gastroenterol* 2014; **20**: 9497-9505 [PMID: 25071344 DOI: 10.3748/wjg.v20.i28.9497]

World Journal of Gastroenterology

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

World J Gastroenterol 2021 July 14; 27(26): 4248-4251

DOI: 10.3748/wjg.v27.i26.4248

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LETTER TO THE EDITOR

Impact of COVID-19 on the clinical status of patients with Wilson disease

Yu-Pei Zhuang, Hao-Jie Zhong

ORCID number: Yu-Pei Zhuang 0000-0002-9652-3898; Hao-Jie Zhong 0000-0001-6586-756X.

Author contributions: Zhong HJ designed the research; Zhuang YP analyzed the data; Zhuang YP and Zhong HJ performed the research and wrote the letter.

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

Manuscript source: Unsolicited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: China

Peer-review report's scientific

Yu-Pei Zhuang, Hao-Jie Zhong, Department of Gastroenterology, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou 510080, Guangdong Province, China

Hao-Jie Zhong, School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510000, Guangdong Province, China

Hao-Jie Zhong, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou 510000, Guangdong Province, China

Corresponding author: Hao-Jie Zhong, MD, PhD, Doctor, Department of Gastroenterology, The First Affiliated Hospital of Guangdong Pharmaceutical University, No. 19 Nonglingxia Road, Guangzhou 510080, Guangdong Province, China. jaxzhong@126.com

Abstract

The coronavirus disease 2019 (COVID-19) pandemic has greatly impacted health systems. Many guidelines on chronic liver diseases have been released to optimize the use of medical resources and patient management. However, most of these guidelines have been established through expert consensus because the existing data do not provide strong evidence for developing effective recommendations. As Wilson disease (WD) is a rare chronic liver disease, the impact of COVID-19 on the clinical status of patients with WD is unclear. The present study showed a marked shortage of medical resources for clinically managing patients with WD during the pandemic. Although patients with WD who consistently took anticopper therapy showed no significant differences in hepatic and extrahepatic markers before and after the pandemic, their complication incidences, especially the infection incidence, were significantly increased during the study period. Therefore, patients with WD should be encouraged to adhere to anticopper therapy and be closely monitored to prevent infections and other complications. The present study provides a clinical basis for further managing WD during the pandemic.

Key Words: Coronavirus disease 2019; Wilson disease; Clinical status; Complications; Infections; Anticopper therapy

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

Received: April 10, 2021
Peer-review started: April 10, 2021
First decision: May 24, 2021
Revised: June 3, 2021
Accepted: June 16, 2021
Article in press: June 16, 2021
Published online: July 14, 2021

P-Reviewer: Boregowda U
S-Editor: Ma YJ
L-Editor: Filipodia
P-Editor: Ma YJ

Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has had a long-lasting impact on the quality of care for patients with cirrhosis. Although many guidelines have been released for the rational use of medical resources, few clinical data are available to support these guidelines. The clinical features of patients with Wilson disease during the COVID-19 pandemic remain unclear. We compared the clinical features of patients with Wilson disease before and after the pandemic to clarify the impact of COVID-19 on these patients and provide a basis for their clinical management.

Citation: Zhuang YP, Zhong HJ. Impact of COVID-19 on the clinical status of patients with Wilson disease. *World J Gastroenterol* 2021; 27(26): 4248-4251
URL: https://www.wjgnet.com/1007-9327/full/v27/i26/4248.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i26.4248

TO THE EDITOR

Many countries have enforced social distancing and strict stay-at-home strategies to reduce the spread of coronavirus disease 2019 (COVID-19). However, these measures often negatively affect patients with other diseases[1,2]. Many guidelines on chronic liver diseases have been released to optimize the use of medical resources and patient management[3]. Most of these guidelines have been established through expert consensus because the existing data do not provide strong evidence for developing effective recommendations.

Given the high copper deposition in the livers of patients with Wilson disease (WD), these patients often develop liver injury and cirrhosis. Because WD has clinical features that are distinct from those of liver diseases caused by other etiologies and medical resources have been in short supply during the pandemic, the clinical features of patients with WD should be examined to improve their management. Therefore, we conducted a before–after study to investigate the clinical features of these patients before and during COVID-19.

We reviewed the medical records of patients with WD who were hospitalized for routine office visits or emergency visits at the First Affiliated Hospital of Guangdong Pharmaceutical University from 1 January 2018 to 3 September 2020. In China, the diagnostic criteria for WD are similar to those of the diagnostic scoring system for WD. During the COVID-19 pandemic, the number of WD inpatient visits dropped from 198 to 95, indicating a 52.02% decrease from the number of WD inpatient visits during the same period in 2019. These data indicate that the ongoing pandemic has led to a marked shortage of medical resources for clinically managing patients with WD. Medical data on 68 patients with WD who were hospitalized at our hospital during and before the pandemic were analyzed. All of these patients underwent anticopper therapy during the pandemic. Most of them (83.82%) had developed cirrhosis before the pandemic, and none had COVID-19.

The hepatic and extrahepatic status of patients who consistently used anticopper therapy during the pandemic did not significantly deteriorate (Table 1). However, owing to lifestyle changes and delayed screening for complications during the pandemic, the complication incidence increased significantly in these patients during the study period (23.53% *vs* 11.76%, $P = 0.021$). Notably, most complications (22/24) occurred in patients with WD-associated cirrhosis. Among the complications, infections were the most prevalent (11.8% *vs* 1.5%, $P = 0.016$). Although the community mitigation measures for COVID-19 are thought to reduce the incidence of respiratory infections in the general population[4], our data showed that the incidence of respiratory infections in patients with WD increased during the pandemic (7.4% *vs* 0%, $P = 0.063$).

Following the COVID-19 outbreak, the Chinese government implemented strong strict measures, and most citizens, except those involved in essential services, were ordered to stay at home. These measures helped keep the pandemic under control in China. However, the lockdown and movement restrictions often led to reduced physical activity, prolonged sedentary behaviors, imbalanced nutritional intake, poor mental health and delayed routine follow-up visits in these patients[5]. These changes were associated with cirrhosis-associated immune dysfunction and accounted for the

Table 1 Clinical features and complications in patients with Wilson disease before and after coronavirus disease 2019

	Before COVID-19 (*n* = 68)	After COVID-19 (*n* = 68)	*P* value
Demographic characteristics			
Age (yr)	28.00 (23.00–33.00)		-
Male sex	37 (54.41)		-
Hepatic features			
Elevated ALT (> 40 U/L)	16 (23.53)	12 (17.65)	0.424
Elevated AST (> 35 U/L)	13 (19.12)	17 (25.00)	0.388
Elevated bilirubin (> 17.1 μmol/L)	15 (22.06)	13 (19.12)	0.754
Hypoproteinemia (albumin < 35 g/L)	10 (14.71)	12 (17.65)	0.774
Elevated PT (> 15 s)	11 (16.18)	13 (19.12)	0.791
Elevated INR (> 1.5)	1 (1.47)	2 (2.94)	1.000
Child-Pugh			1.000
A	64 (94.12)	65 (95.59)	
B/C	4 (5.88)	3 (4.41)	
Cirrhosis	57 (83.82)	57 (83.82)	1.000
Extrahepatic features			
Neurological manifestations	50 (73.5)	49 (72.1)	1.000
Psychiatric manifestations	3 (4.4)	4 (5.9)	1.000
Kayser-Fleischer ring	32 (47.1)	35 (51.5)	0.375
Splenomegaly/splenectomy	45 (66.2)	47 (69.1)	0.688
Complications			
Any complication	8 (11.76)	16 (23.53)	0.021
Ascites	2 (1.5)	5 (7.4)	0.375
Infections	1 (1.5)	8 (11.8)	0.016
Respiratory infection	0 (0)	5 (7.4)	0.063
Urinary infection	0 (0)	1 (1.5)	1.000
Gastrointestinal infection	1 (1.5)	2 (2.9)	1.000
SBP	0 (0)	1 (1.5)	1.000
PVT	0 (0)	0 (0)	-
Gastroesophageal varices	5 (7.4)	7 (10.3)	0.500
Variceal bleeding	0 (0)	1 (1.5)	1.000
Hepatic encephalopathy	0 (0)	0 (0)	-
Renal impairment	0 (0)	0 (0)	-
Liver failure	0 (0)	0 (0)	-
HCC	0 (0)	0 (0)	-

Data are presented as medians (interquartile ranges) or *n* (%). ALT: Alanine transaminase; AST: Aspartate transaminase; HCC: Hepatocellular carcinoma; INR: International normalized ratio; PT: Prothrombin time; PVT: Portal vein thrombosis; SBP: Spontaneous bacterial peritonitis; COVID-19: Coronavirus disease 2019.

high infection risk[6].

In conclusion, the hepatic and extrahepatic status of patients with WD who adhered strictly to their anticopper therapy during the COVID-19 pandemic did not significantly worsen, but the complication incidence — especially the infection incidence — increased significantly. Therefore, patients with WD should be encouraged to adhere

to anticopper therapy and be closely monitored to prevent infections and other complications.

REFERENCES

1 **Mafham MM**, Spata E, Goldacre R, Gair D, Curnow P, Bray M, Hollings S, Roebuck C, Gale CP, Mamas MA, Deanfield JE, de Belder MA, Luescher TF, Denwood T, Landray MJ, Emberson JR, Collins R, Morris EJA, Casadei B, Baigent C. COVID-19 pandemic and admission rates for and management of acute coronary syndromes in England. *Lancet* 2020; **396**: 381-389 [PMID: 32679111 DOI: 10.1016/S0140-6736(20)31356-8]

2 **Villarreal-Garza C**, Aranda-Gutierrez A, Ferrigno AS, Platas A, Aloi-Timeus I, Mesa-Chavez F, Ayensa-Alonso A. The challenges of breast cancer care in Mexico during health-care reforms and COVID-19. *Lancet Oncol* 2021; **22**: 170-171 [PMID: 33539740 DOI: 10.1016/S1470-2045(20)30609-4]

3 **Bollipo S**, Kapuria D, Rabiee A, Ben-Yakov G, Lui RN, Lee HW, Kumar G, Siau K, Turnes J, Dhanasekaran R. One world, one pandemic, many guidelines: management of liver diseases during COVID-19. *Gut* 2020; **69**: 1369-1372 [PMID: 32499304 DOI: 10.1136/gutjnl-2020-321553]

4 **Olsen SJ**, Azziz-Baumgartner E, Budd AP, Brammer L, Sullivan S, Pineda RF, Cohen C, Fry AM. Decreased Influenza Activity During the COVID-19 Pandemic - United States, Australia, Chile, and South Africa, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 1305-1309 [PMID: 32941415 DOI: 10.15585/mmwr.mm6937a6]

5 **Pinto AJ**, Dunstan DW, Owen N, Bonfá E, Gualano B. Combating physical inactivity during the COVID-19 pandemic. *Nat Rev Rheumatol* 2020; **16**: 347-348 [PMID: 32355296 DOI: 10.1038/s41584-020-0427-z]

6 **Xiang M**, Zhang Z, Kuwahara K. Impact of COVID-19 pandemic on children and adolescents' lifestyle behavior larger than expected. *Prog Cardiovasc Dis* 2020; **63**: 531-532 [PMID: 32360513 DOI: 10.1016/j.pcad.2020.04.013]

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